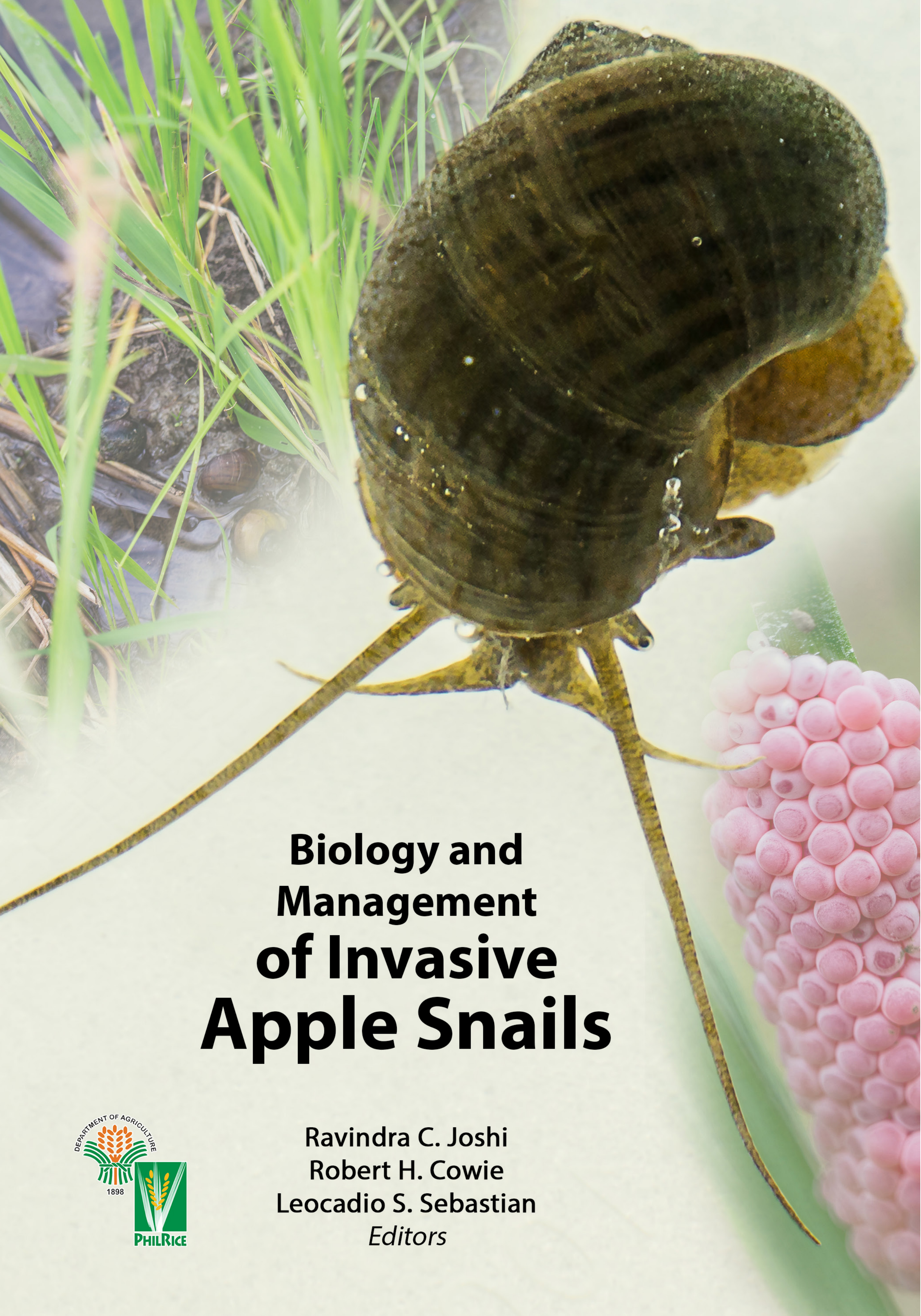

Biology and Management of Invasive Apple Snails

Ravindra C. Joshi
Robert H. Cowie
Leocadio S. Sebastian
Editors

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DEPARTMENT OF AGRICULTURE
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Foreword

The concern with invasive non-native species that exploded in the public consciousness beginning in the 1980s has focused especially on striking predators (e.g., Burmese python, lionfish), highly visible plants that overgrow native vegetation (e.g., kudzu, killer alga [*Caulerpa taxifolia*]), and biting and stinging insects that threaten human health (e.g., red imported fire ant, yellow fever [and zika] mosquito). Invasive snails rarely attract newspaper headlines and television spots. They are slow, they don't bite humans, and most are inconspicuous. Many have not been studied extensively, and the lay public cannot readily recognise features that distinguish them from native snails.

However, several are of great consequence, and these include apple snails of the genus *Pomacea*. In particular, *Pomacea canaliculata* was one of three snails canonized among 100 of the world's worst invasive species by the World Conservation Union (IUCN) in 2000. This listing was a response to the widespread introduction in Asia of this South American species, which causes extensive damage to both agriculture and native ecosystems. As a sign of how poorly understood some apple snails are, a *Pomacea* invasion into the southeastern United States was long thought to be of *P. canaliculata* but is now known to be of a different South American species, *P. maculata*. Further, once it was recognized as a distinct species, *P. maculata* was originally referred to as *P. insularum* and came to be known popularly in the USA as the "island apple snail" as an ongoing controversy arose about its impact on the snail-eating Everglades snail kite, a listed endangered species in the USA that originally specialized on eating the native *P. paludosa*. The invasion of *P. maculata* in the southeastern USA probably results from its introduction through the aquarium trade, which is also responsible for the establishment in Florida of *P. diffusa*, yet another South American species popular among aquarium enthusiasts. Furthermore, confusion about the identity of the invasive *Pomacea* species in Asia, introduced primarily as a human food resource, has only recently been resolved: instead of just one widespread species there are two, the same *P. canaliculata* and *P. maculata*.

Among this welter of introductions and confusion, it is heartening and timely to have this comprehensive, up-to-date volume on the history of apple snail invasions, their various impacts, their biology (including proper systematics), and what to do about them. *Biology and Management of Invasive Apple Snails* serves not only as an authoritative source for those trying to understand *Pomacea* impacts, how to manage them, and how to prevent further introductions, but as a model for invasion scientists generally as they begin to comprehend and grapple with the complexity of many of the most important invasions by all kinds of animals and plants. It highlights how important good basic science is to slowing the wave of invasions transforming the ecology of much of the earth.

I hope this book, published by the Philippine Rice Research Institute (PhilRice), will be widely used by researchers, extension workers, museum scientists, policy makers, consumers, and farmers, so that the threat of invasive apple snails will become part of history.

Daniel Simberloff, Ph.D.
University of Tennessee
United States of America

Foreword

Invasive apple snails (locally known as *Golden Kuhol*) were introduced to the Philippines in the early 1980s. They came either directly from their native South America or from elsewhere in Asia, perhaps Taiwan, and their first beachhead in Asia. The people who brought the snails saw them as a new and inexpensive source of protein for poor families as well as a potentially lucrative source of revenue, especially if exported as exotic "escargot". But the interest as food was short-lived and the export markets did not develop. The snails ended up in rice paddies, with their populations expanding into millions. By mid-980s, the apple snail became one big pest of rice not only in the Philippines but also throughout much of Southeast and Eastern Asia adding to the already great losses caused by a suite of other, mainly insect, pests. The financial losses incurred were immense (e.g., US$425 million to US$1.2 billion in the Philippines in 1990), primarily because of the loss of production and also due to the costs associated with attempts to control the ravenous snails. Farmers' health was impacted by the rampant use of inappropriate pesticides, and food and nutrition security were threatened by production losses.

Huge efforts were made in many countries to develop control measures, some more effective than others, but all incurring considerable costs in terms of financial outlay as well as backbreaking labour. In some countries these measures met some success, albeit with ongoing costs, while in others, the snails continued to ravage wetland crops as well as having potentially serious environmental and biodiversity impacts. Extensive applied research was undertaken during the first two decades following introduction of the snails. In the Philippines, PhilRice led in the basic and applied researches to manage the golden apple snail. In 2006, PhilRice published the multi-authored book, *Global Advances in Ecology and Management of Golden Apple Snails*, edited by Drs. Ravindra C. Joshi and Leocadio S. Sebastian.

Since then, new research had been done. The identities of the snails are now known definitively – *Pomacea canaliculata and Pomacea maculate* – such that the term "golden apple snail" is losing favour as it does not distinguish the two species. The present volume, which is not a second edition of the 2006 book but a compilation of new research, advances in management, and updated country reports, is also edited by Dr. Joshi and Dr. Sebastian, but now in collaboration with the widely acknowledged apple snail expert Dr. Robert H. Cowie.

As the Executive Director of PhilRice, it is with great pleasure, satisfaction, and honour that I write this foreword as an introduction to what will no doubt become a key important work, published by PhilRice. This book brings together both basic and applied research on apple snails in efforts to further advance management and control of these major invasive and noxious pests, and to support the farmers, extension workers, and others who are in the front lines of the battle.

Dr. Sailila E. Abdula, Ph.D.
Executive Director, PhilRice

Preface

Apple snails, family Ampullariidae, are so called because many species, notably in the genera *Pomacea* and *Pila*, bear large, round shells. *Pomacea* species are native to South and Central America, parts of the Caribbean, and the southeastern USA, while *Pila* species are native to Africa and Asia. In the year 2000, one species of apple snail, *Pomacea canaliculata*, was listed among the world's 100 most invasive species, largely because it had become a major pest of wetland rice in much of Southeast Asia. However this listing was published at a time when there was still confusion regarding the true identity of the invasive species in Asia; in fact two species are involved, not only *Pomacea canaliculata* but also *Pomacea maculata*. *Pomacea canaliculata* is native to Argentina and Uruguay, while *P. maculata* is more widely distributed from the La Plata region of Argentina to the Amazon basin of Brasil, including Uruguay and Paraguay, and possibly Bolivia, Ecuador and Peru.

These two species have commonly been referred to as golden apple snails, or GAS, often without clarifying specifically which species, perhaps both, was involved, or indeed simply assuming it to be *Pomacea canaliculata*. For clarity, this book avoids this ambiguous common name designation, and hopes that others will move forward with the correct species designation for the apple snails with which they work. Only in this way can research results be truly comparative and useful.

One or both of these species of *Pomacea* have become widely established not only in many parts of Southeast Asia but also in Japan, Taiwan, Guam, Hawaii, Papua New Guinea, the Dominican Republic, Spain and parts of the mainland USA. *Pomacea diffusa* has been introduced to Sri Lanka, and *Pomacea scalaris*, as well as *Pomacea canaliculata*, has been introduced to Taiwan. An additional unidentified species has been introduced to the southeastern USA. Most of these introductions are the result of escape or release from aquaculture operations, or happen through the pet trade. In the Philippines alone, estimates of economic losses associated with apple snails ranged from US$425 million to US$1.2 billion in 1990.

Pomacea species are also important transmitters of *Angiostrongylus cantonensis*, the rat lungworm, which has had major human health consequences, most notably in southern China, where the snails are eaten raw as a delicacy.

There is therefore a clear need to control the proliferation and spread of these pests in ecologically and economically sustainable ways. This requires research on control and management measures, but also a clear understanding of the identities and basic biology of the species involved. In 2006, a previous book, *Global Advances in the Ecology and Management of Golden Apple Snails* (edited by R. C. Joshi and L. S. Sebastian), documented progress in this arena. However, in the decade since publication of that landmark book, research on apple snails has burgeoned and the identities of the species involved has been clarified. The present book reinterprets old problems and presents much of this new knowledge, with the lessons learned and knowledge available in one country or region informing management approaches more widely. We hope that this new book will not only bring together this new knowledge in a single accessible place but also highlight the need to prevent the further spread of these invasive species, especially in the context of a changing climate.

On a personal note, we, the editors, found this book project challenging. We are most grateful to all the authors from around the world for sharing their personal knowledge and experience, in many cases writing in a language with which they were not familiar. We also commend them for their responsiveness to our invitation, their perseverance in rectifying errors and responding to our reviews, and the patience they exhibited under the pressures of a tight production schedule towards the culmination of a project that had taken longer than anticipated. The book would not have been possible without the help of a number of key individuals. We especially thank Dr. Sailila E. Abdula, Executive Director, Philippine Rice Research Institute (PhilRice), for continuing the tradition of excellent publications in PhilRice, and for providing much needed resources for publication of this book. We also acknowledge the good work of Dr. Flordeliza H. Bordey and Dr. Ronan G. Zagado who patiently escorted us through the commercial production of the book, Ms. Perry Irish H. Duran for design of the attractive book cover and Mrs. Elaine E. Joshi for preparing the index.

Finally, we dedicate this book to the many rice, taro and other farmers, who have for decades tried to reduce damage to their crops caused by invasive apple snails.

Editors

About the editors

Dr. Ravindra C. Joshi is the visiting professor at the Pampanga State Agricultural University, the Philippines; visiting adjunct professor of Agriculture at the University of the South Pacific, Fiji; Tropical Agriculture Association (UK) coordinator for the Pacific region; SAFE-Network Pacific Island coordinator, and technical adviser to Deltamed, Spain on invasive apple snail. He was a former chief science research specialist at the Philippine Rice Research Institute (PhilRice) where he first started working on invasive apple snails in 1987. His central research over twenty five years is on invasive alien species (IAS), important to agriculture sector, at the international, regional and national organizations, including the private and academic institutions, in Africa, Asia and Pacific Island countries. He published three manuals on IAS: Global Advances in Ecology and Management of Golden Apple Snails, Rice Black Bug: Taxonomy, Ecology, Management of Invasive Species, and Philippine Rats: Ecology and Management. In addition, he has also published over 150 research articles on IAS and their management. He has a Ph.D. in Entomology from the University of the Philippines Los Baños in 1988. He has worked as a rice entomologist at the International Institute of Tropical Agriculture, Nigeria, and as a crop protection specialist under the auspices of the Cambodia-IRRI-Australia Project. He reviewed the crop protection research programs of the International Centre of Insect Physiology and Ecology in 1988 and of PhilRice in 1998. He also served as a short-term bench consultant to the System-wide Program on Integrated Pest Management (IPM) / Consultative Group on International Agricultural Research Leafminer Flies Technical Working Group, and to the Food and Agriculture Organization's rice-fish IPM project in Surinam and Guyana. He was also the site coordinator in Solomon Islands with the World Vegetable Center; former senior adviser/consultant to the offices of minister and permanent secretary of Agriculture in Solomon Islands and Fiji, to advise on policy and research areas, and as former Non-OECD Representative to the CG Fund Council (formerly CGIAR) to represent the Pacific Island Countries and Territories.

Dr. Robert H. Cowie is a native of England. He has a degree in Zoology from Cambridge University and a Ph.D. from Liverpool University. Most of his career has been devoted to research on diverse aspects of the biology of land and freshwater snails, and in particular the diversity and impacts of snails as invasive species. However, for four years he worked on the biology and control of crop and forestry damaging termites in developing countries, primarily in Africa, publishing a popular book recounting his experiences during this period. He moved to Hawaii in 1990 to take a position as curator of the mollusc collections of the Bishop Museum, the pre-eminent museum of the natural and cultural history of the Pacific islands. Leaving the Museum in 2001 he took up his present position as a research professor at the University of Hawaii.

Dr. Cowie became involved with research on apple snails almost immediately on arriving in Hawaii, as a species of *Pomacea*, at that time unidentified, had been recently introduced and was already a serious pest of wetland taro. Determining the identity of this species, which turned out to be *Pomacea canaliculata*, led to a developing interest in the systematics of the entire family Ampullariidae. In collaboration with others, this work, including a contribution to the earlier book on apple snail biology and management, published in 2006, has clarified the taxonomy of invasive apple snails globally. In addition to his systematics research, however, Dr. Cowie's interests range across many other aspects of apple snail biology. His research has been widely cited and Dr. Cowie is recognized as one of the world experts in the field.

Dr. Leocadio S. Sebastian was co-editor of the book "Global Advances in Ecology and Management of Golden Apple Snails" published by PhilRice in 2006. He is currently the Regional Program Leader, CCAFS- Southeast Asia (and concurrently, IRRI Country Representative in Vietnam from September 2013-June 2017). His primary responsibility is to ensure coherence among CCAFS R4D activities from field to regional level, and play a key role in achieving outcomes and impacts of CCAFS's work at the national and regional levels. He leads the integration of CCAFS agenda into the regional agenda and national programs in CCAFS focus countries. Prior to his current position, he was the regional director for Asia, Pacific and Oceania (APO) of Bioversity International (2008-2013) and the executive director, Philippine Rice Research Institute (PhilRice – 2000-2008). He is a rice breeder by training, with a Ph.D. in Plant Breeding from the Cornell University, Ithaca, New York obtained through a Rockefeller Foundation Fellowship. He is a multi-awarded plant breeder and research administrator who espouses and ardently practices creativity, innovation, partnerships, and engagement by integrating the whole spectrum of research, development, and extension (RD&E), and mobilizing governmental, inter-governmental, international, and civil society networks for collaboration to attain greater impact.

Contents

GENERAL ASPECTS OF APPLE SNAIL BIOLOGY

Non-native apple snails: systematics, distribution, invasion history and reasons for introduction

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Abstract

The freshwater snail family Ampullariidae includes nine extant genera. Species of *Pomacea* in particular, but also species of *Pila* and *Marisa*, have become invasive where they have been introduced. Introduction of *Pomacea* spp. to Asia around 1980, initially to Taiwan, followed by their rapid range expansion and development as serious agricultural pests, especially of wetland rice, led to a dramatic increase in research not only in the means to control them but also in the basic systematics necessary to identify them. Ampullariid systematics has always been confused but the advent of molecular approaches, combined with modern morphological study, including extensive study of type material, determined that the two key invasive species in Asia, as well as in the USA and elsewhere, are *Pomacea canaliculata* and *Pomacea maculata*. Additional introduced species, both in Asia and elsewhere, include *Pomacea scalaris* (Taiwan), *Pomacea diffusa* (Sri Lanka, Australia, USA) and *Marisa cornuarietis* (Caribbean islands, Spain, USA). *Pila scutata* may have been transported widely in Asia but its native or introduced status in many Asian countries is not clear; however, it has been introduced to and is established in Hawaii. The main reasons for introduction of these species have included introduction as a human food resource, as a domestic aquarium snail, for biocontrol of other snails that act as vectors of the parasites causing schistosomiasis, and for control of aquatic weeds.

Additional keywords: Ampullariidae, Gastropoda, identification, introduced, *Pomacea*

Introduction

Ampullariidae are freshwater snails predominantly distributed in humid tropical and sub-tropical habitats in Africa, South and Central America and Asia. They include the largest of all freshwater snails (up to 17 cm in maximum dimension) and are a major component of the native freshwater mollusc faunas of many regions (Hayes *et al*., 2015). Among the nine genera usually recognised (Hayes *et al*., 2015), species of the two genera *Pomacea* and *Pila* in particular are frequently known as 'apple snails' because many of them bear large, round, sometimes greenish shells.

This contribution summarises recent advances in understanding of the systematics of invasive ampullariids in particular, since the treatment of Cowie *et al*. (2006) (see also Hayes *et al*., 2015). It also summarises the history of the introduction of apple snails, most notably *Pomacea canaliculata* and *Pomacea maculata*, and current knowledge of their invasive ranges. Much has been written about apple snails as pests, and their management. This large body of work was reviewed by Cowie (2002) and treated extensively by the contributions in the book edited by Joshi & Sebastian (2006), with a more recent review focussing specifically on impacts on wetlands (Horgan *et al*., 2014a). We now know that at least some of these publications may not have correctly identified the species involved, as it has only been possible to identify them definitively since the advent of molecular approaches (Cowie *et al*., 2006; Rawlings *et al*., 2007; Hayes *et al*., 2008). However, we do not attempt to re-review these and more recent studies, as they generally have not added to our broad understanding of apple snails as pests and have not offered any novel breakthroughs in terms of their management. The basic biology of apple snails (ecology, physiology, behaviour, etc.) was reviewed by Cowie (2002) and recently and extensively by Hayes *et al*. (2015), who concentrated primarily on the extensive body of research that has been undertaken since around the start of the 21st century throughout both the native and the invaded ranges of apple snails; these aspects are also not reviewed here.

Systematics

Classification

Ampullariids are basal members of the Caenogastropoda. The family Ampullariidae is in the superfamily Ampullarioidea. The family name Pilidae is a junior synonym of

Ampullariidae (Cowie, 1997; ICZN, 1999) and should not be used. The Cyclophoroidea and Viviparoidea are closely related within the group Architaenioglossa (Bouchet & Rocroi, 2005). The Campaniloidea may also be closely related. Although marine ancestry of the Architaenioglossa is assumed by most, relationships among the four superfamilies and the resolution of the base of the Caenogastropoda remain unresolved (Hayes *et al*., 2015).

Diversity, taxonomy, nomenclature

There are nine genera of extant ampullariids with almost 200 species currently considered valid (Table 1), although it is likely that with additional research this number may be reduced to around 120 (Cowie, 2015; Hayes *et al*., 2015). The great majority of the species are referred to just three genera: *Pila* (*Ampullaria* and *Ampullarius* are junior synonyms and should not be used – Cowie, 1997; ICZN 1999), *Lanistes* and *Pomacea*. The remaining six genera each contain only one or a few species.

Species of *Pomacea* especially, introduced to southern and eastern Asia and islands of the Pacific, have become major agricultural pests, notably in rice and taro but also other crops (Cowie, 2002; Joshi & Sebastian, 2006). *Pomacea* species have also been introduced to the continental USA (Rawlings *et al*., 2007), Europe (Andree & López, 2013), Israel (Roll *et al*., 2009), Australia (Hayes *et al*., 2008), to some Pacific islands, notably the Hawaiian Islands (Tran *et al*., 2008), and to non-native locations within South and Central America (Hayes *et al*., 2008).

The name 'golden apple snail' has been used widely in Asia for introduced *Pomacea* (Lai *et al*., 2005; Joshi & Sebastian, 2006), implying a single species, although it had been identified or misidentified as numerous different species, often incorrectly spelled or in combination with an incorrect genus name, also often misspelled (see Cowie *et al*., 2006). It had also been suggested, in some cases based on misidentifications, that more than one species was present in Asia (e.g. Keawjam & Upatham, 1990; Mochida, 1991; Yipp *et al*., 1991). And the name 'golden apple snail' has even been considered as referring to 'an ill-defined group including several species such as *Pomacea insularis* [sic], *P. canaliculata*, *P. vigas* [sic], *Ampullaria gigas*, and *Pila leopoldvillensis* (Simpson *et al*., 1994; Roger, 1996).

We now know that more than one species has indeed been introduced to Asia. Cowie *et al*. (2006) provided preliminary molecular and morphological data identifying the 'golden apple snail' as two species, *Pomacea canaliculata* and *P. maculata*

Table 1. Numbers of nomenclaturally available species-group names and numbers of taxa of Ampullariidae (excluding fossil taxa). From Hayes *et al*. (2015)

Genus	Distribution	Currently valid species[a]	Estimated actual species diversity[b]	Subspecies, varieties, etc.[a]	Synonyms[a]
Afropomus	Africa	1	1	1	-
Asolene	South America	8	8	2	14
Felipponea	South America	3	3	-	-
Forbesopomus	Asia	1	1	-	-
Lanistes	Africa	43	20	16	34
Marisa	South America	2	3	-	6
Pila	Africa and Asia	3	30	17	83
Pomacea	South, Central and North America	97	50	22	109
Saulea	Africa	1	1	-	-
Total		187	117	58	246

[a] For the New World, from Cowie & Thiengo (2003), and taking into account changes subsequent to that publication, as tabulated by (Cowie, 2015); and for the Old World, Cowie (2015); homonyms considered by Cowie & Thiengo (2003) and Cowie (2015) to represent taxonomically valid species and infraspecific taxa are counted as such. [b] From Berthold (1991), Hayes & Cowie (unpublished).

(as *P. insularum*, which is now a junior synonym of *P. maculata*, according to Hayes *et al*., 2012). They also noted *P. diffusa* in Sri Lanka and *P. scalaris* in Taiwan. Hayes *et al*. (2008) confirmed these identifications, with *P. canaliculata* widespread in Southeast Asia, *P. maculata* somewhat less widespread, and *P. diffusa* and *P. scalaris* only in Sri Lanka and Taiwan, respectively. Subsequently, an additional 'group', perhaps a distinct but as yet unidentified species, has been detected in phylogenetic analyses focussed on *P. canaliculata* and *P. maculata* in China (Lv *et al*., 2013; Q. Yang, pers. comm., 2016). Furthermore, hybrids between *P. canaliculata* and *P. maculata* have been detected in both the invaded and native ranges (Hayes *et al*., 2012; Matsukura *et al*., 2013; Q. Yang, pers. comm., 2016). As the common name 'golden apple snail' encompasses at least two species, to avoid future confusion regarding which species is being referred to, it is preferable to avoid using the term.

Hayes *et al.* (2008) clarified the identities of the invasives in Southeast Asia, and subsequently (Hayes *et al.*, 2012) redescribed *P. canaliculata* and *P. maculata*, distinguishing them in an integrative taxonomic framework that included anatomical, biogeographic and phylogenetic systematics data (Table 2; Figs. 1, 2). These two species are not sister taxa, and in fact are not especially closely related to each other (Hayes *et al*., 2009a). Hayes *et al*. (2012) synonymised a number of species with *P. canaliculata*, and designated a neotype for this species, as the original type material is considered lost. A single specimen was designated as the neotype of both *P. maculata* and *P. gigas* (a name also previously used for the species in Southeast Asia) and as the lectotype of *P. insularum* (selected from among syntypes in the Natural History Museum, UK), thereby rendering all three nominal species objective synonyms. *Pomacea maculata* is thus the correct, valid scientific name for the invasive species formerly known as *P. insularum*.

The comparatively recent resolution of this taxonomic confusion means that many publications prior to 2012 failed to distinguish *P. maculata* and *P. canaliculata* in Southeast Asia. For example, the snails from Cambodia, illustrated by Cowie (2002) as *P. canaliculata*, are in fact *P. maculata*, and some of the information given by Cowie (2002) for *P. canaliculata* is undoubtedly confounded with information for *P. maculata*.

In addition to these species of *Pomacea* in Asia, the African species *Pila leopoldvillensis*, considered a synonym of *Pila wernei* by Cowie (2015), has been reported in the Philippines (Barcelo & Barcelo, 1991) and Taiwan (Wu & Lee, 2005). Barcelo & Barcelo (1991) used *P. leopoldvillensis* as the name for the 'golden snail', noting that it originated in the Amazon River basin and laid pink eggs, even though

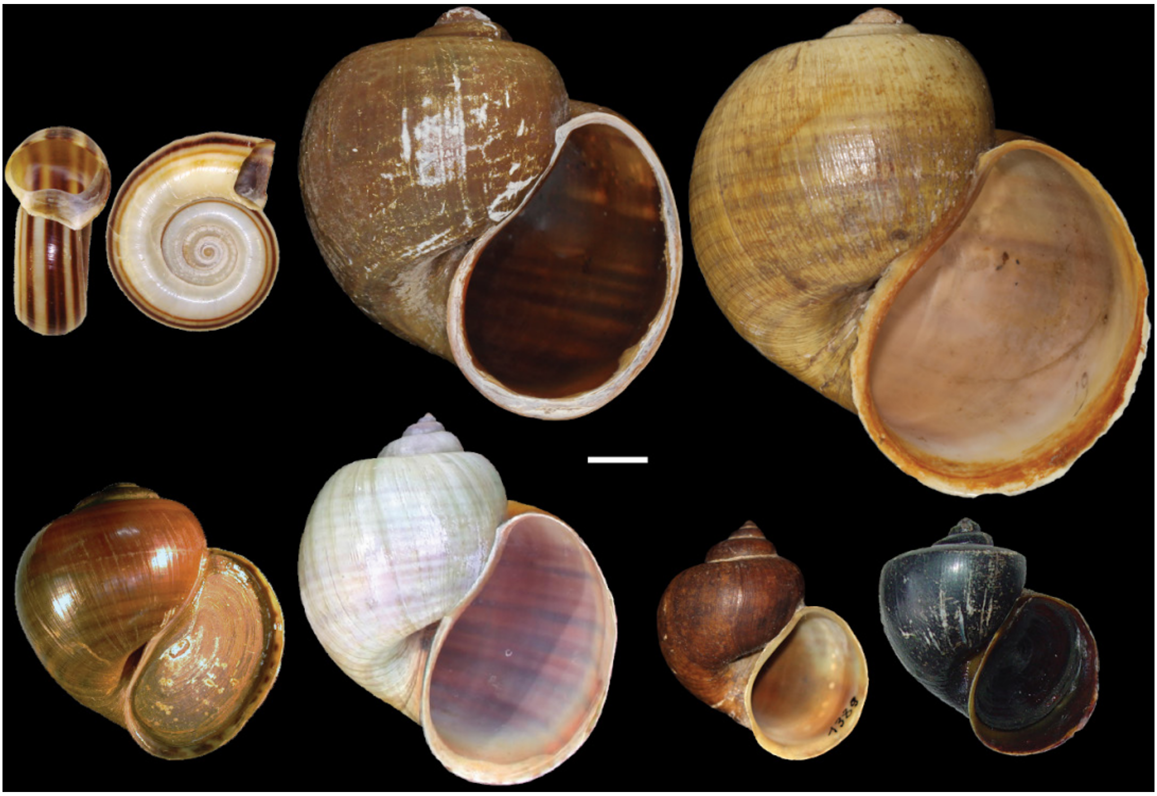

Fig. 1. Shells of introduced ampullariids. Top row, left to right: *Marisa cornuarietis*, *Pomacea canaliculata*, *P. maculata*; bottom row, left to right: *Pila scutata*, *Pomacea* sp. (incorrectly identified as *P. haustrum* by Rawlings *et al*., 2007), *P. diffusa*, *P. scalaris*. Scale bar: 1 cm. Shell morphology and colour is for many species a poor character on which to base identification, as there is considerable intra-specific variation.

Pila species are African and Asian and are not known to lay pink eggs, with most species reported to lay opaque white or cream colored eggs. The name '*leopoldvillensis*' refers to the capital (now Kinshasa) of the Democratic Republic of the Congo, the type locality of this species (Cowie, 2015). No doubt this was a misidentification of *Pomacea canaliculata*, as it was probably the only *Pomacea* species present in the Philippines at that time (Hayes *et al*., 2008; Matsukura *et al*., 2013). On the other hand, the illustration of Wu & Lee (2005), labelled as *Pila leopoldvillensis*, indeed appears to be of a species of *Pila*. There are no native Taiwanese ampullariids (Pace, 1973) and it seems much more likely that it is an Asian species, possibly the widely distributed *Pila scutata*. Roll *et al*. (2009) reported *Pomacea bridgesii* (almost certainly misidentified *P. diffusa*), *P. canaliculata*, *P. maculata* (as '*insularum*') and *P. paludosa* associated with human dominated habitats in Israel, but the identifications need verification.

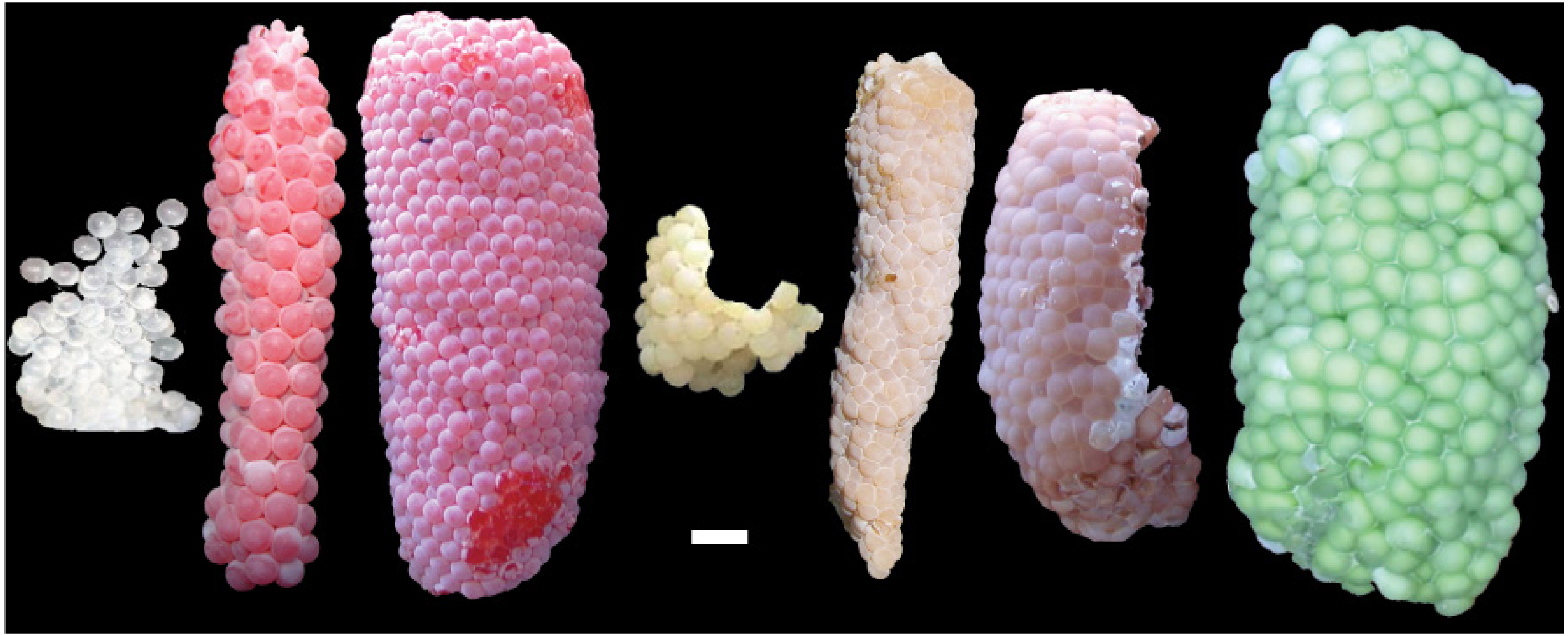

Fig. 2. Egg masses of introduced ampullariids. From left: *Marisa cornuarietis*, *Pomacea canaliculata*, *Pomacea maculata*, *Pila scutata*, *Pomacea. diffusa*, *Pomacea scalaris*, *Pomacea* sp. (incorrectly identified as *Pomacea haustrum* by Rawlings *et al*., 2007). Scale bar: 5 mm. Eggs of *M. cornuarietis* are laid under water; those of *Pila scutata* are laid on the substrate surface just above the water line; and those of the *Pomacea* species are laid above water on emergent vegetation, rocks, walls, pilings, etc. Egg morphology and colour are often useful for distinguishing species.

Table 2. Summary of features used to distinguish *Pomacea maculata* and *P. canaliculata* by Hayes *et al*. (2012).

Feature	*Pomacea maculata*	*Pomacea canaliculata*
Adult Shell		
Maximum adult shell height	> 165 mm	~ 60 mm
Body whorl shoulder	Angulate	Rounded
Inner pallial lip	Pigmented yellow-orange-red	Unpigmented
Alimentary System		
Radula	Rachidian base concave	Rachidian base convex
Buccal ganglion	Six nerves	Five nerves
Mid-oesophagus	Tall, highly branched longitudinal folds	Low, simple longitudinal folds
Gastric caecae	Large	Small
Rectal gland	Large	Small
Reno-Pericardial System		
Kidney anterior chamber	Narrow set of anterior leaflets	Broad set of anterior leaflets
Ampulla	Pericardial artery lacking	Pericardial artery present
Reproductive System		
Dorsal penis sheath glands	Smooth apical gland tissue lacking; medial gland absent; basal gland present	Smooth apical gland tissue present, medial gland present
Bursa copulatrix	Large	Small
Eggs		
Average clutch size	~1500 eggs	~260 eggs
Average egg diameter	< 2.00 mm	> 3.00 mm
Mean hatchling width, height	1.19 mm, 1.25 mm	2.60 mm, 2.75 mm
Mean hatchling first whorl width	0.81 mm	2.41 mm

Three species have been introduced to the Hawaiian Islands: *Pomacea canaliculata*, *Pomacea diffusa* and *Pila scutata*. Cowie (1995a) also recorded *Pomacea paludosa* but this was a misidentification, and the species identified by Cowie (1995a) as *P. bridgesii* is correctly identified as *P. diffusa* (Cowie *et al.*, 2007). *Pomacea diffusa* was originally described as a smaller subspecies of *P. bridgesii* (see Cowie & Thiengo, 2003), but these two species are genetically distinct and their type specimens are sufficiently different that they are now considered separate species (Cowie *et al.*, 2006; Rawlings *et al.*, 2007; Hayes *et al.*, 2008, 2009a). Indeed, all global records of *P. bridgesii* as an introduced species are probably correctly referred to *P. diffusa*. *Pila conica* is a junior synonym of *Pila scutata* (Low *et al.*, 2013). Thus, *Pila scutata* is the correct name for the species identified as *P. conica* by Cowie (1995a).

In the continental USA, introduced *Pomacea* were initially identified primarily as *Pomacea canaliculata* and given the common name 'channeled apple snail' (American spelling of 'channelled' as the name was coined in the USA), an anglicisation of the specific epithet (Howells *et al.*, 2006). However, Rawlings *et al.* (2007) distinguished three species within what had been identified previously as 'channeled apple snails' (i.e. *P. canaliculata*), namely: 1) *P. canaliculata*, 2) *P. maculata* (as *P. insularum*, and which had therefore been given the common name 'island apple snail' as '*insularum*' is Latin for 'of islands') and 3) *Pomacea* sp. (misidentified as *P. haustrum*) (Hayes *et al.*, 2012). Rawlings *et al.* (2007) also confirmed the presence of *Pomacea diffusa* and *Marisa cornuarietis* in addition to *Pomacea paludosa*, the only apple snail species native to North America.

In Europe, apple snails have been introduced to two locations, both in Spain. *Pomacea maculata* (as '*insularum*') seems well established in the Ebro Delta (López *et al.*, 2010; MMAMRM, 2011; EFSA Panel on Plant Health, 2012; Horgan *et al.*, 2014a; Andree & López, 2013). In addition, Andree & López (2013) reported DNA sequences, from empty shells collected in the field, that were consistent with *P. canaliculata*, so it appears that both species have been introduced. Arias & Torralba-Burrial (2014) recorded *Marisa cornuarietis* from a single locality in northern Spain.

Distributions

The native and non-native ranges of ampullariid species are summarised in Table 3. The following sections provide additional information and analysis of the more significant introductions.

Native ranges of introduced ampullariid species

The taxonomic confusion surrounding *Pomacea canaliculata* has obscured its true natural range until recently. Many species that molecular studies have shown to be distinct (Hayes *et al*., 2008, 2009a), have been confused with *P. canaliculata* in the past, to the extent that some authors suggested that many of these nominal species might be synonyms of *P. canaliculata* and therefore that its range extended throughout much of South America (see Hylton Scott, 1958; Cazzaniga, 2002, 2006; Cowie, 2002; Wu & Xie, 2006). The natural range of *P. canaliculata* is now known to be much more restricted (Hayes *et al*., 2012), consisting of the Lower Paraná, Uruguay and La Plata basins, although based on habitat similarity and watershed connections it is possible that it may also occur in the lower reaches of the Upper Paraná and parts of southern Brasil. It is not present in the Amazon basin. Its southern limit in Argentina seems to be limited by temperature and this may limit its spread to higher latitudes in its invaded range (Seuffert *et al*., 2010, 2012).

Pomacea maculata has a much wider native range in South America extending from the lower Paraná River basin in the Rio de la Plata region of Argentina and Uruguay, through Paraguay and northwards in Brasil through the Pantanal to north of Manaus in Amazonia, overlapping with the range of *P. canaliculata* in the south, and perhaps extending west into Bolivia, Ecuador and Peru (Hayes *et al*., 2008, 2009a, b, 2012; Thiengo *et al*., 2011).

Perhaps the third most widely introduced *Pomacea* species is *P. diffusa*. In much of the literature, this species had been identified incorrectly as *Pomacea bridgesii* (see above). *Pomacea diffusa* is widely distributed through much of the Amazon basin, whereas *P. bridgesii* is restricted to Bolivia and the western Amazon basin (Pain, 1960; Rawlings *et al*., 2007; Hayes *et al*., 2008; Hayes, Cowie & Thiengo, unpublished).

Pomacea scalaris ranges from Buenos Aires in Argentina northwards through the Pantanal to Cuiabá in Mato Grosso state (Hayes *et al*., 2008) and perhaps more widely.

Table 3. Native and non-native ranges of introduced ampullariids in the wild (records in captivity and records in artificial thermal outflows not listed). Some, especially earlier, references listed for *Pomacea canaliculata* may in fact be based on *P. maculata*.

Species	Native range	Non-native range	First record or known date of introduction	Representative references for non-native range
Marisa cornuarietis	Colombia Venezuela	Costa Rica	?	Nguma *et al*., 1982
		Cuba	1950	Hunt, 1958
		Dominican Republic	1986	Vargas *et al*., 1991; Perera & Walls, 1996
		Egypt[a]	1972	Demian & Kamel, 1973; Brown, 1994
		French Guyana[b]	2005	Mansur, pers. comm., in Massemin *et al*., 2009
		Grenada	2009	Barker, 2016
		Guadeloupe	1973	Pointier & David, 2004
		Guyana	?	Nguma *et al*., 1982; Massemin *et al*., 2009
		Martinique	1987	Pointier, 2001
		Panama	?	Nguma *et al*., 1982
		Puerto Rico	1952	Harry & Cumbie, 1956; Hunt, 1958; Peebles *et al*., 1972; Jobin *et al*., 1977; Nguma *et al*., 1982; Perera & Walls, 1996
		St. Kitts	1950s	Ferguson et al., 1960
		Spain	2012	Arias & Torralba-Burrial, 2014
		Sudan[a]	1981	Haridi *et al*., 1985; Brown, 1994
		Surinam	?	Nguma *et al*., 1982
		Tanzania[a]	1977	Nguma *et al*., 1982; Brown, 1994
		USA (continental)	1957	Hunt, 1958; Neck, 1984, Cowie, 2002; Howells *et al*., 2006; Rawlings *et al*., 2007
Pila scutata	Southeast Asia[c]	Guam	1984	Smith, 1992; Cowie, 2002
		Hawaii	1966	Cowie, 1995; Tran *et al*., 2008
		Palau[d]	1984-1985	Eldredge, 1994, Cowie, 2002
		Taiwan[e]	1975	Wu & Lee, 2005

Pomacea canaliculata	Argentina Uruguay Paraguay southern Brasil	Bangladesh[f]	2006	Ranamukhaarachchi & Wikramasinghe, 2006; Wu & Xie, 2006
		Cambodia[g]	2006	Ranamukhaarachchi & Wikramasinghe, 2006
		Chile	2008	Letelier & Soto-Acuña, 2008
		China	1981-1985	Hayes *et al*., 2008; Kwong *et al*., 2008
		Dominican Republic	1991	Rosario & Moquete, 2006
		Ecuador	2005	Horgan *et al*., 2014b
		Egypt[f]	2006	Wu & Xie, 2006
		Guam	1989	Smith, 1992; Hayes *et al*., 2008
		Hawaii	1989	Cowie, 1995; Hayes *et al*., 2008; Tran *et al*., 2008
		India[f]	2006	Ranamukhaarachchi & Wikramasinghe, 2006; Wu & Xie, 2006
		Indonesia	1981-1984	Mochida, 1991; Hayes *et al*., 2008
		Japan	1981	Mochida, 1991; Hayes *et al*., 2008
		Laos	1991-1994	Douangboupha & Khamphoukeo, 2006; Halwart & Bartley, 2006; Hayes *et al*., 2008
		Malaysia	1987-1992	Mochida, 1991; Halwart, 1994; Naylor, 1996; Yahaya *et al*., 2006; Hayes *et al*., 2008
		Mexico	2009	Campos *et al*., 2013
		Myanmar (Burma)	2008	Wu & Xie, 2006; Hayes *et al*., 2008
		Papua New Guinea	1991	Laup, 1991; Hayes *et al*., 2008
		Philippines	1980	Mochida, 1991; Hayes *et al*., 2008
		Singapore	1991	Ng, 1991; Ng *et al*., 2014
		South Africa[h]	before 1991	Berthold, 1991
		South Korea	1981-1986	Mochida, 1991; Hayes *et al*., 2008
		Spain	2001	Andree & López, 2013
		Taiwan	1979-1981	Mochida, 1991; Cheng & Kao, 2006; Yang *et al*., 2006; Halwart & Bartley, 2006; Hayes *et al*., 2008; Wu *et al*., 2010
		Thailand	1982-1990	Mochida, 1991; Halwart & Bartley, 2006; Hayes *et al*., 2008

		Trinidad	2014	Mohammed, 2015
		USA (continental)	1997	Cerutti, 1998; Rawlings *et al*., 2007
		Vietnam	~1988	Cuong, 2006; Halwart & Bartley, 2006; Hayes *et al*., 2008
Pomacea diffusa[i]	Amazonia	Australia	2004	Hayes *et al*., 2008; Ponder *et al*., 2016
		Brasil (Pará)	2008	Hayes *et al*., 2008
		Brasil (Pernambuco)	2006	Hayes, unpublished
		Brasil (Rio de Janeiro)	2008	Hayes *et al*., 2008
		Colombia	2008	Hayes, unpublished
		French Guiana	~1930	Tillier, 1980 (as *Ampullaria sordida*); Massemin *et al*., 2009
		Hawaii	1962	Cowie, 1995
		New Zealand[j]	2010	Collier *et al*., 2011
		Panama	2008	Hayes *et al*., 2008
		Sri Lanka	early 1980s	Epa, 2006; Hayes *et al*., 2008; Wijesekara, 2010
		USA (continental)	1950s-1960s	Howells *et al*., 2006; Rawlings *et al*., 2007
		Venezuela	2009	Hayes, unpublished
Pomacea sp.[k]	Amazonia	USA	2007	Rawlings *et al*., 2007
Pomacea maculata	Argentina to Amazonia	Cambodia	before 1995	Cowie, 1995b; Hayes *et al*., 2008
		China	2006-2007	Lv *et al*., 2013
		Israel	2008	Roll *et al*., 2009
		Japan	2008/2013	Matsukura *et al*., 2008, 2013
		Malaysia	2008	Hayes *et al*., 2008
		Pakistan[l]	2009	Baloch, 2017
		Philippines	2013	Matsukura *et al*., 2013
		Singapore	2008	Hayes *et al*., 2008; Ng *et al*., 2014
		South Korea	2008	Hayes *et al*., 2008; Matsukura *et al*., 2013
		Spain	2009	López *et al*., 2010; MMAMRM, 2011; European Food Safety Authority; 2012; Andree & López, 2013; Horgan *et al*., 2014a

		Thailand	1990	Hayes *et al.*, 2008
		USA (continental)	1989	Martin *et al.*, 2012; Byers *et al.*, 2013
		Vietnam	2008	Hayes *et al.*, 2008
Pomacea scalaris	Argentina Southern Brasil	Taiwan	1989	Hayes *et al.*, 2008; Wu *et al.*, 2010, 2011

[a] not known whether widely established; [b] presence remains to be confirmed; [c] native and non-native range difficult to disentangle (see text); [d] thought to have been eradicated; [e] tentative identification based on the record of *Pila leopoldvillensis* by Wu & Lee (2005); [f] unconfirmed; [g] unconfirmed; may refer to *P. maculata*, which is present in Cambodia; [h] identified as *Pomacea lineata* but probably *P. canaliculata*; possibly not established; [i] the record in Puerto Rico of Horgan *et al.* (2014a) appears to be in error; [j] a single specimen, may not be established; [k] identified incorrectly as *P. haustrum* by Rawlings *et al.* (2007); [l] originally identified as *P. canaliculata* (see Horgan *et al.*, 2014a).

The true native range of *Pila scutata* is difficult to determine (Tan *et al.*, 2013). In Singapore, it has declined drastically (Tan *et al.*, 2013; Ng *et al.*, 2014), but it used to be widespread and seemed to be strongly associated with anthropogenically disturbed habitats. It has been considered native in Singapore, with the first record in 1847 (not definitive) or 1885 (Low *et al.*, 2013), but has also been recorded from Indonesia, Peninsular Malaysia, Myanmar, Borneo, Vietnam, Cambodia, Laos and the Philippines, and with doubt from Thailand and China (Low *et al.*, 2013; Tan *et al.*, 2013) and Taiwan (as *Pila leopoldvillensis*, see above). However, whether it occurred naturally in these countries or whether it became widespread as a widely introduced popular food item, is not clear (Tan *et al.*, 2013).

The native range of *Marisa cornuarietis* may only encompass Venezuela and Colombia (Nguma *et al.*, 1982). However, it occurs widely in other parts of northern South America (Table 2) but it is not clear whether its presence in at least some of these areas is natural or the result of introductions. It is often confused with two congenerics, *M. planogyra*, which occurs throughout the Pantanal of central west Brasil, and *M. rotula*, which occurs north of the eastern Andean cordillera in Colombia and Panama.

Non-native ranges

The most widespread species in Asia is *Pomacea canaliculata* (Table 3). *Pomacea maculata* is also widespread in Asia, but not to the extent of *P. canaliculata* (Hayes *et al.*, 2008; Lv *et al.*, 2013). In the USA, *P. maculata* is present in the southeast, contrasting with the initial distribution of *P. canaliculata* in the west, although *P. canaliculata* is also now present in Florida (Rawlings *et al.*, 2007). An unidentified species of *Pomacea*, incorrectly identified by Rawlings *et al*. (2007) as *P. haustrum*, is also present in Florida (Hayes *et al*., 2012). Within South America, *P. canaliculata* has been reported beyond its native range, in Chile and Ecuador (Letelier & Soto-Acuña, 2008; Horgan *et al*., 2014b. Both *P. maculata* and *P. canaliculata* are present in Spain (Andree & López, 2013).

In Hawaii, *Pomacea canaliculata* is widely distributed and locally abundant on five of the main islands (Lach & Cowie, 1999; Cowie *et al*., 2007). *Pomacea diffusa*, also reported from Hawaii, was not widespread or abundant and has not been seen since 1998 (Cowie *et al*., 2007, unpublished) and may no longer be present. The Asian *Pila scutata* has been recorded from the islands of Maui, Molokai and Oahu (Cowie, 1995a), although it was not recorded on Maui and Molokai by Cowie *et al*. (2007) and Tran *et al*. (2008). It has since been confirmed as still present on Oahu (Cowie, Hayes, C. Tran & K. Matsukura, unpublished). In the Pacific, it was also introduced to Guam (Smith, 1992) and Palau, where it was eradicated (Eldredge, 1994), and is probably the species identified as the African *Pila leopoldvillensis* in Taiwan (see above). It is widespread in Asia but its native range in Asia is not known definitively and it is possible that part of this wide Asian distribution results from introductions (see above). *Pomacea scalaris* has been introduced to Taiwan, its only known non-native location.

Pomacea diffusa is also established in Sri Lanka (Nugaliyadde *et al*., 2001; Wijesekara, 2010), and in non-native regions of South and Central America (Hayes *et al*., 2008) and the southeastern USA (Rawlings *et al*., 2007). Introduced populations of *P. diffusa* exhibit very little genetic variation, with a single COI haplotype shared by individuals from Australia, Sri Lanka, New Zealand, Panama and parts of Brasil where the species has been introduced, and by specimens from pet stores in Hawaii, Florida and Iran (Hayes *et al*., 2008; Thiengo *et. al*., 2011) as well as Singapore, Hong Kong and Washington DC (Hayes, unpublished).

As noted above, the native range of *Marisa cornuarietis* may only encompass Venezuela and Colombia, with its presence in other parts of northern South America (Table 3) due to human activities, although it is yet possible that it occurs in these areas naturally. It is widely introduced elsewhere, especially in the Caribbean, as well as in the USA, especially in the southeast (Rawlings *et al*., 2007), and Spain (Arias & Torralba-Burrial, 2014). It is present in Hungary but only in an urban section of a stream close to the outflow from a thermal spa (Frisóczki *et al*., 2016).

Records of other introduced ampullariid species are the results of misidentifications (Table 4).

Table 4. A selection of misidentifications, and the corresponding correct names, of introduced *Pomacea* species. This list does not include the numerous early misidentifications of *Pomacea maculata* as *P. canaliculata* (see text).

Region	**Misidentification**	**Correct name**	**Author(s)**
Asia (Hong Kong)	*Ampullaria levior*	*Pomacea canaliculata*	Yipp *et al*., 1992
Asia (Indonesia)	*Pomacea paludosa*	*Pomacea canaliculata*	Hendarsih-Suharto *et al*., 2006
Asia (Philippines)	*Pomacea cuprina*	Probably *Pomacea canaliculata*	Mochida, 1991
Asia (Sri Lanka)	*Pomacea bridgesii*	*Pomacea diffusa*	Nugaliyadde *et al*., 2001
Asia (Taiwan)	*Pila leopoldvillensis*	Probably *Pila scutata*	Barcelo & Barcelo, 1991; Wu & Lee, 2005
Asia (Taiwan)	*Pomacea lineata*	*Pomacea canaliculata*	Cheng, 1989
Asia (Vietnam)	*Pomacea bridgesii*	*Pomacea canaliculata* or *Pomacea maculata*	Cuong, 2006
Hawaii	*Pomacea paludosa*	*Pila conica*	Cowie, 1995
Hawaii	*Pomacea bridgesii*	*Pomacea diffusa*	Cowie, 1995
USA (Texas)	*Pomacea canaliculata*	*Pomacea maculata*	Neck, 1986; Neck & Schulz, 1992
USA (Florida)	*Pomacea haustrum*	*Pomacea* sp.	Rawlings *et al*., 2007

Introduction history and reasons

Introductions for food

Some ampullariids are used as human food in their native ranges, mostly in Asia but also in South America and Africa. However, deliberate introduction as a novel human food resource is probably the most important cause of their spread and establishment, although the aquarium trade is of major importance in some regions (see below).

In the Pacific, *Pila scutata* was introduced without authorisation, either accidentally or deliberately as a food item to both Hawaii (first recorded 1966; Cowie, 1995a) and Guam (first recorded 1984; Smith, 1992), probably from the Philippines (Tran *et al*., 2008). It was also introduced to Palau in 1984 or 1985, probably for the same purpose, but was eradicated by 1987 (Eldredge, 1994). But it is the South American *Pomacea* species that have attracted most attention, notably in southern and eastern Asia, where they have become major agricultural pests.

At some time between 1979 and 1981, what became widely assumed to be a single species of *Pomacea* was introduced to Asia, initially from Argentina to Taiwan (Mochida, 1991), although it may have been introduced earlier in the 1970s to the Philippines, China and Vietnam (Wu & Xie, 2006). Undoubtedly this was *Pomacea canaliculata*, the only widespread species in Taiwan. *Pomacea scalaris*, also present in Taiwan, may have been introduced accidentally, perhaps with the original introduction(s) of *P. canaliculata* as their native ranges overlap (Wu & Lee, 2005; Wu *et al*., 2011). The initial introduction of *P. canaliculata* to Taiwan was illegal, its purpose being to develop the species for both local consumption and export to the gourmet restaurant trade. The subsequent spread of these snails in Asia and the Pacific, distributed primarily for the same purposes, has been summarised by Cowie (2002), Halwart & Bradley (2006), Wu & Xie (2006) and others (generally not distinguishing *P. canaliculata* and *P. maculata*). Halwart and Bradley (2006) listed the origins of many of the Asian introductions as ‘Amazon basin’, which is incorrect except possibly for *Pomacea diffusa*. In 1981 snails were taken from Taiwan to Japan, Korea (Lee & Oh, 2006), China and Indonesia. By 1982 they had been introduced to the Philippines and introductions to the Philippines continued from various sources as snail-farming was promoted by governmental and non-governmental organisations. By 1983 about 500 snail businesses had opened in Japan; they were present in Okinawa by at least 1984. *Pomacea maculata* may have been first introduced around this time, from Argentina and southern Brasil (Hayes *et al*., 2008). Later, the snails were taken to parts of Malaysia (Sarawak and Peninsular Malaysia, 1987), Vietnam (1988 or 1989), Thailand (1989 or 1990) and Laos (1992). They were present in Hong Kong and Singapore by 1991 and Cambodia by at least 1994. In the Pacific they were in Hawaii by 1989 or perhaps earlier (Cowie *et al*., 2007), Guam by 1989 (perhaps introduced from Taiwan or more likely the Philippines; Tran *et al*., 2008), and Papua New Guinea in 1990 (Orapa, 2006), probably introduced from the Philippines.

Prior to the clarification by Hayes *et al*. (2008), most of these reports assumed that a single species was involved, usually identified as *Pomacea canaliculata*. Hayes *et al*. (2008) concluded, based on mitochondrial DNA (mtDNA) diversity, that the Asian populations of both *P. canaliculata* and *P. maculata* resulted from multiple introductions. Tran *et al*. (2008) showed that only a single haplotype was present in Hawaiian *P.*

canaliculata, suggesting a single introduction or multiple introductions from a single location, probably the Philippines.

The species identified in Taiwan as the African *Pila leopoldvillensis* is in fact probably Asian *Pila scutata* (see above). It was imported into Taiwan in 1975 for culture for food prior to the introduction of the South American *Pomacea canaliculata* (Wu & Lee, 2005), but seems to have disappeared, perhaps outcompeted by *P. canaliculata* (*P. maculata* has not been reported from Taiwan).

Pomacea canaliculata was recorded in California in 1998, perhaps introduced from Hawaii for food (Rawlings *et al*., 2007). By 2007 it was in Arizona and Florida, perhaps introduced from California.

In Asia particularly, the snails' economic potential was over-estimated and while many, mostly small aquaculture operations arose, relatively few persisted (Acosta & Pullin, 1991). In Taiwan, the local market failed because consumers disliked the snails' taste and texture (Yang *et al*., 2006). Stringent health regulations in developed nations largely precluded its importation (Naylor, 1996). Snails escaped or were deliberately released, becoming widespread and abundant, and major crop pests, in many countries. They nonetheless continue to be considered a delicacy in some regions, notably in southern China, where they are eaten raw and where they have become important transmitters of *Angiostrongylus cantonensis*, the rat lungworm, with major human health consequences (Lv *et al*., 2011).

The aquarium trade

Ampullariids are popular domestic aquarium snails (Perera & Walls, 1996; Wilstermann-Hildebrand, 2009: Ng *et al*., 2016). Various species have therefore been introduced around the world, perhaps also accidentally with aquarium plants. *Pomacea diffusa*, usually referred to as *P. bridgesii* until their distinction was clarified by Hayes *et al*. (2008), is perhaps the most widely available ampullariid in the aquarium pet trade (Perera & Walls, 1996), although a number of other species are also available (Horgan *et al*., 2014a; Ng *et al*., 2016). In the USA, *Pomacea diffusa* was probably introduced to Florida in the early 1960s and is now also established in Alabama (Rawlings *et al*., 2007). It is produced commercially on a large scale in Florida (Perera & Walls, 1996). The market has expanded since the discovery and development of bright yellow, orange and other colour variants of *P. diffusa* and to some degree other *Pomacea* species (Perera & Walls, 1996). *Pomacea diffusa* has been intercepted by customs officials in Singapore.

It is established in Australia and Sri Lanka (Epa, 2006; Hayes *et al*., 2008; Ponder *et al*., 2016) and was reported in the wild in Hawaii (Cowie, 1995a), although it may no longer be present (see above). *Pomacea canaliculata* (including brightly coloured forms) in California and Arizona was probably introduced for food (Rawlings *et al*., 2007) but the aquarium trade may also have been involved. *Pomacea maculata* has been detected in the trade in Belgium (Hayes *et al*., 2008) and Singapore (Ng et al., 2016) and its presence in Spain probably originated in the trade. Its presence in the southeastern USA may have been a result of pet trade introductions. *Pomacea diffusa* has also been sold for food in Belgium, as 'sea snails' (Thiengo, personal observation).

Keawjam & Upatham (1990) considered the *Pomacea* in Thailand to have been imported by the aquarium trade, but it is also probable that they were introduced for food, as elsewhere in Southeast Asia. *Pomacea canaliculata* is available in the aquarium trade in Singapore (Ng *et al*., 2016) and in Hawaii, locally collected *Pomacea canaliculata* are available in aquarium stores, and purchase followed by release for culture as food items may have been one reason for its spread in Hawaii (Cowie, 2002), although the original source of the aquarium snails was probably local, following their initial introduction for food. *Asolene spixii* has been seen in pet stores in Hawaii but has not yet been found in the wild. *Pomacea lineata* (probably misidentified *P. canaliculata*) has been introduced to South Africa.

Marisa cornuarietis has been introduced to several countries (e.g. the USA) (Perera & Walls, 1996) and Spain (Arias & Torralba-Burrial, 2014) and is well known in the aquarium trade (e.g., Ng *et al*., 2016).

Biological control

In the Caribbean *Pomacea glauca* and more widely *Marisa cornuarietis* have been introduced in attempts to control the snail vectors of *Schistosoma* spp., the cause of human schistosomiasis, through competition and predation (Peebles *et al*., 1972; Pointier *et al*., 1991; Perera & Walls, 1996; Pointier & David, 2004), and *M. cornuarietis* has been tested in field experiments in Egypt and Tanzania for the same purpose (Nguma *et al*., 1982), although it seems not to have become established in the wild in Africa.

Many ampullariids feed voraciously on aquatic plants, this being one reason for their success in controlling other snail species by reducing the available food. They have therefore been used or suggested for aquatic weed control in both natural wetlands and irrigated rice, e.g. *Marisa cornuarietis* in Florida and Puerto Rico (Simberloff & Stiling, 1996), *Pomacea canaliculata* in Asia (Joshi & Sebastian, 2006; Wada, 1997), although there are concerns in Asia that this might lead to farmers introducing snails to areas they have not yet reached (Wada, 2006).

Conclusions

Since the review of Cowie (2002) and book edited by Joshi & Sebastian (2006) there have been considerable advances in understanding the identities of invasive and other introduced apple snail species. Most of these advances have been the result of molecular analysis, both in the native and non-native ranges. Extensive bibliographic and museum research has brought rigour to the formerly highly confused nomenclature. And detailed morphological study, in conjunction with molecular analysis, has provided the basis not only for distinguishing the key invasive species, but also for modern systematic revision, at least of the New World taxa.

As for many species introduced deliberately, the benefits initially perceived are often outweighed by the negative impacts. The primary reason for introducing apple snails has been for human food, but they also continue to be spread through the aquarium trade. They are still used for food in many places, although not to the extent originally envisaged. But the damage to agriculture and the less well understood but potentially serious damage to non-agricultural systems, outweigh arguments for their further introduction for food. Once introduced and established, control measures become necessary. As for most introductions of the species outside their native ranges, the introduction of apple snails, even for ostensibly legitimate reasons of human health and well-being, is fraught with dangers and should be prevented.

References

Acosta, B.O. & Pullin, R.S.V. 1991. *Environmental Impact of the Golden Snail (*Pomacea *sp.) on Rice Farming Systems in the Philippines*. Freshwater Aquaculture Center, Central Luzon State University, Nueva Ecija; ICLARM, Manila.

Andree, K.B. & López, M.A. 2013. Species identification from archived snail shells via genetic analysis: a method for DNA extraction from empty shells. *Molluscan Research* 3: 1-5.

Arias, A. & Torralba-Burrial, A. 2014. First European record of the giant ramshorn snail *Marisa cornuarietis* (Linnaeus, 1758) (Gastropoda: Ampullariidae) from northern Spain. *Limnetica* 33(1):65-72.

Baloch, W.A. 2017.Country report (Pakistan) on non-native apple snails. In: *Biology and Management of Invasive Apple Snails* (Joshi, R.C., Cowie, R.H. & Sebastian, L.S, ed.), p. 213-219. Philippine Rice Research Institute, Nueva Ecija.

Barcelo, P.M. & Barcelo, J.R. 1991. The potential diet of snail (*Pila leopoldvillensis*) meal as protein supplement in broiler diets. *Tropicultura* 9: 11-13.

Berthold, T. 1991. Vergleichende Anatomie, Phylogenie und historische Biogeographie der Ampullariidae (Mollusca, Gastropoda). *Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg* (NF) 29: 1-256.

Bouchet, P. & Rocroi, J.-P. 2005. Classification and nomenclator of gastropod families. *Malacologia* 47: 1-397.

Brown, D.S. 1994. *Freshwater Snails of Africa and their Medical Importance*, Taylor & Francis, London.

Barker, G.M. 2016. *Marisa cornuarietis* (giant ramshorn). In: *Invasive Species Compendium*. CAB International, Wallingford, UK. www.cabi.org/isc. Accessed 3 March 2017.

Campos, E., Ruiz-Campos, G. & Delgadillo, J. 2013. Primer registro del caracol manzano exótico *Pomacea canaliculata* (Gastropoda: Ampullariidae) en México, con comentarios sobre su propagación en el bajo río Colorado. *Revista Mexicana de Biodiversidad* 84: 671-675.

Cazzaniga, N.J. 2002. Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda: Ampullariidae). *Biocell* 26: 71-81.

Cazzaniga, N.J. 2006. *Pomacea canaliculata*: harmless and useless in its natural realm (Argentina). In: *Global Advances in Ecology and Management of Golden Apple*

Snails (Joshi, R.C. & Sebastian, L.S., ed.), p. 37-60. Philippine Rice Research Institute, Nueva Ecija, Philippines.

Cerutti, R. 1998. An infestation of *Pomacea canaliculata* (Lamarck, 1804 [sic]) in Lake Miramar, San Diego, California. *The Festivus* 30: 25-27, 29.

Cheng, E.Y. 1989. Control strategy for the introduced snail *Pomacea lineata*, in rice paddy. *British Crop Protection Council Monograph* 41: 69-75.

Cheng, E.Y. & Kao, C.-H. 2006. Control of golden apple snail *Pomacea canaliculata* (Lamarck), in Taiwan. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 155-167. Philippine Rice Research Institute, Nueva Ecija.

Collier, K.J., Demetras, N.J., Duggan, I.C. & Johnston, T.M. 2011. Wild record of an apple snail in the Waikato River, Hamilton, New Zealand, and their incidence in freshwater aquaria. *New Zealand Natural Sciences* 36: 1-9.

Cowie, R.H. 1995. *Report on a Visit to Cambodia to Advise on Apple Snails as Potential Rice Pests*. Report prepared for Cambodia-IRRI-Australia Project, Phnom Penh. Bishop Museum, Honolulu.

Cowie, R.H. 1995. Identity, distribution and impacts of introduced Ampullariidae and Viviparidae in the Hawaiian Islands. *Journal of Medical and Applied Malacology* 5 [for 1993]: 61-67.

Cowie, R.H. 1997. *Pila* Röding, 1798 and *Pomacea* Perry, 1810 (Mollusca, Gastropoda): proposed placement on the Official List, and Ampullariidae Gray, 1824: proposed confirmation as the nomenclaturally valid synonym of Pilidae Preston, 1915. *Bulletin of Zoological Nomenclature* 54(2): 83-88.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CAB International, Wallingford.

Cowie, R.H. 2015. The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa* 3940(1): 1-92.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastropoda: Ampullariidae: *Asolene*, *Felipponea*, *Marisa*, *Pomacea*, *Pomella*): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-23. Philippine Rice Research Institute, Nueva Ecija.

Cowie, R.H., Hayes, K.A., Tran, C.T. & Levin, P. 2007. Distribution of the invasive apple snail *Pomacea canaliculata* (Lamarck) in the Hawaiian Islands (Gastropoda: Ampullariidae). *Bishop Museum Occasional Papers* 96: 48-51.

Cuong, D.C. 2006. The golden apple snail in Vietnam. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 243-254. Philippine Rice Research Institute, Nueva Ecija.

Demian, E.S. & Kamel, E.G. 1973. Effect of *Marisa cornuarietis* on *Bulinus truncatus* populations under semi-field conditions in Egypt. *Malacologia* 14: 439.

Douangboupha, B. & Khamphoukeo, K. 2006. Golden apple snail damage and management practices in rice farmers' fields in the Lao PDR. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 275-280. Philippine Rice Research Institute, Nueva Ecija.

EFSA Panel on Plant Health. 2012. Statement on the identity of apple snails. *EFSA Journal* 10(4): 2645.

Eldredge, L.G. 1994. *Perspectives in Aquatic Exotic Species Management in the Pacific Islands. Volume 1. Introductions of Commercially Significant Aquatic Organisms to the Pacific islands*. South Pacific Commission, Noumea.

Epa, U.P.K. 2006. Alien invasive snail, *Pomacea* sp., in Sri Lanka. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 319-324. Philippine Rice Research Institute, Nueva Ecija.

Frisóczki, B., Vig, Z., Horotán, K. & Varga, J. 2016. A new alien snail species from the Eger stream, Hungary (Mollusca, Ampullariidae). *Opuscula Zoologica (Budapest)* 47(2): 197-201.

Ferguson, F.F., Richards, C.S., Sebastian, S.T. & Buchanan, I.C. 1960. Natural abatement of schistosomiasis mansoni in St. Kitts, British West Indies. *Public Health* 74(7): 261-265.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40(2): 199-206.

Halwart, M. & Bartley, D.M. 2006. International mechanisms for the control and responsible use of alien species in aquatic ecosystems, with special reference to the golden apple snail. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 449-458. . Philippine Rice Research Institute, Nueva Ecija

Haridi, A.A., El Safi, S.H. & Jobin, W.R. 1985. Survival, growth and reproduction of the imported ampullarid snail *Marisa cornuarietis* in Central Sudan. *Journal of Tropical Medicine and Hygiene 88(2):* 135-144.

Harry, H.W. & Cumbie, B.G. 1956. Stream gradient as a criterion of lotic habitats suitable for *Australorbis glabratus* in Puerto Rico *American Journal of Tropical Medicine and Hygiene* 5(5): 921-928.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H. & Thiengo, S.C. 2009a. A global phylogeny of apple snails: Gondwanan origin, generic relationships and the influence of outgroup choice (Caenogastropoda: Ampullariidae). *Biological Journal of the Linnean Society* 98: 61-76.

Hayes, K.A., Cowie, R.H., Jørgensen, A., Schultheiß, R., Albrecht, C. & Thiengo, S.C. 2009b. Molluscan models in evolutionary biology: apple snails (Gastropoda: Ampullariidae) as a system for addressing fundamental questions. *American Malacological Bulletin* 27(1/2): 47-58.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Hendarsih-Suharto, Marwoto, R.M., Heryanto, Mulyadi & Siwi, S.S. 2006. The golden apple snail, *Pomacea* spp., in Indonesia. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 231-242. Philippine Rice Research Institute, Nueva Ecija.

Horgan, F.G., Stuart, A.M. & Kudavidanage, E.P. 2014a. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica* 54: 90-100.

Horgan, F.G., Felix, M.I, Portalanza, D.E., Sánchez, L., Moya Rios, W.M., Farah, S.E., Wither, J.A., Andrade, C.I. & Espin, E.B. 2014b. Responses by farmers to the apple snail invasion of Ecuador's rice fields and attitudes toward predatory snail kites. *Crop Protection* 62: 135-143.

Howells, R.G., Burlakova, L.E., Karatayev, A.Y., Marfurt, R.K. & Burks, R.L. 2006. Native and introduced Ampullariidae in North America: history, status, and ecology. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 73-112. Philippine Rice Research Institute, Nueva Ecija.

Hunt, B.P. 1958. Introduction of *Marisa* into Florida. *The Nautilus* 72: 53-55.

Hylton Scott, M.I. 1958. Estudio morfológico y taxonómico de los ampulláridos de la República Argentina. *Revista del Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'* 3: 233-333.

ICZN [International Commission on Zoological Nomenclature]. 1999. Opinion 1913. *Pila* Röding, 1798 and *Pomacea* Perry, 1810 (Mollusca, Gastropoda): placed on the Official List, and Ampullariidae Gray, 1824: confirmed as the nomenclaturally valid synonym of Pilidae Preston, 1915. *Bulletin of Zoological Nomenclature* 56: 74-76.

Jobin, W.R., Brown, R.A., Vélex, S.P. & Ferguson, F.F. 1977. Biological control of *Biomphalaria glabrata* in major reservoirs of Puerto Rico. *American Journal of Tropical Medicine and Hygiene* 26(5): 1018-1024.

Joshi, R.C. & Sebastian, L.S. (ed.). 2006. *Global Advances in Ecology and Management of Golden Apple Snails*, Philippine Rice Research Institute, Nueva Ecija.

Keawjam, R.S. & Upatham, E.S. 1990. Shell morphology, reproductive anatomy and genetic patterns of three species of apple snails of the genus *Pomacea* in Thailand. Journal of Medical and Applied Malacology 2: 49-62.

Kwong, K.-L., Wong, P.-K., Lau, S.S.S. & Qiu, J.-W. 2008. Determinants of the distribution of apple snails in Hong Kong two decades after their initial invasion. Malacologia 50: 293-302.Lach, L. & Cowie, R.H. 1999. The spread of the introduced freshwater apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) on Oʻahu, Hawaiʻi. Bishop Museum Occasional Papers 58: 66-71.

Lai, P.-Y., Chang, Y.F. & Cowie, R.H. (ed.). 2005. Proceedings – APEC Symposium *on the Management of the Golden Apple Snail*. National Pingtung University of Science and Technology, Pingtung.

Laup, S. 1991. Golden apple snail and its eradication in Papua New Guinea. In: *Proceedings of a Seminar on Pests and Diseases of Food Crops – Urgent Problems and Practical Solutions* (Kumar, R., ed.), p. 55-62. Department of Agriculture and Livestock, Konedobu.

Lee, T.-G. & Oh, K.C. 2006. Golden apple snails in Korea. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 516-525. Philippine Rice Research Institute, Nueva Ecija.

Letelier, S. & Soto-Acuña, S. 2008. Registro de *Pomacea* sp. (Gastropoda: Ampullaridae) en Chile. *Amici Molluscarum* 16: 6-13.

López, M.A., Altaba, C.R., Andree, K.B. & López, V. 2010. First invasion of the apple snail *Pomacea insularum* in Europe. *Tentacle* 18: 27-29.

Low, M.E.Y., Tan, S.K. & Ng, T.H. 2013. *Pila conica* (Wood, 1828), or *Pila scutata* (Mousson, 1848)? The correct name for the native apple snail of Singapore (Gastropoda: Ampullariidae). *Nature in Singapore* 6: 55-60.

Lv, S., Zhang, Y., Steinmann, P., Yang, G.-J., Yang, K., Zhou, X.-N. & Utzinger, J. 2011. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biology* 56: 717-734.

Lv, S., Zhang, Y., Liu, H.-X., Hu, L., Liu, Q., Wei, F.-R., Guo, Y.-H., Steinmann, P., Hu, W., Zhou, X.-N. & Utzinger, J. 2013. Phylogenetic evidence for multiple and secondary introductions of invasive snails: *Pomacea* species in the People's Republic of China. *Diversity and Distributions* 19: 147-156.

Massemin, D., Lamy, D., Pointier, J.-P. & Gargominy, O. 2009. *Coquillages et Escargots de Guyane. Seashells and Snails from French Guiana*, Biotope, Mèze (Collection Parténope); Muséum national d'Histoire naturelle, Paris.

Matsukura, K., Okuda, M., Kubota, K. & Wada, T. 2008. Genetic divergence of the genus *Pomacea* (Gastropoda: Ampullariidae) distributed in Japan, and a simple molecular method to distinguish *P. canaliculata* and *P. insularum*. *Applied Entomology and Zoology* 43(4): 535-540.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

MMAMRM [Ministerio de Medio Ambiente y Medio Rural y Marino]. 2011. *Pest Risk*

Analysis on the Introduction of Pomacea insularum *(d'Orbigny, 1835) into the EU*. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-62.

Mohammed, R.S. 2015. Potential New establishment of an exotic snail, *Pomacea canaliculata* (Lamarck 1819 [sic]) (Gastropoda: Ampullariidae) in Trinidad. *Living World, Journal of The Trinidad and Tobago Field Naturalists' Club* 2015: 67-68.

Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Neck, R.W. 1984. Occurrence of the striped ram's horn snail, *Marisa cornuarietis*, in central Texas (Ampullariidae). *The Nautilus* 98: 119-120.

Neck, R.W. 1986. A second record of an introduced apple snail, *Pomacea canaliculata*, from the lower Rio Grande valley of Texas. *Texas Conchologist* 22(3): 54-57.

Neck, R.W. & Schultz, J.G. 1992. First record of a living channeled applesnail, *Pomacea canaliculata* (Pilidae), from Texas. *Texas Journal of Science* 44: 115-116.

Ng, P.K.L. 1991. *A Guide to Freshwater Life in Singapore*. Singapore Science Centre, Singapore.

Ng, T.H., Tan, S.K. & Low, M.E.Y. 2014. Singapore Mollusca: 7. The family Ampullariidae (Gastropoda: Caenogastropoda: Ampullarioidea). *Nature in Singapore* 7: 31-47.

Ng, T.H., Tan, S.K., Wong, W.H., Meier, R., Chan, S.-Y., Tan, H.H. & Yeo, D.C.H. 2016. Molluscs for sale: assessment of freshwater gastropods and bivalves in the ornamental pet trade. *PLoS ONE* 11(8): e0161130. doi:10.1371/journal.pone.0161130

Nguma, J.F.M., McCullough, F.S. & Masha, E. 1982. Elimination of *Biomphalaria pfeifferi*, *Bulinus tropicus* and *Lymnaea natalensis* by the ampullariid snail, *Marisa cornuarietis*, in a man-made dam in northern Tanzania. *Acta Tropica* 39: 85-90.

Nugaliyadde, L., Jayasundera, D.M., Amarasinghe, A.A.L., Yusa, Y. & Hidaka, T. 2001. Distribution of an alien snail, *Pomacea bridgesii,* in fresh water habitats and its potential threat to faunal diversity and rice cultivation in Sri Lanka. *Annals of the Sri Lanka Department of Agriculture* 3: 375-376.

Orapa, W. 2006. Golden apple snail in Papua New Guinea. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 528-529. Philippine Rice Research Institute, Nueva Ecija.

Pace, G.L. 1973. The freshwater snails of Taiwan (Formosa). *Malacological Review* Supplement 1: 1-118.

Pain, T. 1960. *Pomacea* (Ampullariidae) of the Amazon river system. *Journal of Conchology* 24: 421-432.

Peebles, C.R., Oliver-González, J. & Ferguson, F.F. 1972. Apparent adverse effect of *Marisa cornuarietis* upon *Lymnaea columella* and *Biomphalaria glabrata* in an ornamental pond in Puerto Rico. *Proceedings, Hawaiian Entomological Society* 21: 247-256.

Perera, G. & Walls, J.G. 1996) *Apple Snails in the Aquarium*, T.F.H. Publications, Inc., Neptune City, New Jersey.

Pointier, J.-P. 2001. Invading freshwater snails and biological control in Martinique island, French West Indies. *Memórias do Instituto Oswaldo Cruz* 96 (Supplement): 67-74.

Pointier, J.-P. & David, P. 2004. Biological control of *Biomphalaria glabrata*, the intermediate host of schistosomes, by *Marisa cornuarietis* in ponds of Guadeloupe: long-term impact on the local snail fauna and aquatic flora. *Biological Control* 29: 81-89.

Pointier, J.-P., Théron, A., Imbert-Establet, D. & Borel, G. 1991. Eradication of a sylvatic focus of *Schistosoma mansoni* using biological control by competitor snails. *Biological Control* 1: 244-247.

Ponder, W.F., Hallan, A., Shea, M. & Clark, S.A. 2016. *Australian Freshwater Molluscs*. http://keys.lucidcentral.org/keys/v3/freshwater_molluscs/

Ranamukhaarachchi, S.L. & Wikramasinghe, S. 2006. Golden apple snails in the world: introduction, impact, and control measures. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 133-151. Philippine Rice Research Institute, Nueva Ecija.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology* 7: 97.

Roger, P.A. 1996. *Biology and Management of the Floodwater Ecosystem in Ricefields*. International Rice Research Institute, Los Baños, Philippines.

Roll, U., Dayan, T., Simberloff, D. & Mienis, H.K. 2009. Non-indigenous land and freshwater gastropods in Israel. *Biological Invasions* 11: 1963-1972.

Rosario, J. & Moquete, C. 2006. The aquatic snail *Ampullaria canaliculata* L. – plague of irrigated lowland rice in the Dominican Republic. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 514-515. Philippine Rice Research Institute, Nueva Ecija.

Seuffert, M.E., Burela, S. & Martín, P. 2010. Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *Journal of Thermal Biology* 35: 77-84.

Seuffert, M.E., Saveanu, L. & Martín, P.R. 2012. Threshold temperatures and degree-day estimates for embryonic development of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Malacologia* 55(2): 209-217.

Simberloff, D. & Stiling, P. 1996. Risks of species introduced for biological control. *Biological Conservation* 78: 185-192.

Simpson, I.C., Roger, P.A., Oficial, R. & Grant, I.F. 1994. Effect of nitrogen fertiliser and pesticide management on floodwater ecology in a wetland ricefield III. Dynamics of benthic molluscs. *Biology and Fertility of Soils* 18: 219-227.

Smith, B.D. 1992. Introduction and dispersal of apple snails (Ampullariidae) on Guam. *Pacific Science Association Information Bulletin* 44: 12-14.

Tan, S.K., Lee, Y.-L. & Ng, T.H. 2013. The status of the apple snail, *Pila scutata* (Gastropoda: Ampullariidae) in Singapore. *Nature in Singapore* 6: 135-141.

Thiengo, S.C., Hayes, K.A., Mattos, A.C., Fernandez, M.A. & Cowie, R.H. 2011. A família Ampullariidae no Brasil: aspectos morfológicos, biológicos e taxonômicos. In: *Tópicos em Malacologia: Ecos do XIX Encontro Brasileiro de Malacologia*. (Fernandez, M.A., dos Santos, S.B., Pimenta, A.D. & Thiengo, S.C.), p. 95-111. Sociedade Brasileira de Malacologia, Rio de Janeiro.

Tillier, S. 1980. Gastéropodes terrestres et fluviatiles de Guyane Française. *Mémoires du Muséum National d'Histoire Naturelle. Nouvelle Série, Série A, Zoologie* 118: 1-190.

Tran, C.T., Hayes, K.A. & Cowie, R.H. 2008. Lack of mitochondrial DNA diversity in invasive apple snails (Ampullariidae) in Hawaii. *Malacologia* 50: 351-357.

Vargas, M., Gomez, J. & Perera, G. 1991. Geographic expansion of *Marisa cornuarietis* and *Tarebia granifera* in the Dominican Republic. *Journal of Medical and Applied Malacology* 3: 69-72.Wada, T. 1997. Introduction of the apple snail *Pomacea canaliculata* and its impact on rice agriculture. In: *Proceedings. International Workshop on Biological Invasions of Ecosystems by Pests and Beneficial Organisms*, p. 170-180. National Institute of Agro-Environmental Sciences, Ministry of Agriculture, Forestry and Fisheries, Tsukuba.

Wada, T. 2006. Impact and control of introduced apple snail, *Pomacea canaliculata* (Lamarck), in Japan. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 181-197. Philippine Rice

Research Institute, Nueva Ecija.

Wijesekara, A. 2010. Invasive alien species of agricultural importance in Sri Lanka: have we managed them properly? In: *Invasive Alien Species in Sri Lanka – Strengthening Capacity to Control Their Introduction and Spread* (Marambe, B., Silva, P., Wijesundara, S. & Atapattu, N., ed.), p. 115-122. Biodiversity Secretariat of the Ministry of Environment, Sri Lanka.

Wilstermann-Hildebrand, M. 2009. *Apfel-Schnecken. Die Familie der Ampullariidae*. Natur und Tier - Verlag GmbH, Münster.

Wu, J.-Y., Meng, P.-J., Liu, M.-Y., Chiu, Y.-W. & Liu, L.-L. 2010. A high incidence of imposex in *Pomacea* apple snails in Taiwan: a decade after triphenyltin was banned. *Zoological Studies* 49: 85-93.

Wu, M. & Xie, Y. 2006. The golden apple snail (*Pomacea canaliculata*) in China. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 285-298. Philippine Rice Research Institute, Nueva Ecija.

Wu, W.-L. & Lee, Y.-C. 2005. The biology and population analysis of the golden apple snail in Taiwan. In: *Proceedings – APEC Symposium on the management of the golden apple snail* (Lai, P.-Y., Chang, Y.-F. & Cowie, R.H., ed.), 25-33. National Pingtung University of Science and Technology, Pingtung.

Wu, J.-Y., Wu, Y.-T., Li, M.-C., Chiu, Y.-W., Liu, M.-Y. & Liu, L.-L. 2011. Reproduction and juvenile growth of the invasive apple snails *Pomacea canaliculata* and *Pomacea scalaris* (Gastropoda: Ampullariidae) in Taiwan. *Zoological Studies* 50: 61-68.

Yahaya, H., Nordin, M., Muhamad Hisham, M.N. & Sivapragasam, A. 2006. Golden apple snails in Malaysia. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 215-230. Philippine Rice Research Institute, Nueva Ecija.

Yang, P.-S., Chen, Y.-H., Lee, W.-C. & Chen, Y.-H. 2006. Golden apple snail management and prevention in Taiwan. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 169-179. Philippine Rice Research Institute, Nueva Ecija.

Yipp, M.W., Cha, M.W. & Liang, X.Y. 1991. A preliminary impact assessment of the introduction of two species of *Ampullaria* (Gastropoda: Ampullariidae) into Hong Kong. In: *Proceedings of the tenth International Malacological Congress, Tübingen, 27 August-2 September 1989* (Meier-Brook, C., ed.), p. 393-397. UNITAS Malacologia, Tübingen.

Identification of *Pomacea* species using molecular techniques

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Abstract

Recent development of molecular approaches has prompted new taxonomic analyses and identification of species in the genus *Pomacea*. Following the sequencing of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene of many *Pomacea* species, this marker was used by several research groups to reveal the diversity and distributions of certain key species in both native and invaded areas. Nuclear DNA sequences such as elongation factor 1α and the H3 histone subunit together with mitochondrial sequences permitted assessment of relationships among closely related species. These molecular techniques contributed to improved taxonomic understanding of this genus, and clarification of the distribution and invasion pathways of some important species. Nonetheless, there remains some risk of misidentification when relying on molecular techniques.

Additional keywords: Ampullariidae, Asia, DNA sequencing, invasive species, Mollusca, *Pomacea canaliculata*, *Pomacea maculata*

Introduction

Taxonomy and identification of organisms have traditionally been based on morphological features. Identification by diagnostic morphology is usually easy and reliable once the diagnostic traits of the target species are clarified. The remarkable development of molecular biology in recent decades, however, has brought a novel approach to taxonomy. A molecular approach allows us to distinguish organisms that could not be distinguished morphologically, to clarify phylogenetic distances among related species and to examine the possibility of genetic exchange between closely related species. Nowadays, DNA barcoding, sequencing a short genetic marker specific to individual species, is widely used to distinguish a large number of species in almost all groups of organisms (e.g. CBOL Plant Working Group, 2009; Teletchea, 2009; Schlick-Steiner *et al*., 2010).

The taxonomy of the genus *Pomacea* has historically been extremely confused because of intraspecific variation and environmental influences on the variation of morphological traits (Cazzaniga, 2002; Estebenet & Martín, 2003). The number of nominal *Pomacea* species was thought by Cazzaniga (2002) to be approximately 50, with fewer than this actually thought to be valid because of unrecognized synonyms. However, rigorous nomenclatural compilations recognized almost 100 valid species (Cowie & Thiengo, 2003; Cowie, 2015; Hayes *et al*., 2015). Although recent morphological and molecular analysis concluded that *P. insularum*, *P. gigas* and a number of other species were synonyms of either *P. maculata* or of *P. canaliculata* (Hayes *et al*., 2012), other as yet un-named species have yet to be described (R.H. Cowie, pers. comm.). Nonetheless, Hayes *et al*. (2015) still estimated the real number of valid species of *Pomacea* to be around 50. The taxonomy of *Pomacea* will gradually become better understood through further morphological and molecular biological analyses.

Following the initial clarification of the identities of the non-native species of *Pomacea* in North America, using mitochondrial DNA sequences (Rawlings *et al*., 2007), molecular approaches addressing the taxonomy, identification, interspecific relationships and geographic origins of *Pomacea* species have developed remarkably (e.g. Hayes *et al*., 2008, 2009; Tran *et al*., 2008). Here, we first review recent works on the taxonomy, diversity and distribution of *Pomacea* species based on DNA sequences. We then provide brief technical guidance for DNA analysis of *Pomacea*, commenting on both the advantages and disadvantages.

Identification of *Pomacea* in Asia

Establishment of techniques for analysis of mitochondrial cytochrome *c* oxidase subunit I (COI) DNA sequences in *Pomacea* species contributed greatly to understanding the current status and process of expansion of the invasive species in Asia.

Identification of invasive species is one of the most fundamental needs when attempting to manage them. However, even 25 years after the first invasion of *Pomacea* in East and Southeast Asian countries, rigorous identification of the invasive snails was not possible because identification of *Pomacea* species based on shell morphology, particularly among closely related species, was difficult and in some cases impossible (Cowie *et al*., 2006). Analysis of COI sequences of *Pomacea* species from throughout the region revealed that two species, *P. canaliculata* and *P. maculata*, are widely distributed from Malaysia to Japan (Hayes *et al*., 2008; Matsukura *et al*., 2008), and that *P. scalaris* occurred in Taiwan (Hayes *et al*., 2008). This result resolved the presumption in a number of papers, based on morphology, that there was more than one species of *Pomacea* introduced widely in Asia (e.g. Mochida, 1991; Keawjam & Upatham, 1986), belonging to the "*canaliculata* group", which includes *P. canaliculata* and closely related species (Cazzaniga, 2002; Hayes *et al*., 2009). For example, *Pomacea* introduced to Japan had been thought to be a single species (*P. canaliculata*) until 2008 but analysis of COI sequences showed that both *P. maculata* and *P. canaliculata* were present (Matsukura *et al*., 2008). In addition, the molecular analysis revealed that *P. canaliculata* is dominant and widespread in most parts of southern and central Japan, whereas *P. maculata* was observed in only three of ten prefectures in the southern region. Revealing the current distributions of *Pomacea* spp. in Japan has prompted establishment of appropriate management strategies in prefectures in the infested regions.

Analysis of COI sequences of *P. canaliculata* and *P. maculata* in East and Southeast Asia also revealed that these snails were introduced probably on multiple occasions from their native regions because the COI sequences were significantly divergent and contained several haplotypes found in native populations in Brazil and Argentina (Hayes *et al*., 2008). Clarification of the invasive process from native to invaded areas is important in permitting attempts to limit further invasion and expansion of the invasive snails. Further molecular analysis (e.g. SNP and microsatellite analysis) of both native and invasive populations could reveal additional details of the invasion process and help to predict the risk of further spread in the invaded areas.

Clarifying the inter-specific relationship between *P. canaliculata* and *P. maculata*

Analysis of nuclear DNA sequences can be an effective approach for assessing relationships between closely related species, whereas analysis of mitochondrial DNA sequences, often in combination with nuclear DNA, is more often used to examine phylogenetic relationships among species within a genus, family or other taxonomic group of interest. Unlike mitochondrial DNA, nuclear DNA is derived from both parents, and therefore, analysis of nuclear DNA can detect genetic exchange and its frequency between species.

The confused taxonomy of *Pomacea* has been improved remarkably since the advent of molecular approaches using mitochondrial DNA; however, some relationships are still unclear, particularly among closely related species. Matsukura *et al*. (2013) examined the nuclear elongation factor 1α (EF1α) sequences of *P. canaliculata* and *P. maculata* collected from East and Southeast Asia. Phylogenetic analysis of these sequences demonstrated significant divergence of the sequences between the two species, as Hayes *et al*. (2009) had also reported. However, some snails had both *P. canaliculata* and *P. maculata* EF1α sequences. This result indicates that there is genetic exchange between *P. canaliculata* and *P. maculata*. The hybrid snails were widely distributed in the region (Matsukura *et al*., 2013). Similar genetic exchange between the two species also seems to occur in their native South America (Hayes *et al*., 2012; Matsukura *et al*., 2013). Knowledge of the origin of the hybrid snails and their adaptive significance would add to our understanding of diversity and distribution in the genus. Similar clarification of relationships among other closely related *Pomacea* species would be valuable.

Technical guidance for DNA analysis of *Pomacea*

Analysis protocols (DNA extraction, amplification by PCR and analysis) for *Pomacea*, which basically follow general protocols used in animals, differ (but not considerably) among research groups (e.g. Thaewnon-ngiw *et al*., 2004; Rawlings *et al*., 2007; Matsukura *et al*., 2008; Dong *et al*., 2011; Cooke *et al*., 2012). In this section, technical guidance for *Pomacea* DNA analysis is provided. For more details see Matsukura *et al*. (2008) and Matsukura *et al*. (2013).

DNA extraction

Foot muscle of *Pomacea* is usually used for DNA extraction (Thaewnon-ngiw *et al*., 2004; Rawlings *et al*., 2007; Matsukura *et al*., 2008) to prevent contamination of DNA by symbiotic microorganisms and gut contents (DNA of prey can be detected in the early stages of digestion). The phenol-chloroform-protease K method (Thaewnon-ngiw *et al*., 2004; Rawlings *et al*., 2007), Qiagen's Dneasy extraction kit (Rawlings *et al*., 2007; Dong *et al*., 2011; Cooke *et al*., 2012) and GE Healthcare's Nucleon PhytoPure DNA extraction kit (Matsukura *et al*., 2008) have all been used for DNA extraction, and are all appropriate for obtaining pure whole genomic DNA from *Pomacea*. Whole genomic DNA can be extracted from ethanol-preserved samples as well as fresh material (Hayes *et al*., 2008; Dong *et al*., 2011).

Primer sets for **Pomacea**

Mitochondrial COI sequences are used to distinguish *Pomacea*, as widely used for other organisms. The primer set for COI in diverse metazoan invertebrates designed by Folmer *et al*. (1994) can be used for *Pomacea*. PCR using this primer set amplifies an approximately 700 bp fragment of COI of *Pomacea*, and the sequences are sufficiently divergent to distinguish most *Pomacea* species (Rawlings *et al*., 2007). In addition to COI, the mitochondrial rRNA region (Rawlings *et al*., 2007), nucleotide 18S rDNA, the H3 histone subunit and EF1α (Hayes *et al*., 2009), and inter-simple sequence repeats (Dong *et al*., 2011) have all been used to reveal inter-specific and inter-population relationships in *Pomacea* (Table 1).

Amplified DNA fragments are usually sequenced for further analysis such as for identification and phylogenetic analysis. To distinguish certain species, simple molecular methods have been developed to save the cost of sequencing. *Pomacea canaliculata*, *P. maculata* and "*P. bridgesii*" (in fact almost certainly *P. diffusa*) are distinguishable by multiplex PCR of mitochondrial COI using primer sets (Table 1) specific to each species (Matsukura *et al*., 2008; Cooke *et al*., 2012). EF1α sequences in conjunction with a restriction enzyme method using *Apa*LI can distinguish *P. canaliculata* and *P. maculata* and detect hybridization between them (Fig. 1) (Matsukura *et al*., 2013).

Table 1. Primer sets (forward and reverse) for phylogenetic analysis or identification in *Pomacea*.

Target region	Species	Sequence		Annealing temp. (°C)	Reference [a]
mitochondrial					
cytochrome *c* oxidase subunit I	almost all [b]	fwd:	GGT CAA CAA ATC ATA AAG ATA TTG		Rawlings *et al*. (2007)
		rev:	TAA ACT TCA GGG TGA CCA AAA AAT CA	48-55	
	P. canaliculata [c]	fwd:	TGG GGT ATG ATC AGG CC	55	Matsukura *et al*. (2008)
	P. maculata [c]	fwd:	ATC TGC TGC TGT TGA AAG	55	Matsukura *et al*. (2008)
	P. bridgesii [c, d]	fwd:	CTC TAT CCT AGG TGC GGT AAA TT	55	Cooke *et al*. (2012)
16S ribosomal DNA		fwd:	CGC CTG TTT ATC AAA AAC AT		Hayes *et al*. (2009)
		rev:	CTC CGG TTT GAA CTC AGA TC	48	
nuclear					
elongation factor 1α	almost all	fwd:	TGT GAA TAA GAT GGA CAG CA		Hayes *et al*. (2009)
		rev:	AAT CCT AAC CTC CAA TTT TGT	50	
H3 histone subunit	almost all	fwd:	ATG GCT CGT ACC AAG CAG ACV GC		Hayes et al. (2009)
		rev:	ATA TCC TTR GGC ATR ATR GTG AC	60	
18S ribosomal DNA	almost all	fwd:	TAC CTG GTT GAT CCT GCC AGT AG		Hayes *et al*. (2009)
		rev:	GAA TTA CCG CGG CTG CTG G	48	
inter-simple sequence repeat	*P. canaliculata*	$(AG)_8C$, $(GA)_8C$, $(GAA)_6$, $(CA)_8RY$		46-52	Dong *et al*. (2011)

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[a] references are to the first published study that used each primer set for analysis of *Pomacea*

[b] see Hayes *et al*. (2009) for species for which the primer set is available

[c] reverse primer was commonly used with other species analysed by Rawlings *et al*. (2007) and Hayes *et al*. (2009)

[d] in fact almost certainly *P. diffusa*

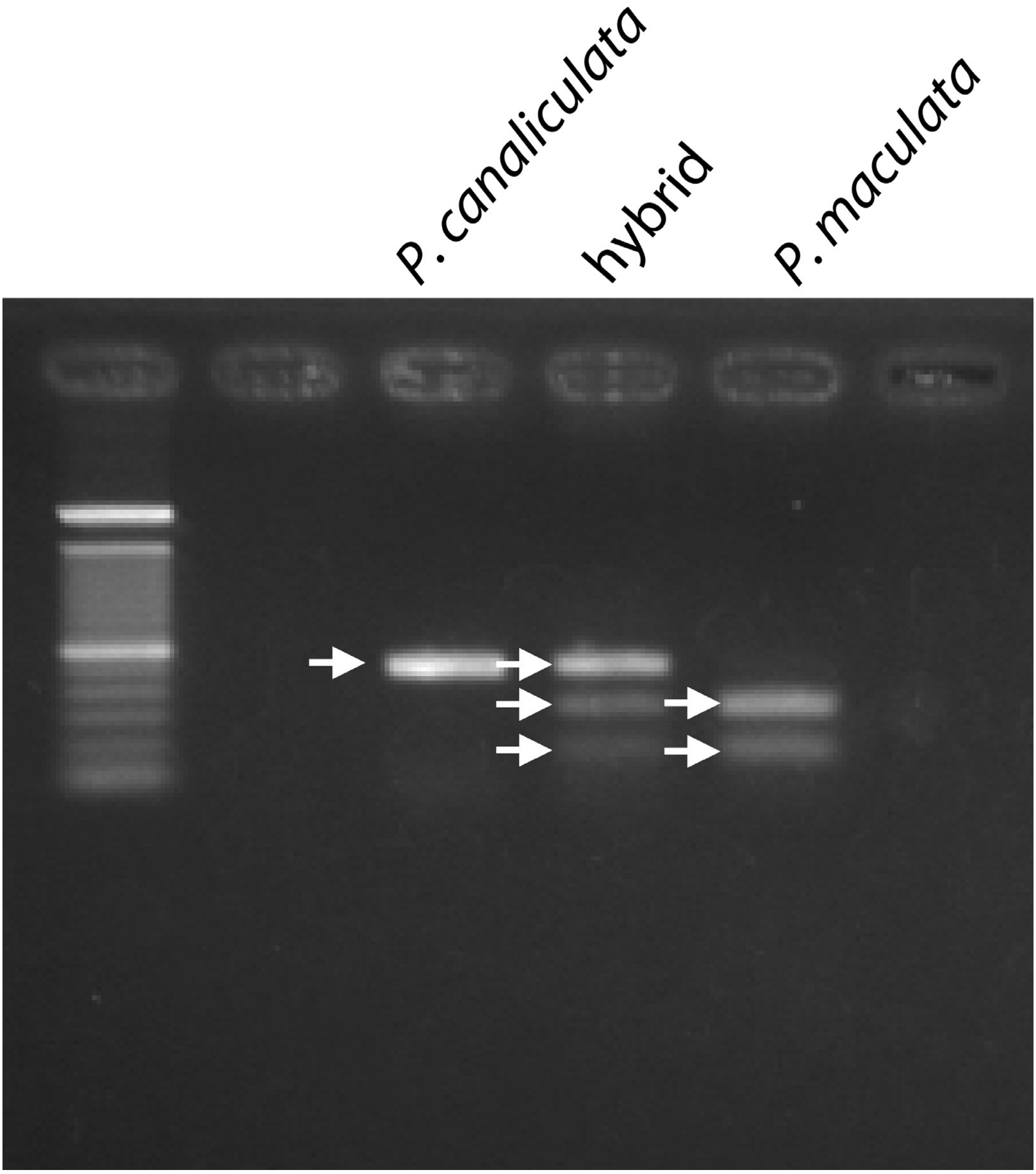

Fig. 1. Identification of nuclear EF1α type of *Pomacea canaliculata*, *P. maculata* and their hybrids using a restriction enzyme approach. *Pomacea canaliculata* exhibits a single fragment of ca. 520 bp, whereas *P. maculata* shows two fragments of 190 and 330 bp). Hybrid snails show all three fragments.

Advantages and disadvantages of DNA analysis for identification of *Pomacea*

Before the development of molecular approaches, the taxonomy of *Pomacea* was extremely confused and therefore misidentification often occurred (Cazzaniga, 2002). Divergent mitochondrial COI sequences among key *Pomacea* species (Rawlings *et al*., 2007; Hayes *et al*., 2008, 2009, 2012) have played a central role in improving *Pomacea* taxonomy. Because DNA analysis is a routine technique in modern biology, identification of *Pomacea* using molecular techniques has been (and will be) performed in several laboratories. Molecular approaches, therefore, will be important in clarification of the current distributions of the key invasive species (*P. canaliculata*, *P. maculata* etc.) as well as the pathways and processes of invasion in the genus.

Although identification using COI sequences is a valuable approach, it is not perfect. If there is genetic exchange among species, identification via mitochondrial sequences may risk misidentification. For example, Matsukura *et al*. (2013) reported discordance between mitochondrial COI and nuclear EF1α types between *P. canaliculata* and *P. maculata* (i.e. some snails had EF1α of *P. maculata* despite having COI of *P. canaliculata*, and vice versa). Careful identification together with analysis of nuclear sequences and morphological consideration is recommended for species that may hybridize with other species, or that need taxonomic reconsideration.

Also, although distinguishing two or more known species using these simple methods is feasible, if another congener is included unknowingly among the individuals to be tested, it could be misidentified as one or other of the known species, or its DNA may simply not be amplified.

References

Cazzaniga, N.J. 2002. Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda: Ampullariidae). *Biocell* 26: 71-81.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 12794-12797.

Cooke, G.M., King, A.G., Miller, L. & Johnson, R.N. 2012. A rapid molecular method to detect the invasive golden apple snail *Pomacea canaliculata* (Lamarck, 1822). *Conservation Genetics Resources* 4: 591-593.

Cowie, R.H. 2015. The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa* 3940: 1-92.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastropoda: Ampullariidae: *Asolene*, *Felipponea*, *Marisa*, *Pomacea*, Pomella): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In*: Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-23. Philippine Rice Research Institute, Nueva Ecija.

Dong, S., Shentu, X., Pan, Y., Bai, X., Yu, X. & Wang, H. 2011. Evaluation of genetic diversity in the golden apple snail, *Pomacea canaliculata* (Lamarck), from different geographical populations in China by inter simple sequence repeat (ISSR). *African Journal of Biotechnology* 10: 1777-1783.

Estebenet, A.L. & Martín, P.R. 2003. Shell interpopulation variation and its origin in *Pomacea canaliculata* (Gastropoda: Ampullariidae) from southern pampas, Argentina. *Journal of Molluscan Studies* 65: 301-310.

Folmer, O., Black, M., Hoeh, W., Lutz, W. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008 Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Thiengo, S.C. & Cowie, R.H. 2009 A global phylogeny of apple snails: Gondwanan origin, generic relationships and the influence of outgroup choice (Caenogastropoda: Ampullariidae). *Biological Journal of the Linnean Society* 98: 61-76.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Keawjam, R.S. & Upatham, E.S. 1990. Shell morphology, reproductive anatomy and genetic patterns of three species of apple snails of the genus *Pomacea* in Thailand, *Journal of Medical and Applied Malacology* 2: 45-57.

Matsukura, K., Okuda, M., Kubota, K. & Wada, T. 2008. Genetic divergence of the genus *Pomacea* (Gastropoda: Ampullariidae) distributed in Japan, and a simple molecular method to distinguish between *P. canaliculata* and *P. insularum*. *Applied Entomology and Zoology* 43: 535-540.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-62.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology* 7: 97.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421-438.

Teletchea, F. 2009. Molecular identification methods of fish species: reassessment and possible applications. *Reviews in Fish Biology and Fisheries* 19: 265-293.

Thaewnon-ngiw, B., Klinbunga, S., Phanwichien, K., Sangduen, N., Lauhachinda, N. & Menasveta, P. 2004. Genetic diversity and molecular markers in introduced and Thai native apple snails (*Pomacea* and *Pila*). *Journal of Biochemistry and Molecular Biology* 37: 493-502.

Tran, C.T., Hayes, K.A. & Cowie, R.H. 2008. Lack of mitochondrial DNA diversity in invasive apple snails (Ampullariidae) in Hawaii. *Malacologia* 50: 351-357.

Invasive apple snails are threatening natural ecosystems in Southeast Asia, Europe and North America

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Abstract

Ever since it was found that apple snails have an appetite for rice seedlings, there have been numerous studies on how to control several *Pomacea* species in rice fields. Unfortunately, less effort has been devoted to investigating the invasive snails' impact on natural wetlands and other aquatic ecosystems. This is of major concern, as wetlands are highly productive ecosystems that harbour great biodiversity and biological production that is important in the rural economy of many countries, especially in Southeast Asia. The plants in these wetlands that now are threatened by herbivorous apple snails do not only provide food, animal fodder and habitat for a number of organisms, but they ensure minimum water quality maintenance, since nutrients in sewage water from nearby households are assimilated in the growing plant tissues. Later, when the plants are harvested, the nutrients are removed from the system. In many rural areas, this is the only wastewater treatment available. This contribution presents findings from a project that investigated how apple snails affect natural wetland ecosystems in Southeast Asia and the more recent invasions in southern Europe and North America, as well as discussing future research needs. The results clearly show that apple snails are a serious threat to these important ecosystems. At higher densities, the snails have the capacity to completely eradicate most palatable plant species. Besides the loss of plants that used to provide income or habitat, this may lead to high in-water nutrient levels and algal blooms and, therefore, lower water quality. Several *Pomacea* species are omnivorous and may therefore affect biodiversity directly through predation. The concluding recommendation is that apple snail research should include a more holistic approach in which rice fields and wetlands are considered together. We know that apple snails recolonize rice fields through irrigation canals that are connected to wetlands and, of course, from more distant water systems during floods. Likewise, pesticides used in rice fields flow in the

opposite direction to the detriment of many organisms, including humans, in the natural environment.

Additional keywords: algal blooms, Ampullariidae, aquatic plants, herbivory, natural predators, natural wetlands, pesticides, stable shift, water quality

Introduction

The invasive apple snail *Pomacea canaliculata* is a well studied rice pest in Southeast Asia, but fewer studies have quantified the effects of this snail and similar *Pomacea* species on the natural environment in this region or elsewhere. In recent years, however, there has been more focus on how invasive *Pomacea* snails interact with native biota and how they affect invaded ecosystems (see Horgan *et al*., 2012). Increased attention to the ecosystem effects of these invaders is welcome and important, especially as apple snail densities in natural wetlands and shallow lakes may be as high as those in rice fields, and because these snails consume large quantities of many different plant species besides rice. Apple snails will feed on living invertebrates including worms, microcrustaceans, bryozoans and other snails (Wood *et al*., 2006; Horgan *et al*., 2014), including conspecific eggs and hatchlings (Horn *et al*., 2008). They may also feed on fish eggs (Carlsson, personal observation). They are even able to feed on vegetal and animal material floating at the surface by pedal surface collection (Saveanu & Martín, 2013). This ability to use multiple food sources suggests that *Pomacea* snails may remain at high population densities even after they have consumed most aquatic plants, making plant recovery difficult. It is also likely that predation on invertebrates becomes increasingly important in the relative absence of aquatic plants.

Pomacea snails are still invading new habitats. In 2009 the Ebro delta in Spain was invaded by *Pomacea maculata*, where it has the potential to become a major pest of rice (EFSA, 2012, 2013; Horgan *et al*., 2014). This is the first record of apple snails establishing in the wild in Europe and major efforts are being undertaken to eradicate the snail in Spain and to halt further spread in Europe (EFSA, 2012, 2013). Wetlands in both Southeast Asia and Europe are often located close to or even connected to rice fields. This means that snail control efforts that are focused only on rice fields are likely to be short-lived, as new snails enter the fields from nearby wetlands through irrigation canals and during floods. Pesticides that are used in rice fields to kill apple snails are likely to be transported in the opposite direction and enter the natural environment, with negative consequences for many organisms, including people. It is therefore important that snail control efforts and studies focus on both rice fields and natural waters, such as wetlands. Invasive species are a major threat not only to biodiversity but also to ecosystem services of great economic and cultural value (Buchan & Padilla, 1999; Ewel *et al*., 1999; Mack *et al*., 2000; Strayer, 2001).

Role of aquatic plants in wetland ecosystems

Aquatic plants are central to most biotic and abiotic processes in wetlands, shallow lakes and the littoral zones of rivers and deeper lakes. The plants maintain biodiversity by providing varied and structurally complex habitats that offer refuge from predation for macroinvertebrates, zooplankton and juvenile fish (Diehl, 1988, 1992; Persson & Crowder 1998); and they represent food, or the substrate for food (periphyton), that is consumed by macroinvertebrates (James *et al*., 2000), fish and waterfowl (Lodge *et al*., 1998). In Southeast Asia, large areas are covered by wetlands that are more or less affected by human activity, and the aquatic plant community is central to many functions in these productive and important ecosystems (Fig. l). These wetlands host a large number of animal and plant species that are important for the rural poor who herd ducks, harvest aquatic plants and catch molluscs, crustaceans and fish for food and animal fodder. The wetlands are also important as natural biofilters that may ensure minimum water quality. Nutrients in wastewater from nearby houses and villages are assimilated in growing plant tissues and, in a climate that favours year-round plant growth, continuous harvest of these fast-growing aquatic plants removes assimilated nutrients from the wetland system. In some wetlands that receive domestic wastewater, this plant removal may be the only wastewater treatment available. Aquatic plants in wetlands are thus an important part of rural everyday life in Southeast Asia and many resident and transient organisms depend on wetland plants at some life stage.

Fig. 1. Wetland not yet invaded by *Pomacea*.

What happens when apple snails invade?

Pomacea canaliculata and to a lesser extent *P. maculata* (formerly known as *P. insularum*; Hayes *et al*., 2012) are the best known invasive apple snails. They are omnivorous animals that feed on a great variety of plants, detritus, periphyton and animal matter in both native and invaded habitats. However, feeding, activity, growth and reproduction increase with temperature, and mean temperatures in large parts of Southeast Asia are much higher than those in the species' natural range in South America, especially for *P. canaliculata*, which has a more southerly distribution than *P. maculata* (Hayes *et al*., 2015). Invaded wetlands in Southeast Asia and other warm regions therefore become infested with active, fast growing and fast reproducing snails. To make matters worse, *Pomacea* snails also seem to prefer naive aquatic plants, like those found in North America (and probably in other invaded habitats) compared to South American plants that have coevolved with the snails. The best explanation of this is that the North

American and other naive aquatic plants lack the chemical and physical defenses that deter the snails in South America (Morrison & Hay, 2011). This suggests that the aquatic plants in both Asia and Europe lack a coevolutionary history with *Pomacea*, which makes them highly susceptible to herbivory. For these reasons the ecosystem resilience to a *Pomacea* invasion is expected to be low in warmer parts of Southeast Asia, Europe and the southern United States.

Invasive *Pomacea* populations therefore become pests not only of rice but also of other plants of direct economic importance such as taro (*Colocasia esculenta*), morning glory (*lpomoea aquatica*), lotus (*Nelumbo nucifera*), mat rush (*Juncus decipiens*), Chinese mat grass (*Cyperus monophyllus*), wild rice (*Zizania latifolia*), Japanese parsley (*Oenanthe stolonifera*), water chestnut (*Trapa bicornis*) and azolla (*Azolla* spp.) (Mochida, 1991), as well as many others (Carlsson, unpublished). It is crucial to quantify this consumption to understand how the aquatic plant community is affected by this new and intense herbivory. During experiments in the Lao PDR (Carlsson & Lacoursière, 2005), we allowed snails (in this case probably *P. canaliculata*: R.H. Cowie, pers. comm.) to consume duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*) and morning glory (*Ipomoea aquatica*) in field enclosures in a wetland. These plants were selected for their local dominance, economic value (Choowaew, 1993) and tolerance of polluted waters, which allows them to be used in wastewater treatment (Tripathi *et al*., 1991). Water hyacinth is, however, a South American plant that may be an invasive pest itself. In this experiment snails at a density typical for the area (10 snails/m^2) consumed an amount of duckweed and water hyacinth that, if these plants were to be consumed at the same rate in the wetlands, would equal up to 500 kg wet mass per hectare per day. The snails were excluded from other food sources and this could exaggerate consumption, but we found it highly likely that herbivory of this magnitude would seriously affect the wetland ecosystem. If the plants that people harvest decline drastically when the invasive snails consume them instead, both the local economy and the removal of nutrients that have been assimilated in the plants are threatened. This is a major difference between plant harvest and apple snail herbivory, since the snails only recycle the nutrients within the wetland. To get a better picture of how the apple snails affect wetland ecosystems, we therefore surveyed natural wetlands in Thailand that were not recipients of domestic wastewater but that had varying densities of apple snails (Carlsson *et al*. 2004a), in this case probably *P. maculata* (R.H. Cowie, pers. comm.). After this we performed a complementary field experiment to unravel the mechanisms behind the patterns found. In the survey, snail densities, aquatic plant cover, aquatic plant species richness, nutrient (total phosphorus and total nitrogen) concentrations and chlorophyll a (as an estimate of phytoplankton biomass) were determined for each of 14 wetlands in the same catchment area. The survey showed that high densities of apple snails were associated with an almost complete absence of aquatic plants, high nutrient concentrations and high phytoplankton biomass (Carlsson *et al.*, 2004a; Fig. 2).

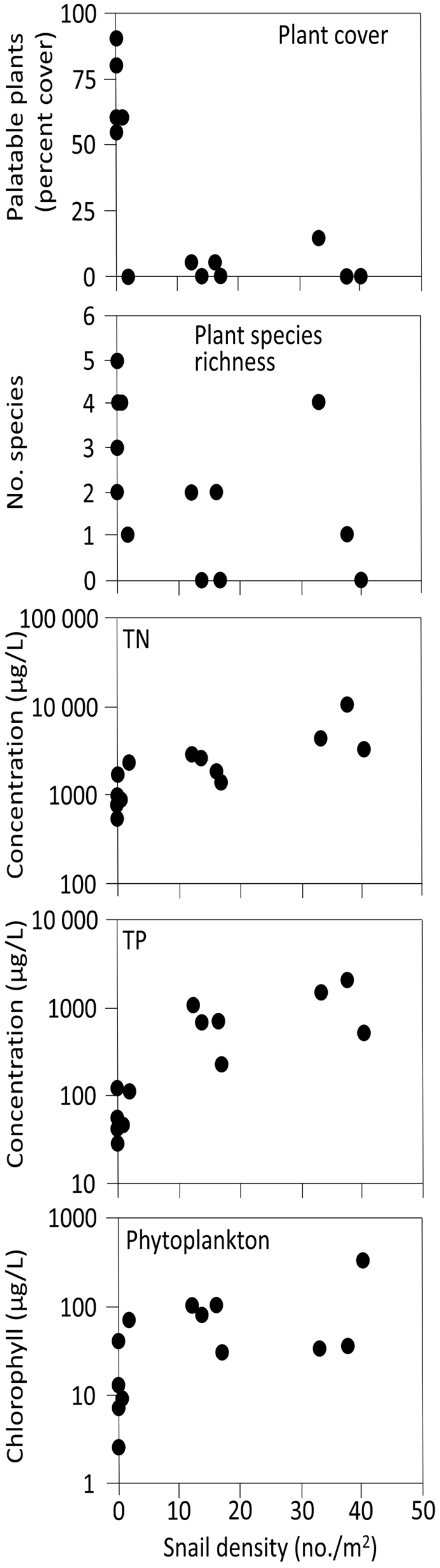

Fig. 2. Correlations among sites between snail density and macrophyte plant cover, species richness, total nitrogen (TN), total phosphorus (TP), and phytoplankton chlorophyll a in Thai wetlands (n = 14). Note the log axes on the TN, TP and chlorophyll graphs. From Carlsson et al. (2004a).

This snapshot suggests that increasing apple snail densities led to eradication of aquatic plants, which in turn strongly modifies the flux of nutrients and shifts primary production from plants to phytoplankton. Shifts from a plant-dominated, clear-water state to a turbid, algal-dominated state (Scheffer *et al.*, 1993) have attracted much interest in ecology. These alternative states are quite stable, since both the vegetated and turbid states are self-reinforcing (Scheffer *et al.*, 1993).

In the follow-up experiment, apple snail herbivory again had a strong negative effect on plant biomass, and this decrease in aquatic plants led to severe algal blooms; i.e. snails had an indirect positive effect on phytoplankton biomass (Carlsson *et al.*, 2004a; Fig.3). Calculations further showed that the amount of phosphorous recycled by snail herbivory was sufficient to promote the observed increase in phytoplankton. Although several previous studies had shown that herbivores such as birds (van Donk, 1998), fish (Hansson *et al.*, 1987), and crayfish (Lodge *et al.*, 1998) feed intensely on aquatic plants, it had not been shown that herbivores can induce shifts between alternative stable states in freshwater systems.

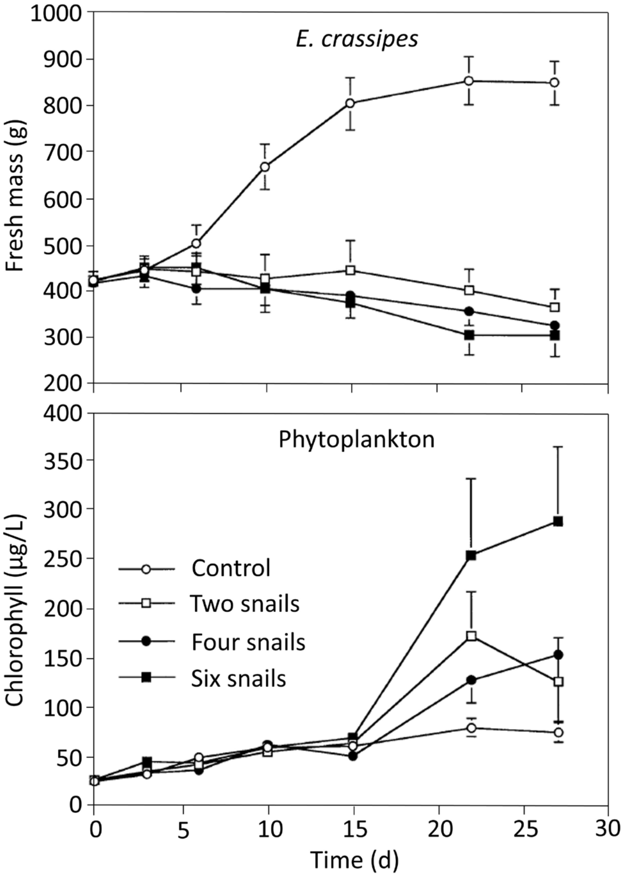

Fig. 3. Temporal development in phytoplankton chlorophyll a in experimental enclosures at snail densities of zero, two, four and six individuals per m^2. Mean values ± SE are given (n = 6). From Carlsson et al. (2004a).

More importantly, we now know that the ongoing invasion by *Pomacea canaliculata* and *P. maculata*, until quite recently recognized mainly as one of the most serious threats to rice production, alters both the state and functions of invaded wetland ecosystems to the detriment of all organisms that depend on aquatic plants. The ongoing invasions by these snails is thus both an economic and an ecological catastrophe.

Natural predators of the invasive snails

Ever since it was discovered that the intentionally introduced snails spread from aquaculture and started to consume rice seedlings, strenuous efforts by farmers, scientists and governments in Southeast Asia have been made to control them. Farmers handpick the snails and their eggs, transplant older rice seedlings (less susceptible to attack), and try to keep water levels in rice fields to a minimum, since the snails stop feeding when the water level drops below the height of their shells (Yusa & Wada, 1999). Various ducks and fish (mainly common carp, *Cyprinus carpio*) consume apple snails and are therefore herded through or bred in the rice fields (Fernando & Halwart, 2000; Teo, 2001). Besides these and other labour-intensive techniques of snail control, the snail invasion has led to indiscriminate use of registered and unregistered pesticides (Mochida, 1991; Naylor, 1996; Yusa & Wada, 1999), with serious non-target effects on human health and the associated environment (Anderson, 1993; Halwart, 1994). Pesticides applied in the water are further of limited use since they kill both invasive snails and non-target organisms, but not the snails' eggs that are deposited out of the water. Hatching snails (after 2-3 weeks) are therefore unlikely to meet many predators or competitors in the water, and since the effects of the pesticides have probably worn off, the field has to be treated again. Unfortunately, not only pesticides but all the above control strategies are futile during the floods that occur frequently in many Southeast Asian countries. This is because snails in adjacent channels and wetlands rapidly recolonize the rice fields at rising water levels (Mochida, 1991; Yusa & Wada, 1999).

Very little is known about how the apple snails interact with the native biota of invaded areas but sustainable and long-term control measures for these invasive snails should, because of their intimate connection, involve both rice fields and natural aquatic ecosystems. In an attempt to do this, we tested a large number of predators that are indigenous to the Lao PDR and that occur in both rice fields and wetlands to see if and how they could affect the snails (Carlsson *et al*. 2004b). Predation is one of the major factors influencing prey population dynamics and community structure (e.g. Sih, 1987;

Lima & Dill, 1990), and predators can affect prey resource use through both lethal and nonlethal effects (McIntosh & Townsend, 1996; Turner *et al*., 2000). *Pomacea* are key prey organisms for a number of predator taxa in their native regions (Donnay & Beissinger, 1993) and respond with predator avoidance to chemical cues released by many different predators (Snyder & Snyder, 1971). They also respond to chemical cues released from injured conspecifics (Ichinose, 2002).

Important predators could therefore reduce the impact of the snails on both rice and natural aquatic plants through both lethal and nonlethal effects. Invasive species are often thought to be free from natural enemies in their new habitat, but in some areas of Southeast Asia, hunting and fishing pressures are so high that they could mask potential effects of predators on the snail. This is because predators rarely reach population or individual sizes at which they are able to affect the unwanted snails. Predator removal can have large and cascading effects on ecosystems (e.g. Silliman & Bertness, 2002).

Feeding trials (Carlsson *et al*., 2004b) revealed that at least four indigenous fish species (*Clarias batrachus*, *Anabas testudineus*, *Ompok bimaculatus* and *Osphronemus exodon*), the turtle *Malayemys subtrijuga* and the freshwater crab *Esanthelphusa nimoafi* (Yeo, 2004) consumed apple snails of a shell height of 2-24 mm. The results therefore add to the numbers of indigenous and non-indigenous organisms (accidentally introduced) that (at least in the laboratory) consume the snail (Yusa & Wada, 1999; Yusa, 2001; Ichinose *et al*., 2002, and references in these papers).

Unfortunately few studies have quantified predation and other interactions between the invasive snail and native predators in natural habitats of Southeast Asia (or elsewhere), as pointed out by Yusa *et al*. (2000). It is therefore too early to conclude that there are no effective indigenous predators of the invasive snails. An invasive population is a growing resource for those predators that learn to us it (Carlsson *et al*., 2009). Halwart (1994) suggested that, in order to develop biological control programs for the snails in Southeast Asia, we need to focus on predators and parasitoids of the snail in its native South America. Further introductions of non-native species for biological control may, however, have unpredictable and unwanted effects on non-target organisms and natural ecosystems. This approach should therefore remain as a very last resort.

Conclusion

The vast majority of studies on *Pomacea* in Southeast Asia and other invaded regions since the first introductions in the early 1980s have been carried out in rice fields in the context of rice crop protection. In recent years, however, increased attention has been paid to the environmental impacts of these snails. This is important since *Pomacea* densities in invaded wetlands in parts of Southeast Asia and elsewhere are as high as those reported from snail-infested rice fields (Hirai, 1988; Halwart, 1991; Tanaka *et al*., 1999). Further, in invaded wetlands, these high snail densities may be associated with an almost complete absence of aquatic plants, high nutrient concentrations and high biomass of phytoplankton (Carlsson *et al*., 2004a). Invasion of *Pomacea* thus has an impact at the ecosystem level, as it causes a complete shift in both ecosystem state and function of invaded wetlands. Modifications of energy and material fluxes by non-native species may result in loss of biodiversity and ecosystem services of great economic value (Costanza *et al*., 1997), and reduced biodiversity and biomass of aquatic plants affect the nutrient retention capacities of wetlands considerably (Engelhardt & Ritchie, 2001). This is of great concern considering the enormous areas that are invaded in Southeast Asia and those that are becoming invaded elsewhere.

The snails' eggs are aerial and tolerant to changes in humidity and temperature. Egg batches are frequently found attached to aquatic plants that are later transported to both nearby and distant food markets. Egg batches are also deposited on boat hulls, which may be very potent vectors (Fig. 4). Buchan & Padilla (1999) found that the secondary *Dreissena polymorpha* (zebra mussel) colonization of North America, which followed the original introduction to the continent, corresponded highly with recreational boat movements.

Pomacea snails are still spreading within the invaded countries (e.g. Hayes *et al*., 2008; Lv *et al*., 2011), and further spread to countries not yet invaded may still be expected (Baker, 1998). *Pomacea canaliculata* has become abundant in Hawaii (Lach & Cowie, 1999; Cowie *et al*., 2007); both it and *P. maculata* are spreading in some areas of southern North America (Rawlings *et al*., 2007); and *P. maculata* has recently been found in the Ebro delta in Spain (EFSA, 2012, 2013; Horgan *et al*., 2014).

The ongoing spread stresses the urgent need for more research focusing on dispersal mechanisms, population dynamics and effects of *Pomacea* snails in different invaded habitats. Such research could lead to more sustainable and effective control of these invasive snails if, and only if, both managed systems, such as rice fields, and natural

Fig. 4. Boat hulls serve as potent *Pomacea* dispersal vectors, Lao PDR.

systems are considered together. Recent attempts to collect *Pomacea* snails on a larger scale in both rice fields and wetlands and to use this huge resource of protein and calcium as valuable raw material in the animal fodder industry seem promising. The growing list of potential indigenous predators of the snails that seem to adapt to use the new and abundant food resource also deserves more study and attention (Carlsson *et al*., 2009). It is possible that further in-depth studies on how these predators affect the invasive snails in areas of Southeast Asia and elsewhere, where hunting and fishing pressures are intense, may lead to the conclusion that snail control may be one of many economic reasons to conserve biodiversity.

References

Anderson, B. 1993. The Philippine snail disaster. *The Ecologist* 23: 70-72.

Baker, G.H. 1998. The golden apple snail, *Pomacea canaliculata* (Lamarck) (Mollusca: Ampullariidae), a potential invader of fresh water habitats in Australia. In: *Pest management – Future challenges. Sixth Australasian Applied Entomological Research Conference* (Zalucki M.P., Drew, R.A.I & White, G.G., ed.), p. 21-26. University of Queensland, Brisbane.

Buchan, L.A.J. & Padilla, D.K. 1999. Estimating the probability of long-distance overland dispersal of invading aquatic species. *Ecological Applications* 1: 254-265.

Carlsson, N.O.L., Brönmark, C. & Hansson, L.-A. 2004a. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575-1580.

Carlsson, N.O.L, Kestrup, Å, Mårtensson, M. & Nyström, P. 2004b. Lethal and non-lethal effect of multiple indigenous predators on the invasive golden apple snail (*Pomacea canaliculata*). *Freshwater Biology* 49: 1269-1279.

Carlsson, N.O.L. & Lacoursière, J.O. 2005. Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. *Biological Invasions* 7: 233-241.

Carlsson, N.O.L., Sarnelle, O. & Strayer, D.L. 2009. Native predators and exotic prey – an acquired taste? *Frontiers in Ecology and the Environment* 7: 525-532.

Choowaew, S. 1993. Inventory and management of wetlands in the lower Mekong basin. *Asian Journal of Environmental Management* 1: 1-10.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. & van den Belt, M. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387: 234-242.

Cowie, R.H., Hayes, K.A., Tran, C.T. & Levin, P. 2007. Distribution of the invasive apple snail *Pomacea canaliculata* (Lamarck) in the Hawaiian Islands (Gastropoda: Ampullariidae). *Bishop Museum Occasional Papers* 96: 48-51.

Diehl, S. 1988. Foraging efficiency of three freshwater fish: effects of structural complexity and light. *Oikos* 53: 203-214.

Diehl, S. 1992. Fish predation and benthic community structure: the role of omnivory and habitat complexity. *Ecology* 73: 1646-1661.

Donnay, T.J. & Beissinger, S.R. 1993. Apple snail (*Pomacea doliodes* [*sic*]) and freshwater crab (*Dilocarcinus dentatus*) population fluctuations in the Llanos of Venezuela. *Biotropica* 25: 206-214.

EFSA [European Food Safety Authority] Panel on Plant Health. 2012. Scientific Opinion on the evaluation of the pest risk analysis on *Pomacea insularum*, the island apple snail, prepared by the Spanish Ministry of Environment and Rural and Marine Affairs. EF*SA Journal* 10(1): 2552 [57 p].

EFSA [European Food Safety Authority] Panel on Plant Health. 2013. Assessment of the potential establishment of the apple snail in the EU. *EFSA Journal* 11(12): 3487 [50 p].

Engelhardt, K.A.M., & Ritchie, M.E. 2001. Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature* 411: 687-689.

Ewel, J.J., O'Dowd, D.J., Bergelson, J., Daehler, C.C., D'Antonio, C.M., Gómez, L.D., Gordon, D.R., Hobbs, R.J., Holt, A., Hopper, K.R., Hughes, C.E., LaHart, M., Leakey, R.R.B., Lee, W.G., Loope, L.L., Lorence, D.H., Louda, S.M., Lugo, A.E., McEvoy, P.B., Richardson, D.M. & Vitousek, P.M. 1999. Deliberate introductions of species: research needs. *Bioscience* 49: 619-630.

Fernando, C.H. & Halwart, M. 2000. Possibilities for the integration of fish farming into irrigation systems. *Fisheries Management and Ecology* 7: 45-54.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hansson, L.-A., Johansson, L. & Persson, L. 1987. Effects of fish grazing on nutrient release and succession of primary producers. *Limnology and Oceanography* 32: 723-729.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Horn, K.C., Johnson, S.D., Boles, K.M., Moore, A., Siemann, E. & Gabler, C.A. 2008. Factors affecting hatching success of golden apple snail eggs: effects of water immersion and cannibalism. *Wetlands* 28(2): 544-549.

Hirai, Y. 1988. Apple snail in Japan: the present status and management. *Japan Agricultural Research Quarterly* 22: 161-165.

Horgan, F.G., Stuart, A.M. & Kudavidanage, E.P. 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica* 54: 90-100.

Ichinose, K. 2002. Influence of age and body size on alarm responses in a freshwater snail *Pomacea canaliculata*. *Journal of Chemical Ecology* 28: 2017-2028.

Ichinose, K., Tochihara, M., Wada, T., Suguiura, N. & Yusa, Y. 2002. Influence of common carp on apple snail in a rice field evaluated by a predator-prey logistic model. *International Journal of Pest Management* 48: 133-138.

James, M.R., Hawes, I. & Weatherhead, M. 2000. Removal of settled sediments and periphyton from macrophytes by grazing invertebrates in the littoral zone of a large oligotrophic lake. *Freshwater Biology* 44: 311-326.

Lach, L. & Cowie, R.H. 1999. The spread of the introduced freshwater apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) on O‘ahu, Hawai‘i. *Bishop Museum Occasional Papers* 58: 66-71.

Lima, S.L. & Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68: 619-640.

Lodge, D.M., Cronin, G., Van Donk, E. & Froelich, A.J. 1998. Impact of herbivory on plant standing crop: comparisons among biomes, between vascular and nonvascular plants, and among freshwater herbivore taxa. In: *The Structuring Role of Submerged Macrophytes in Lakes. Ecological Studies 131* (Jeppesen, E., Søndergaard, Ma. Søndergaard, Mo. & Christoffersen, K., ed.), p. 149-171. Springer, New York.

Lv, S., Zhang, Y., Steinmann, P., Yang, G.J., Yang, K., Zhou, X.N. & Utzinger, J. 2011. The emergence of angiostrongyliasis in the People’s Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biology* 56: 717-734.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

McIntosh, A.R. & Townsend, C.R. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? *Oecologia* 108: 174-181.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-62.

Morrison, W.E., & Hay, M. E. 2011. Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS ONE* 6(3), e17227.

Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Persson, L. & Crowder, L.B. 1998. Fish-habitat interactions mediated via ontogenetic niche shifts. In: *The structuring role of submerged macrophytes in lakes. Ecological Studies 131* (Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. ed.), p. 3-23. Springer, New York.

Rahel, F.J. & Stein, R.A. 1988. Complex predator-prey interactions and predator intimidation, review and prospectus. *Canadian Journal of Zoology* 68: 619-640.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology* 7: 97 [14 p.].

Saveanu, L. & Martín, P.R. 2013. Pedal surface collecting as an alternative feeding mechanism of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Journal of Molluscan Studies* 79: 11-18.

Scheffer, M., Hosper, S.H., Meijer, M.-L., Moss, B. & Jeppesen, E. 1993. Alternative equlibria in shallow lakes. *Trends in Ecology and Evolution* 8: 275-279.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. In: *Predation: Direct and Indirect Impacts on Aquatic Communities* (Kerfoot, C.W. & Sih, A., ed.), p. 203-224. University Press of New England, Hanover, New Hampshire, and London.

Silliman, B.R. & Bertness, M.D. 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences of the USA* 99: 10500-10505.

Snyder, N.F.R. & Snyder, H.A. 1971. Defenses of the Florida apple snail *Pomacea paludosa*. *Behaviour* 40: 175-215.

Strayer, D.L. 2001. Endangered freshwater invertebrates. *Encyclopedia of Biodiversity* 2: 425-439.

Tanaka, K., Watanabe, T., Higuchi, H., Miyamoto, K., Yusa, Y., Kiyonaga, T., Kiyota, H., Suzuki, Y. & Wada, T. 1999. Density-dependent growth and reproduction of the apple snail, *Pomacea canaliculata*: a density manipulation experiment in a paddy field. *Researches on Population Ecology* 41: 253-262.

Teo, S.S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* 20: 599-604.

Tripathi, B.D., Srivastava, J. & Misra, K. 1991. Nitrogen and phosphorous removal-capacity of four chosen aquatic macrophytes in tropical freshwater ponds. *Environmental Conservation* 18: 143-147.

Turner, A.M., Bernot, R.J. & Boes, C.M. 2000. Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. *Oikos* 88: 148-158.

Van Donk, E. 1998. Switches between clear and turbid water states in a biomanipulated lake (1986 - 1996): the role of herbivory on macrophytes. In: *The structuring role of submerged macrophytes in lakes. Ecological Studies 131* (Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. eds.), p. 290-297. Springer, New York.

Wood, T.S., Anurakpongsatorn, P., Chaichana, R., Mahujchariyawong, J. & Satapanajaru, T. 2006. Heavy predation on freshwater bryozoans by the golden apple snail, *Pomacea canaliculata* Lamarck, 1822 (Ampullariidae). *Natural History Journal of Chulalongkorn University* 6: 31-36.

Yeo, D.C.J. 2004. A new species of *Esanthelphusa* (Crustacea: Brachyura: Parathelphusidae) from Laos, and a redescription of *Potamon* (*Parathelphusa*) *dugasti* Rathbun, 1902. *Raffles Bulletin of Zoology* 52: 219-226.

Yusa, Y. 2001. Predation on eggs of the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) by the fire ant *Solenopsis geminata*. *Journal of Molluscan Studies* 67: 275-279.

Yusa, Y. & Wada, T. 1999. Impact of the introduction of apple snails and their control in Japan. *Naga, the ICLARM Quarterly* 22: 9-13.

Yusa, Y., Sugiura, N. & Ichinose, K. 2000. Predation on the apple snail, *Pomacea canaliculata* (Ampullariidae), by the Norway rat, *Rattus norvegicus*, in the field. *The Veliger* 4: 349-353.

Predation on freshwater bryozoans by invasive apple snails

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Abstract

Freshwater bryozoans in much of southern Asia are under assault from invasive apple snails (*Pomacea canaliculata* and perhaps *P. maculata*). In lakes and ponds where the snails are present the normal bryozoan populations are sharply reduced or absent. Laboratory feeding trials on inert substrata show that the snails readily consume sessile bryozoans in apparent preference over other organic material. Bryozoans in the family Plumatellidae appear to be most vulnerable to predation; the much smaller *Hislopia* species largely escape damage from snail grazing. In laboratory feeding trials other snail species native to Thailand, similar in size to the apple snails, ignore bryozoans completely. Other invertebrate species, as well as fish, are known to graze occasionally on bryozoans, but not as thoroughly as apple snails. Among the 42 bryozoan species living within the current invaded range of the apple snails, 24 are considered rare and 17 are known only from preserved specimens.

Additional keywords: Ampullariidae, Asia, Bryozoa, Mollusca, Phylactolaemata, *Pomacea*

Introduction

Most studies on the invasive character of introduced apple snails, primarily *Pomacea canaliculata* but also *P. maculata*, have focused on the damage inflicted on economically important food crops (e.g. Cowie, 2002). This is not surprising. The destruction is considerable, immediate and easily quantified. In many areas around the world invasive apple snails affect the lives of farmers, alter the local economy, change local habitats and raise serious issues of pesticide use (e.g. Naylor, 1996; Carlsson *et al*., 2004).

Whether invasive apple snails also suppress local snail populations has long been suspected (Halwart, 1994), but solid evidence remains elusive. Populations of local snails have been reported to decline as the density of apple snails increases, but whether this is due to competition for limited resources or to human or environmental factors is unclear. Nonetheless, *Pomacea canaliculata* is known to prey on other snail species (Cazzaniga, 1990; Kwong *et al*., 2009) and so at least their carnivory is established.

However, there is no question about the devastating effects of apple snails on populations of plumatellid bryozoans (Wood *et al*., 2006a). Since 2001 we have studied freshwater bryozoans in Thailand. From the first week of a nationwide survey it was apparent that where non-native apple snails were abundant we could not expect to find bryozoans. This observation, repeated at dozens of lakes and ponds, led to a suspicion that was confirmed in the laboratory (Wood *et al*., 2006a). Apple snails target bryozoans and graze on them systematically and thoroughly. The effect is to limit the substrata on

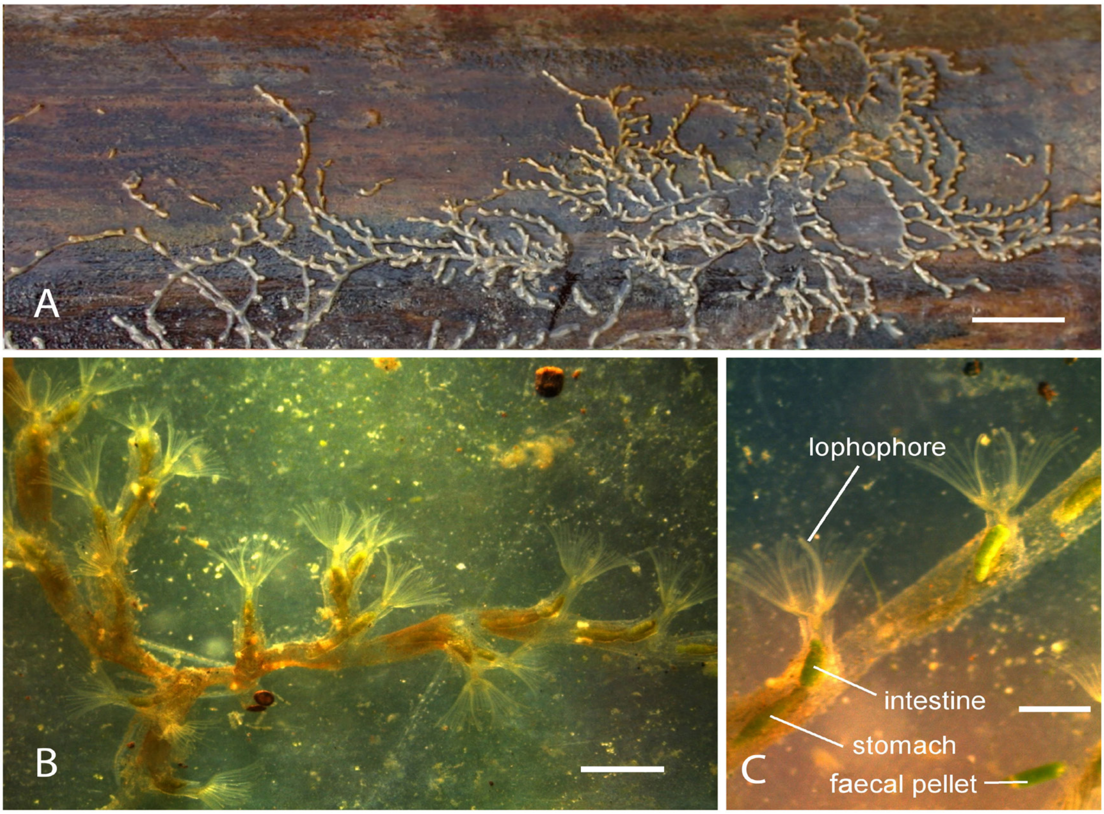

Fig. 1. Morphology of plumatellid bryozoans. A: *Plumatella javanica* on bamboo pulled from the water; scale bar, 1 cm. B: *Plumatella vaihiriae* colony showing zooids with extended lophophores; scale bar, 3 mm. C: closer view of *P. vaihiriae*; scale bar, 1 mm.

which bryozoans can grow, and in many cases to eliminate bryozoan populations from an entire region.

What are bryozoans?

Bryozoans are commonly known as "moss animals" because many species look like moss. Usually brown or green, they are always attached to a submerged object, such as a stick of wood, macrophyte or an article of trash (Fig. 1a). They are abundant, widely distributed and second only to molluscs as the most important filter-feeding invertebrates in fresh waters (Wood & Okamura, 2005).

The bryozoan species most vulnerable to grazing by apple snails are in the family Plumatellidae (Wood *et al*., 2006b). All are composed of branching tubules that spread like thin roots across the substratum (Fig. 1a, b). Sometimes the branches form dense patches with shrubby, upright growths. Apple snails also graze on gelatinous bryozoan species, such as *Asajirella* spp. (Lophopodidae), that grow as globular forms from the size of a pea to structures measuring >10 cm across. The only bryozoans that routinely escape apple snail predation are *Hislopia* spp. (Hislopiidae), which are very small and flat with a tough outer cuticle (Wood *et al*., 2006b).

Bryozoans are modular animals. That is, they grow as colonies composed of multiple, identical units, called zooids. Each zooid is capable of carrying out basic life functions such as food gathering, digestion, respiration and reproduction. In tubular bryozoans the zooids are located at intervals along the tubes. To gather suspended particles of food they use ciliated tentacles organized in an organ called a lophophore (Fig. 1c). The particles are passed down to a large stomach located inside the tube (Fig. 1c). While ingested particles are thoroughly churned about in the stomach most of them appear to remain intact. There is evidence that bryozoan nutrition is derived from bacteria captured along with the particles of detritus (Richelle *et al*., 1994). After variable time in the stomach the particulate material is packed into a so-called intestine, where it is infused with mucus and eventually discharged through the anus as a faecal pellet (Fig. 1c). Faecal pellets accumulating in the sediments are a rich source of food for annelids, insect larvae and other benthic invertebrates.

When threatened or alarmed the bryozoan can withdraw its delicate lophophore into the tube. There are no other behavioural, structural or chemical defence mechanisms that would protect the bryozoan colony from a grazing apple snail. The food value of a colony would include muscle tissue and gut contents. However, there is even more energy to be derived from dormant, lipid-filled capsules called statoblasts that often occur in the tubules. Whether statoblasts can be processed by the snail digestive system is not known.

Statoblasts are a primary means of dispersal. Each is about the size of a full stop (period) on this page. Many hundreds or thousands of them are normally released into the water by a bryozoan colony. With a natural buoyancy they are widely distributed during the flooding season and can also be carried to new sites on the feet and feathers of waterfowl. Statoblasts tolerate desiccation and other unfavourable conditions, and after a period of dormancy they are able to germinate to produce a new colony.

All non-gelatinous bryozoans are attached permanently to the substrate. Although the lophophore can move in and out of the tubes, the colonies as a whole are fixed in place. Fish sometimes nibble at overgrown colonies; various snails, flatworms and immature insects also feed on bryozoans (Wood & Okamura, 2005). However, nothing compares to the wholesale, systematic destruction of entire colonies caused by apple snails.

Further information on these and other aspects of the biology of freshwater bryozoans can be found in the publications of Wood & Okamura (2005), Wood (2009) and Wood *et al*. (2010).

What is the evidence for apple snail grazing on bryozoans?

The indirect evidence is compelling: bryozoans are common residents of lakes and ponds throughout Thailand, except at those sites where apple snails are abundant. In those instances the bryozoans are either missing entirely or else surviving in places inaccessible to snails, such as dangling rope or floating buoys. We have encountered small refugee colonies hidden in deep crevices of floating logs. Sometimes they thrive on the spiny underside of lotus leaves, which apple snails tend to avoid. In general we have learned that whenever we see pink clusters of apple snails eggs we can expect to find very few bryozoans.

More direct evidence of grazing comes from experimental feeding trials in the laboratory. To prepare for these tests we suspended 19 cm x 19 cm glass plates in a pond on the campus of Kasetsart University in Bangkok. After four weeks in the water the plates were covered with a wide variety of organisms, including protozoans, rotifers, flatworms and nematodes. There were also numerous bryozoan colonies, a natural combination of *Plumatella bombayensis*, *P. vorstmani*, *P. vaihiriae* and *Hislopia malayensis*. In the laboratory these plates were cleaned on one side and inverted (clean side up) into a basin of pond water with a single snail. The snails quickly located the

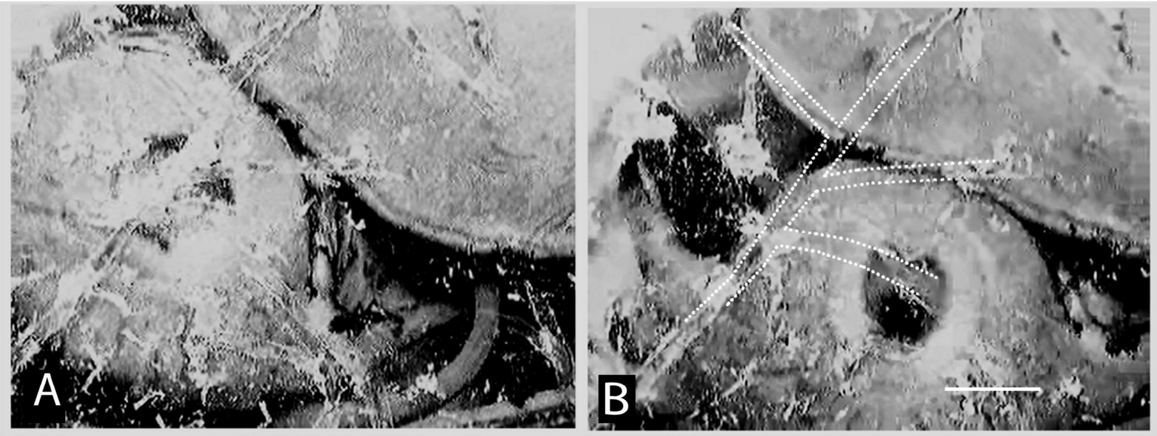

Fig. 2. Photos from a video file showing an apple snail feeding on a plumatellid bryozoan. The animals were on one side of a glass plate and the photos were taken from the opposite side of the plate. A: snail foot is in the upper right, head and mouth are to the left; the bryozoan colony appears as a central hub with 5 branches. B: several seconds later most of the colony is gone, represented here by white dotted lines. Scale bar, 5 mm.

bryozoans and began feeding immediately. If the colony was a single long branch the snail would follow the branch, ignoring the flocculent material on either side and specifically targeting the bryozoans (Fig. 2). The trials were repeated five times, each time with a different snail, and each time giving the same result. All the plumatellid bryozoans were readily consumed; only the very small hislopiid colonies escaped with little apparent damage. In subsequent tests we found apple snails as small as 6 mm shell diameter capable of destroying plumatellid bryozoan colonies.

Are bryozoans affected by native snails?

Our laboratory tests were repeated using native Thai snails: *Pila pesmei*, *Pila ampullacea*, *Pila virescens* (often referred to incorrectly as *Pila polita*; see Cowie, 2015) (Ampullariidae) and *Cipangopaludina chinensis* (Viviparidae). The *C. chinensis* came from the Bangkhen campus of Kasetsart University; the *Pila* spp. were collected in None Thong District in Thailand. We identified the *Pila* spp. using the criteria developed by Keawjam (1986) and matching our specimens with snail shells in the reference collection at Kasetsart University. All our Thai snails were similar in size to the invasive apple snails tested earlier, measuring 3-4 cm at the largest diameter.

Test results were clear and consistent. *Cipangopaludina chinensis* mounted the glass plates and began feeding immediately, but not on bryozoans. With flicking movements of the radula they scooped up all the loose debris on the plate, reaching into tight corners between bryozoan branches but leaving the colonies intact and unharmed. In one trial we allowed two snails to remain on the plate for four hours but noted only slight damage to one of the bryozoan colonies.

The *Pila* species, on the other hand, attempted to avoid the test plate altogether, preferring instead to roam around at the bottom of the basin. When placed directly onto the plate they would quickly escape, passing over the bryozoan colonies and returning to the bottom sediments. Local people tell us that our *Pila* are normally found half-buried in muddy sediments, which could explain the behaviour we observed.

Why does it matter?

Invasive apple snail predation on bryozoans in Asia is so widespread and pernicious that it threatens to eliminate entire species. In view of the serious damage inflicted by the snails on agriculture, the loss of bryozoans may appear relatively unimportant. If there were any group of animals the disappearance of which might seem inconsequential, bryozoans should be the perfect example. Few people have ever heard of bryozoans; fewer still would recognize a bryozoan colony if they saw one. Moreover, bryozoans can create a biofouling nuisance in wastewater treatment and irrigation systems (Wood, 2005). At least one species harbours a fish parasite (Okamura & Wood, 2002). Why should anyone be concerned about the survival of a bryozoan species?

It is difficult to answer this question when so little is known. Consider the following facts. Within the current range of *P. canaliculata* and *P. maculata* in southern Asia there are 42 species of bryozoans (Table 1). Seventeen of these are known from only a single site; seven more are known from fewer than five localities. Our knowledge of these 24 rare species is limited to what can be discerned from imperfectly preserved specimens. No living examples have ever been studied in detail. In fact, 17 of these species have never been seen alive by any living zoologist. Their ecology, physiology, life cycles, etc. are unknown. Table 1 indicates the level of concern for each species based on their abundance, range, our current knowledge and whether they are consumed by invasive apple snails.

A major factor in our ignorance of Asian bryozoans is the absence of any recent studies. Among the many countries where invasive apple snails have been reported, only three (Japan, Thailand, United States) have received significant attention with regard to their bryozoans (e.g. Hirose, 2012; Wood *et al.*, 2006b). Minimal work has been done in

Indonesia (Vorstman, 1928a, b), India (e.g. Annandale, 1911; Rao, 1973; Rao *et al*., 1985) and Cambodia (Hirose & Mawatari, 2007). However, at this time the number of active scientists worldwide studying freshwater bryozoans can be counted on the fingers of one hand.

Freshwater habitats are hotspots of biodiversity. While they occupy less than 1% of the earth's surface they are estimated to support about 10% of all known species (Strayer & Dudgeon, 2010). For several decades these species have been seriously threatened by habitat destruction, pollution of many kinds, the introduction of alien species and other changes. The widespread, destructive grazing of apple snails on freshwater bryozoans is just one more threat to species already under stress. The most common bryozoans appear capable of survival over the long term. We may never know what happens to the rare species.

Table 1. Freshwater bryozoan species in southern Asia. Abundance: 1 – species known from > 20 sites, 2 – known from 5-20 sites, 3 – known from 2-5 sites, 4 – known from a single site. Range: 1-2 – known range extends beyond southern Asia, 3-4 – known range restricted to southern Asia (range of numbers accommodates uncertainty). Knowledge: 1– three or more published studies other than original description, 2 – fewer than three such studies, 3 – nothing known other than original description. Eaten?: 1-2 – probably consumed by invasive apple snails, 3-4 – probably not consumed (range indicates likelihood of consumption based on laboratory studies). The level of concern is the sum of the indices such that 15 represents the level of greatest concern. Undescribed species are distinguished based on material at the Zoological Survey of India (ZSI), Kolkata, with ZSI catalogue numbers provided.

Species	Abundance	Range	Knowledge	Eaten?	Concern
Afrindella philippinensis	4	4	3	4	15
Afrindella testitudinicola	4	4	3	4	15
Asajirella gelatinosa	1	2	1	4	8
Australella indica	4	2	3	3	12
Fredericella indica	1	1	1	4	7
Fredericella sultana	1	1	1	4	7
Gelatinella toanensis	2	2	3	3	10
Hislopia malayensis	1	3	2	2	8
Hislopia natans	3	4	2	3	12
Hyalinella diwaniensis	4	4	3	3	14
Hyalinella lendenfeldi	1	2	3	3	9
Internectella bulgarica	2	2	2	3	9
Lophopodella carteri	1	1	2	4	8
Lophopodella stuhlmanni	3	2	3	3	11

Plumatella agilis	2	1	3	2	8
Plumatella bigemmis	3	4	3	4	14
Plumatella bombayensis	1	4	3	4	12
Plumatella casmiana	1	1	2	4	8
Plumatella chulabhornae	3	4	3	4	14
Plumatella emarginata	1	1	3	4	9
Plumatella ganapati	4	4	3	4	15
Plumatella javanica	1	4	3	4	12
Plumatella longigemmis	3	4	3	4	14
Plumatella minuta	2	2	2	4	10
Plumatella mukaii	2	2	2	4	10
Plumatella siamensis	3	4	3	4	14
Plumatella suwanna	4	4	3	4	15
Plumatella vaihiriae	1	1	2	4	8
Plumatella vorstmani	1	1	1	4	7
Stolella himalayana	4	4	3	4	15
Swarupella andamanensis	3	4	3	4	14
Swarupella divina	4	4	3	4	15
Swarupella kasetsartensis	4	4	3	4	15
Varunella coronifera	4	4	3	4	15
Varunella indorana	4	4	3	4	15
Victorella pavida	2	2	1	4	9
Undescribed, ZSI P1198	4	4	3	4	15
Undescribed, ZSI P1387	4	4	3	4	15
Undescribed, ZSI P161	4	4	3	4	15
Undescribed, ZSI P1787	4	4	3	4	15
Undescribed, ZSI ZEV 2627	4	4	3	4	15
Undescribed, ZSI ZEV 4799	4	4	3	4	15

References

Annandale, N. 1911. *The Fauna of British India, including Ceylon and Burma. Freshwater Sponges, Hydroids & Polyzoa*. Taylor and Francis, London.

Carlsson, N.O.L., Brönmark, C. & Hansson, L.-A. 2004. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575-1580.

Cazzaniga, N.J. 1990. Predation of *Pomacea canaliculata* (Ampullariidae) on adult *Biomphalaria peregrina* (Planorbidae). *Annals of Tropical Medicine and Parasitology* 84: 97-100.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CABI Publishing, Wallingford.

Cowie, R.H. 2015. The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa* 3940(1): 1-92.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hirose, M. & Mawatari, S.F. 2007. Freshwater bryozoans of Tonlé Sap, Cambodia. *Zoological Sciences* 24: 630-641. [in Japanese]

Hirose, M. 2012. Taxonomy and identification of freshwater bryozoans from Japan. *Proceedings of the Japanese Society of Systematic Zoology* 33: 17-32.

Keawjam, R.S. 1986. The apple snails of Thailand: distribution, habitat and shell morphology. *Malacological Review* 19: 61-81.

Kwong, K.-L., Chan, R.K.Y. & Qiu, J.-W. 2009. The potential of the invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia* 51: 343-356.

Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Okamura, B. & Wood, T.S. 2002. Bryozoans as hosts for *Tetracapsula bryosalmonae*, the PKX organism. *Journal of Fish Disease* 25: 1-7.

Rao, K.S. 1973. Studies on freshwater Bryozoa. III. The Bryozoa of the Narmada River system. In: *Living and fossil Bryozoa – recent advances in research* (Larwood, G.P., ed.), p. 529-537. Academic Press, London.

Rao, K.S., Agrawal, V., Diwan, A.P. & Shrivastava, P. 1985. Studies on freshwater Bryozoa V. Observations on central Indian materials. In: *Bryozoa: Ordovician to Recent* (Nielsen, C. & Larwood, G.P. ed.), p. 257-264. Olsen & Olsen, Fredensborg, Denmark.

Richelle, E., Moureau, Z. & Van de Vyver, G. 1994. Bacterial feeding by the freshwater bryozoan *Plumatella fungosa* (Pallas, 1768). *Hydrobiologia* 291: 193-199.

Strayer, D.L. & Dudgeon, D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* 29: 344-358.

Vorstman, A. 1928a. Some freshwater Bryozoa from West Java. *Treubia* 10: 1-14.

Vorstman, A. 1928b. Freshwater Bryozoa from E. Java. *Treubia* 10: 163-165.

Wood, T.S. 2005. The pipeline menace of freshwater bryozoans. *Denisia* 16: 203-208.

Wood, T.S. & Okamura, B. 2005. *A New Key to the Freshwater Bryozoans of Britain, Ireland, and Continental Europe, with Notes on Their Ecology. Scientific Publication No. 63*. Freshwater Biological Association, Ambleside, UK.

Wood, T.S., Anurakpongsatorn, P., Chaichana, R., Mahujchariyawong, J. & Satapanajaru, T. 2006a. Heavy predation on freshwater bryozoans by the golden apple snail, *Pomacea canaliculata* Lamarck, 1822 (Ampullariidae). *The Natural History Journal of Chulalongkorn University* 6: 31-36.

Wood, T.S., Anurakpongsatorn, P. & Mahujchariyawong, J. 2006b. Freshwater bryozoans of Thailand (Ectoprocta and Entoprocta). *The Natural History Journal of Chulalongkorn University* 6: 83-119.

Wood, T.S. 2009. Bryozoans. In: *Ecology and Classification of North American Freshwater Invertebrates* (Thorp, J.H. & Covich, A.P., ed.), p. 437-454. Academic Press, London.

Wood, T.S., Anurakpongsatorn, P. & Mahujchariyawong, J. 2010. *An Introduction to the Freshwater Bryozoans of Thailand*. Kasetsart University Press, Bangkok.

Symbionts and diseases associated with invasive apple snails

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Abstract

This contribution summarizes knowledge of organisms associated with apple snails, mainly *Pomacea* spp., either in a facultative or obligate manner, paying special attention to diseases transmitted via these snails to humans. A wide spectrum of epibionts on the shell and operculum of snails are discussed. Among them algae, ciliates, rotifers, nematodes, flatworms, oligochaetes, dipterans, bryozoans and leeches are facultative, benefitting from the provision of substrate, transport, access to food and protection. Among obligate symbionts, five turbellarian species of the genus *Temnocephala* are known from the branchial cavity, with *T. iheringi* the most common and abundant. The leech *Helobdella ampullariae* also spends its entire life cycle inside the branchial cavity; two copepod species and one mite are found in different sites inside the snails. Details of the nature of the relationships of these specific obligate symbionts are poorly known. Also, extensive studies of an intracellular endosymbiosis are summarized. Apple snails are the first or second hosts of several digenean species, including some bird parasites. A number of human diseases are transmitted by apple snails, angiostrongyliasis being the most important because of the potential seriousness of the disease.

Additional keywords: Ampullariidae, *Angiostrongylus*, commensals, diseases, epibionts, parasites, *Pomacea*, symbiosis

Introduction

The term “apple snail” refers to a number of species of freshwater snails belonging to the family Ampullariidae (Caenogastropoda) inhabiting tropical and subtropical regions (Hayes *et al*., 2015). Some species have invaded parts of the world to which they are not native, altering ecosystems and becoming problematic for a range of human activities.

Because of their morphology (e.g. shape and size of the shell, possession of a mantle cavity), behaviour and peculiar amphibious life habit, apple snails are a suitable habitat for species that associate and interact among themselves and with the snails, whether negatively or positively and including facultative or obligate associations (Fig. 1). Several studies have been carried out in their native ranges on the interactions of these snails with associated species. However, studies dealing with the detailed nature and significance of these relationships are few.

This contribution summarizes current knowledge of organisms associated with apple snails, mainly *Pomacea* spp., either in a facultative or obligate manner. Special attention is paid to diseases transmitted via these snails to humans.

The word symbiosis is used here as a comprehensive concept, setting aside the role played by the two species and the benefits and/or costs derived from their relationships. Symbionts can be specific, associated with one or a few host species, or generalist, associated with a wide range of species (Buckland-Nicks *et al*., 2013). Facultative symbionts (epibionts) temporarily or opportunistically associated with the host species can be easily distinguished from obligate symbionts (parasites, commensals, mutualists) that depend on their host species to live. One instance involves endocytobiosis, i.e. intracellular endosymbiosis (Vega *et al*., 2006).

Facultative symbionts (epibionts)

Epibionts are organisms associated facultatively with the surface of living substrates, in this case the shell and operculum of the snails (Fig. 1). Epibionts such as algae, ciliates and other sessile organisms settle on apple snail shells. This epibiont community could have various effects on the host, including camouflage, increased resistance to water and modification of predator attraction. Epibiosis may be advantageous for epibionts because of host behaviour (e.g. transport of epibionts to more favourable areas, facilitation of access to food, provision of permanent substrate). Other aspects of behaviour can be

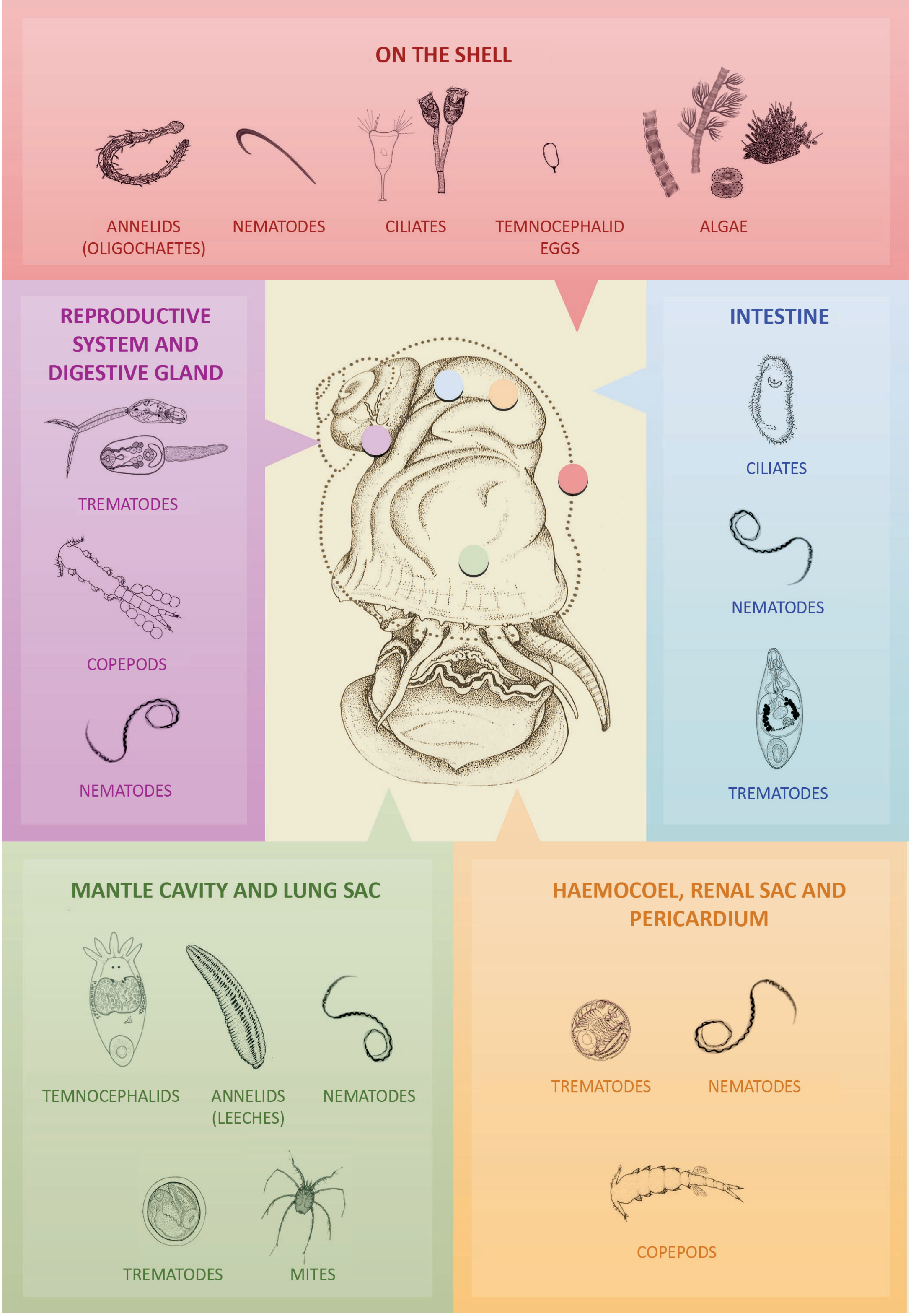

Fig. 1. The main taxonomic groups associated with various parts of the body and shell of apple snails.

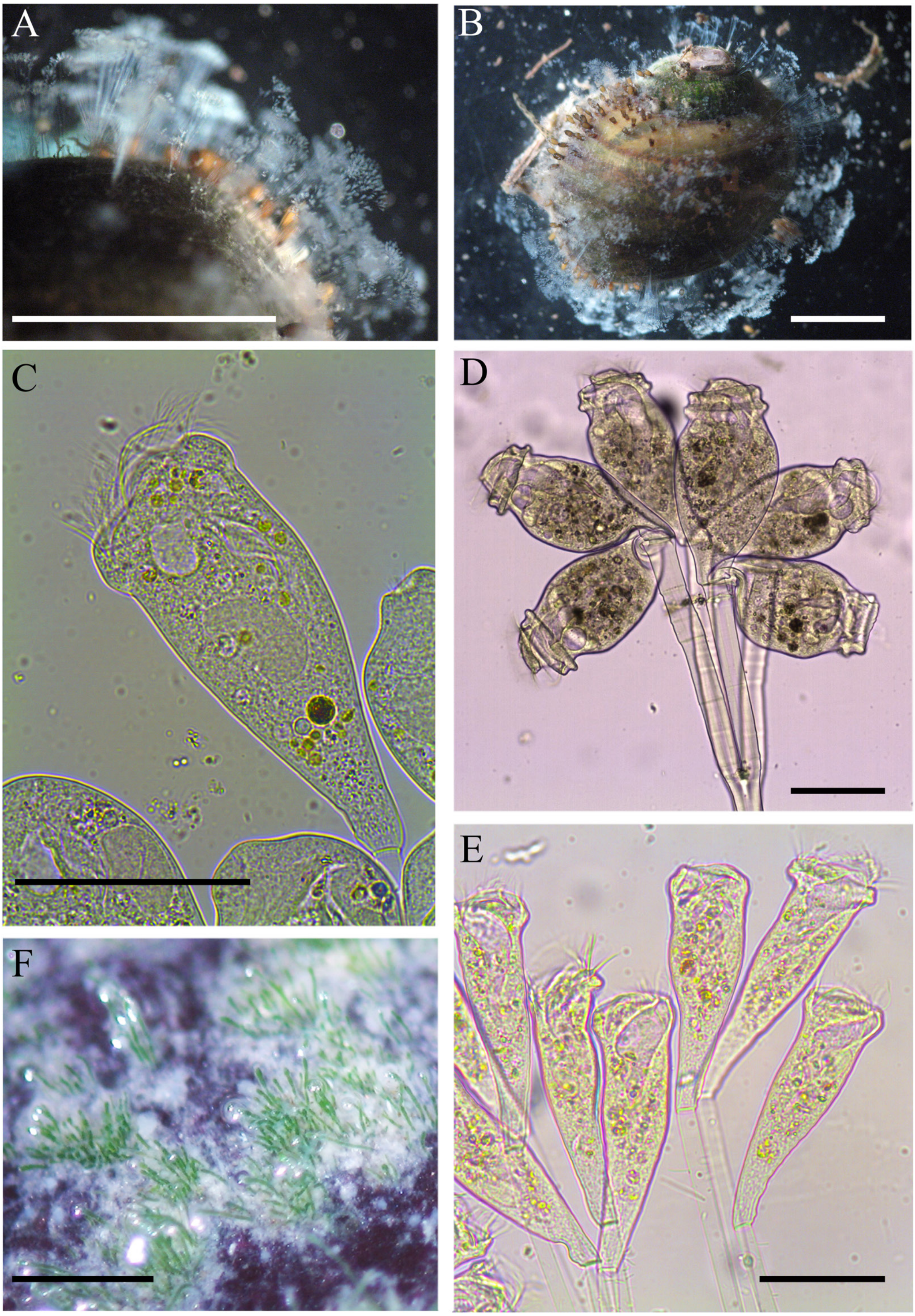

Fig. 2. Epibionts of *Pomacea canaliculata.* A, B: general view of the shell of P. *canaliculata* with ciliates and eggs of *Temnocephala iheringi*. C-E: ciliates, Peritrichida (*Epistylis* sp.). F: Chlorophyceae, Chaetophorales. Scale bars: A, B: 1 cm; C-F: 100 µm.

disadvantageous for epibionts (e.g. burial during unfavourable periods or air exposure due to amphibious habits) (Dias *et al*., 2008).

Observations in different environments in the Río de la Plata River Basin (South America) revealed that in some *Pomacea canaliculata* populations, epibionts cover the shell surface thickly and uniformly while in others the epibiont community is sparse (Fig. 2). When the population density of *P. canaliculata* is high and/or the environment is poor in food, the snails are frequently found grazing on each other's shells.

One of the earliest contributions to knowledge of the diversity of epibionts living on three native species of *Pomacea* commonly inhabiting the Río de la Plata River Basin was by Di Persia & Radici de Cura (1973), who mentioned the presence of a large suite of taxa (Table 1) including many different groups (algae, ciliates, rotifers, nematodes, flatworms, oligochaetes, dipterans, bryozoans, etc.), some of them very common and abundant, others sporadic. In Brasil, Dias *et al*. (2006, 2008, 2010) studied the ciliates adhering to the shell surface of two species of *Pomacea* and Gorni & Alves (2006) studied the oligochaetes inhabiting the umbilicus of *Pomacea bridgesii* (probably correctly identified as *P. diffusa*; R.H. Cowie, pers. comm.). Four species of leeches in the family Glossiphoniidae have been recorded in the umbilicus of *P. canaliculata* and occasionally in the mantle cavity (Damborenea & Gullo, 1996), and in the mantle cavity, foot and on the shell of *P. diffusa* (De-Carli *et al*., 2014). These species benefit from shelter and food provided by the snail during their reproductive period, but they ordinarily live free in the environment and thus form a facultative association with the apple snail.

Table 1 summarizes the main epibiont taxa mentioned as being associated with various species of *Pomacea*.

Commensal symbionts

Temnocephalids (Platyhelminthes: Temnocephalidae) are commensal symbionts frequently associated with apple snails, with which they form an obligate association. Five species of temnocephalids have been described in association with different species of ampullariids, i.e. *Temnocephala colombiensis*, *T. iheringi*, *T. haswelli*, *T. rochensis* and *T. lamothei* (Damborenea & Cannon, 2001; Damborenea & Brusa, 2008; Garcés *et al*., 2013). Among these, *Temnocephala iheringi* has the widest distribution (Table 2). These temnocephalid species have a close and permanent relation with their ampullariid hosts, but no information is available regarding its functional significance. They are found in the mantle cavity and lung sac (Fig. 3), near the gill and by the opening of the lung sac, all year round. They do not live on the shell, but deposit their egg capsules on it, especially in the umbilicus and where the channelled suture meets the aperture. Temnocephalids feed on algae or small animals entering the mantle cavity in the breathing currents generated by the host snail.

There are a number of studies on populations of *Temnocephala iheringi*, especially in the Río de la Plata River Basin and southern Buenos Aires Province (Damborenea, 1996, 1998; Martín *et al*., 2005). During a study of the symbiotic species assemblages associated with *Pomacea canaliculata* in streams connected with the Río de la Plata, Damborenea *et al*. (2006) found a high prevalence of temnocephalids (80-100 % of the snails) in most sampling stations, with average abundance varying among localities, and with the maximum number of temnocephalids within a snail being 167. The distribution

Table 1. Epibionts found on *Pomacea* species.

Epibiont taxon	Host	Country	References	Location
Chlorophyta				
Stigeoclonium sp. *Oedogonium* sp.	*P. canaliculata* *P. maculata* *P. scalaris*	Argentina	Di Persia & Radici de Cura, 1973	shell
Chrysophyta				
Gonphonema sp.	*P. canaliculata* *P. maculata* *P. scalaris*	Argentina	Di Persia & Radici de Cura, 1973	shell
Cyanophyta				
Oscilatoriales	*P. canaliculata* *P. maculata* *P. scalaris*	Argentina	Di Persia & Radici de Cura, 1973	shell
Ciliophora - Peritrichia				
Carchesium polypinum	*P. figulina*	Brasil	Dias *et al*., 2008, 2010	shell
Carchesium sp.	*P. lineata*	Brasil	Dias *et al*., 2006	
Epistylis plicatilis	*P. figulina*	Brasil	Dias *et al*., 2008	
	P. canaliculata	Brasil	Utz, 2007	
Epistylis sp.	*P. canaliculata*	Argentina	this work	
	P. lineata	Brasil	Dias *et al*., 2006	
	P. figulina	Brasil	Dias *et al*., 2008	
Opercularia sp.	*P. figulina*	Brasil	Dias *et al*., 2008	
	P. lineata	Brasil	Dias *et al*., 2006	
Vaginicola sp.	*P. lineata*	Brasil	Dias *et al*., 2006	
Vorticella microstoma	*P. figulina*	Brasil	Dias *et al*., 2008	
Vorticella campanula	*P. figulina*	Brasil	Dias *et al*., 2008	
Vorticella sp.	*P. lineata*	Brasil	Dias *et al*., 2006	

Ciliophora - Suctoria				
Acineta sp.	*P. lineata*	Brasil	Dias *et al*., 2006	shell
Tokophrya fasciculata	*P. figulina*	Brasil	Dias *et al*., 2008	
Tokophrya sp.	*P. lineata*	Brasil	Dias *et al*., 2006	
Annelida - Oligochaeta				
Aelosoma sp.	*P. canaliculata*		Di Persia & Radici de Cura, 1973	shell
Chaetogaster sp.	*P. maculata* *P. scalaris*	Argentina		
Naididae (several species)	*P. bridgesii* (probably *P. diffusa*)	Brasil	Gorni & Alves, 2006	umbilicus of the shell
Annelida - Hirudinea - Glossiphoniidae				
Gloiobdella michaelseni	*P. canaliculata*	Argentina	Damborenea & Gullo, 1996	shell, especially in the umbilicus; sometimes inside the mantle cavity
Helobdella adiastola				
H. simplex				
H. triserialis lineata				
H. triserialis nigricans				
H. triserialis	*P. canaliculata* *P. diffusa*	Brasil	De Carli *et al*., 2014	shell, mantle cavity, foot
Bryozoa				
Hyalinella vahiriae	*P. canaliculata*	Argentina	Cazzaniga, 1988	shell
Mollusca - Bivalvia				
Limnoperna fortunei	*P. canaliculata*	Brasil	Darrigran & Damborenea, 2005	shell (Fig. 5)
Nematoda				
Actinolaimus sp.	*P. canaliculata* *P. maculata* *P. scalaris*	Argentina	Di Persia & Radici de Cura, 1973	among algae growing on the shell

Fig. 5. *Pomacea canaliculata* with *Limnoperna fortunei* attached to the umbilicus. Scale bar: 2 cm. (Photos: Gustavo Darrigran)

Table 2. Symbiont species (commensals and parasites) associated with *Pomacea canaliculata* and other ampullariid species.

Symbiont taxon	Host	Country	References	Location	Type of relationship
Ciliophora - Heterotrichea					
Plagiotoma kempi	*Pila globosa*	India	Bathia, 1936	rectum	parasites or commensals?
Parasicuophora ampullarium	*Pomacea canaliculata*	Uruguay	Gascón, 1975	digestive tract	
Parasicuophora corderoi	*Pomacea canaliculata*	Uruguay	Gascón, 1975	digestive tract	
Platyhelminthes – "Turbelaria" -Temnocephalidae					
Temnocephala colombiensis	*Pomacea* sp.	Colombia	Garcés *et al.*, 2013		
Temnocephala haswelli	*P. canaliculata*	Uruguay	Ponce de León, 1989	youngs and adults in the mantle cavity; egg capsules deposited on the shell	commensals
		Brasil	Seixas *et al*., 2010a		
Temnocephala iheringi	*P. canaliculata*	Argentina	Di Persia & Radici de Cura, 1973; Damborenea, 1992, 1996; Martin *et al*., 2005		
		Brasil	Seixas *et al*., 2010b		
		Uruguay	Dioni, 1967; Ponce de León, 1979		
	Pomacea lineata	Brasil	Pereira & Cocuolo, 1941		
	Asolene platae	Argentina	Hyman, 1955		
	Pomella megastoma	Argentina	Damborenea *et al*., 1997		
Temnocephala lamothei	*Pomella megastoma*	Argentina	Damborenea & Brusa, 2008		
Temnocephala rochensis	*Pomacea canaliculata*	Uruguay	Ponce de León, 1979		
		Brasil	Seixas *et al*., 2010c		
Platyhelminthes - Digenea - cercariae					
Xiphidiocercariae					
Cercaria unidentified (1 species)	*Pomacea canaliculata*	Argentina	Ostrowski de Nuñez, 1979		parasites
Xiphidiocercariae unidentified	*Pomacea canaliculata*	Argentina	Damborenea *et al*., 2006	digestive gland	
Cercariae unidentified (2 species)	*Pomacea crassa*	Venezuela	Uribe, 1925	liver	
Cercariae unidentified (5 species)	*Pomacea glauca*	Venezuela	Nasir, 1965; Nasir & Díaz, 1968a, 1973; Nasir *et al*., 1969		
Cercaria unidentified (1 species)	*Pomacea urceus*	Venezuela	Nasir, 1971		
Cercariae unidentified (5 species)	*Marisa cornuarietis*	Venezuela	Nasir, 1965, 1971; Nasir & Díaz, 1968a		
Stomylotrema gratiosus	*Pomacea maculata*	Brasil	Pinto *et al*., 2015		
Metacercariae					
Echinostoma parcespinosum	*Pomacea canaliculata*	Argentina	Martoreli, 1987; Damborenea *et al*., 2006	mantle cavity	parasites
	Pomacea lineata	Brasil	Machado & Sampaio, 1980		
Dietziella egregia	*Pomacea canaliculata*	Argentina	Digiani & Ostrowski de Nuñez, unpublished; Damborenea *et al*., 2006	renal cavity	
Edietziana malacophilum	*Pomacea paludosa*	Cuba	Pérez Vigueras, 1944		
Echinochasmus zubedakhaname	*Pomacea glauca*	Venezuela	Nasir & Díaz, 1968b		
Stephanoprora heteroglandula	*Pomacea glauca*	Venezuela	Nasir *et al*., 1969		
Echinostoma ilocanum	*Pila luzonica*	Philippines, Cambodia	Sohn *et al*., 2011		
Echinostomatidae unidentified	*Pomacea canaliculata*	Thailand	Keawjam *et al*., 1993	foot	
Cercaria unidentified (1 species)	*Pomacea glauca*	Venezuela	Nasir *et al*., 1969		

Furcocercariae					
Cercaria unidentified (1 species)	*Pomacea canaliculata*	Argentina	Ostrowski de Nuñez, 1978		parasites
Furcocercariae unidentified	*Pomacea canaliculata*	Argentina	Damborenea *et al*., 2006	digestive gland	
Cercaria unidentified (1 species)	*Pomacea glauca*	Venezuela	Nasir *et al*., 1969		
Gymnocephala					
Guaicaipuria pseudoconcilia	*Pomacea glauca*	Venezuela	Nasir *et al*., 1969		parasites
Guaicaipuria parapseudoconcilia	*Pomacea glauca*	Venezuela	Nasir & Silva, 1972		
Cercariae unidentified (5 species)	*Pomacea glauca*	Venezuela	Nasir, 1971; Nasir & Acuña, unpublished; Nasir & Díaz, 1968a, 1973		
Amphistoma					
Amphistoma unidentified	*Pomacea canaliculata*	Thailand	Keawjam *et al*., 1993	foot	parasites
Distomes					
Distomes unidentified	*Pomacea canaliculata*	Thailand	Keawjam *et al*., 1993	heart, kidney and foot muscle	parasites
Macrocerca					
Cercariae unidentified (2 species)	*Pomacea glauca*	Venezuela	Nasir *et al*., 1969	intestine	parasites
Platyhelminthes - Digenea - Paramphistomatidae - adults					
Catadiscus pomaceae	*Pomacea canaliculata*	Argentina	Hamann, 1992	adults in intestine	parasites
Annelida - Hirudinea - Glossiphoniidae					
Helobdella ampullariae	*Pomacea canaliculata*	Argentina	Di Persia & Radici de Cura, 1973; Ringuelet, 1985; Damborenea & Gullo, 1996	young and adults in mantle cavity	commensals or parasites?
	Pomacea maculata	Argentina	Ringuelet, 1949, 1985		
	Pomella megastoma	Argentina	Ringuelet, 1945, 1985		
Maxillipeda - Cyclopoida - Ozmanidae					
Ozmana huarpium	*Pomacea canaliculata*	Argentina	Gammarra *et al*., 2004	in penis sheath, ctenidium and mantle cavity	commensals
Ozmana haemophila	*Pomacea maculata*	Brasil	Ho & Thatcher, 1989	haemocel	
Acari - Hydrachnidia - Unionicolidae					
Unionicola (*Ampullariatax*) *ampullariae*	*Pomacea canaliculata*	Argentina	Di Persia & Radici de Cura, 1973	larvae, nymphs and adults in mantle cavity	commensals
	Pomacea maculata	Argentina	Di Persia & Radici de Cura, 1973		
		Brasil	Rosso de Ferradás & Fernández, 2005		
Nematoda - Secernentea - Metastrongylidae					
Angiostrongylus cantonensis	*Pomacea canaliculata*	China	Lv *et al*., 2009	youngs in "lung"	parasites
	Pomacea paludosa	Cuba	Aguiar *et al*., 1981		
		Hawaii	Briceño Lobo, 1986		
			Wallace & Rosen, 1969a	experimental infestation	
	Pila ampulacea	Thailand	Panyagupta, 1965; Woodruff & Upatham, 1993		
	Pila gracilis	Thailand	Harinasuta *et al*., 1965; Woodruff & Upatham, 1993		
	Pila scutata	Malaysia	Lim *et al*., 1965		
	Pila virescens	Thailand	Harinasuta *et al*., 1965		
Angiostrongylus costaricensis	*Pomacea flagellata*	Costa Rica	Briceño-Lobo, 1986	young in viscerae, foot and mantle; experimental infestation	parasites

Nematoda - Secernentea - Gnathostomatidae					
Gnathostoma spinigerum	*Pomacea canaliculata*	Thailand	Komalamisra, 2009	young (L3) in viscerae and mantle; experimental infestation	parasites
	Pila ampullacea	Thailand	Komalamisra, 2009	foot and viscerae	
Nematoda					
Unidentified	*Pomacea canaliculata*	Argentina	Damborenea *et al*., 2006	larvae in mantle cavity	parasites?

of temnocephalids in the host snail did not differ between host sexes. There is an increase in abundance whenever temperature rises and the snails become more active. The life cycle of the host and the commensal temnocephalids are synchronized in order to enhance reproduction and colonization of new hosts.

Martín *et al*. (2005) studied the factors affecting the distribution and abundance of *T. iheringi* among populations of *P. canaliculata* in southern Buenos Aires Province (Argentina), which is the southern boundary of the snail's native range. They found that only 23 % of the apple snail populations inhabiting streams harboured temnocephalids, but higher frequencies (71 %) were observed in lentic localities. The commensals were found in localities with bicarbonate concentrations below 6.6 meq l^{-1} and could tolerate low water temperatures (4-5 ºC in winter).

In addition to the aforementioned epibiont hirudinean species, there is an additional species, *Helobdella ampullariae* (Fig. 3), that inhabits the mantle cavity of ampullariid snails, establishing an obligatory association (Ringuelet, 1945, 1949; Di Persia & Radici de Cura, 1973). A seventeen-month study of a population of apple snails from Bagliardi Beach, Buenos Aires, Argentina (Damborenea & Gullo, 1996) showed that *H. ampullariae* is associated with the host all year round and completes its life-cycle entirely in this association. Juveniles, adults, adults with cocoons and brooding adults are found inside the mantle cavity of the snails. The reproductive period of the leech is long (December to June), beyond the season in which their hosts are buried. Ringuelet (1945) considered this species as a parasite feeding on the snail, but in fact there is no knowledge of the feeding habits of the leeches.

Two species of copepods are known as symbionts of ampullariids, i.e. *Ozmana haemophila* in the haemocoel of *Pomacea maculata* in the Amazon basin, Brasil (Ho & Thatcher, 1989), and *Ozmana huarpium* in the haemocoel, mantle cavity, ctenidium and penis-sheath groove of *P. canaliculata* in Palermo Park, Buenos Aires city, Argentina, where prevalence of the symbiont is 100 % in both sexes (Gamarra-Luques *et al*., 2004).

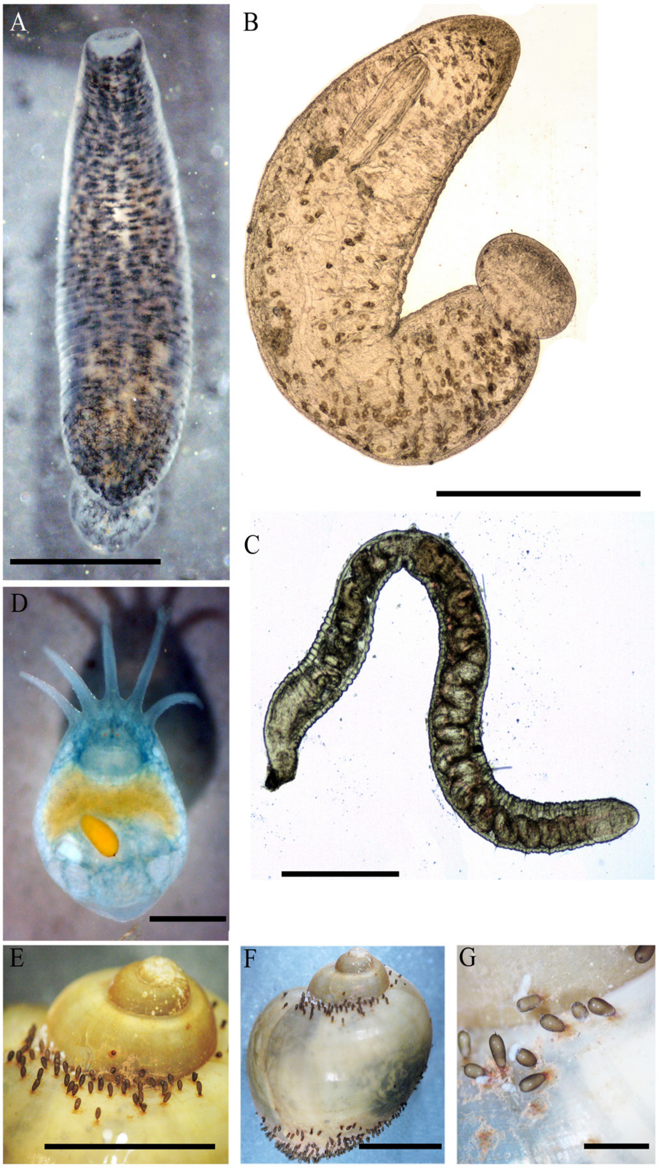

Fig. 3. Commensals of the mantle cavity of *Pomacea canaliculata*. A: general view of *Helobdella ampullariae*. B: detail of *H. ampullariae* under optical microscope. C: Oligochaeta. D: *Temnocephala iheringi*. E-G: eggs of *T. iheringi*. Scale bars, A, B: 2 mm; C, G: 1 mm; D: 500 µm; E: 6 mm, F: 10 mm.

Gamarra-Luques *et al*. (2004) made numerous biological observations on *O. huarpium*, including showing in experiments that it is transmitted to parasite-free hosts during copulation.

A species of unionicolid mite, *Unionicola* (*Ampullariatax*) *ampullariae*, also lives in the mantle cavity of *Pomacea canaliculata* and *P. maculata* (Di Persia & Radici de Cura, 1973; Rosso de Ferradás & Fernández, 2005), *P. maculata* now being the senior synonym of *P. insularum*, which was the name used by these authors (see Hayes *et al*., 2012). Di Persia & Radici de Cura (1973) recorded larvae, nymphs and adults in the mantle cavity and under the mantle of the snails.

Epithelium cells of the midgut gland of some species of ampullariids carry two kinds of pigmented endosymbiont prokaryote corpuscles belonging to the same organism (Castro-Vazquez *et al*., 2002; Koch *et al*., 2006). Corpuscles are also found in faeces and sediment, indicating a life-cycle alternating endosymbiotic and free phases. These endosymbionts have been extensively studied in *P. canaliculata,* although they have also been found in other species, including *P. maculata*, *P. scalaris*, *Asolene pulchella* and *Marisa cornuarientis* (no faeces were found in the latter) (Vega *et al*., 2006). Bacterial

DNA attributed to 16S rRNA demonstrated a close relationship of the corpuscles with representatives of the orders Chroococcales or Pleurocapsales (Cyanobacteria) (Vega *et al*., 2005, 2006). Koch *et al*. (2003) demonstrated that transmission was vertical from mother to offspring.

Godoy *et al*. (2013) identified proteases in the digestive system of *P. canaliculata*. Protease 30kDa was found in the mid-gut and in the endosymbiotic corpuscles, where activity was detected. Endosymbionts are liberated into the stomach vestibule via the ducts of the mid-gut gland. This suggests protease secretion is a possible function, in addition to detoxification, as suggested by the accumulation of various metals in the corpuscles, which are later liberated in the faeces (Vega *et al*., 2012).

Parasitic symbionts

Three species of heterotrichid ciliates have been found in the digestive system of apple snails, i.e. *Parasicuophora ampullariarum* and *P. corderoi* both inhabiting the gut of *Pomacea canaliculata* from Uruguay (Gascón, 1975), and *Plagiotoma kempi* in the rectum of *Pila globosa* from India (Zeliff, 1933). These observations did not establish the nature of the relationship.

Several trematode larvae have been noted associated with apple snails (Fig. 4), but adults of only one species were found in them, i.e. *Catadiscus pomaceae* (Paramphistomidae) in the intestine of *Pomacea canaliculata* (Hamann, 1992). The life cycle of this species remains unknown. Probably, *P. canaliculata* may become

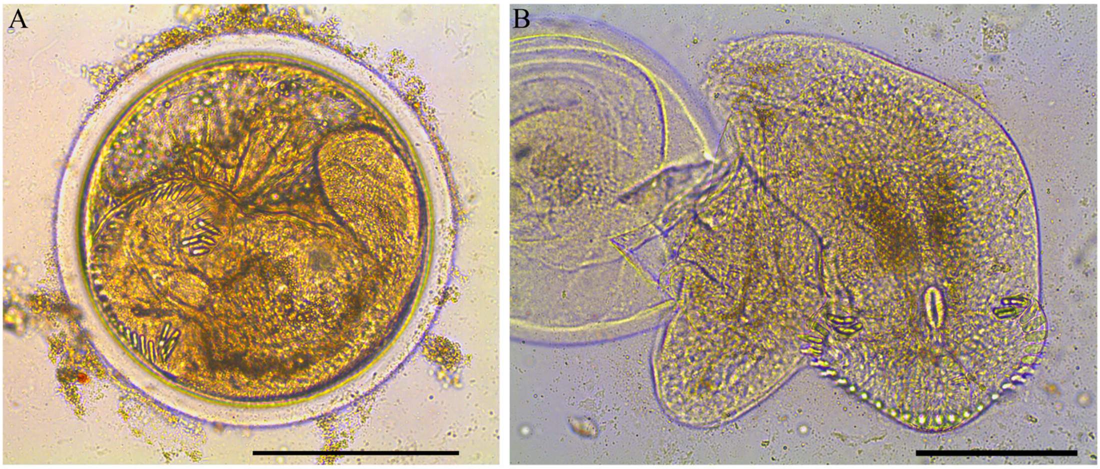

Fig. 4. Microphotographs of metacercariae found in the mantle cavity of *Pomacea canaliculata*. A: encysted metacercariae. B: metacercariae extracted from cyst. Scale bars: 200 μm.

infected by ingesting metacercariae encysted in plants and other substrata, as for other Paramphistomidae (Vega *et al*., 2006).

A large number of trematode larvae have been mentioned associated with apple snails (Table 2). Two redia generations of *Echinostoma parcespinosum* have been found in the digestive gland and gonad of *P. canaliculata* and encysted metacercariae were observed in the mantle cavity and hepatopancreas (Martorelli, 1987). The definitive hosts of this parasite are rails (in the birds' intestines). The apple snail, which is the first intermediate host, can also be the second intermediate host (among other freshwater snails). In the latter case, the life cycle of *E. parcespinosum* may be shortened without the need for another host, as cercariae have been recorded encysted within rediae (Martorelli, 1987). This peculiarity was also observed in Brasil in *Pomacea lineata* (Machado & Sampaio, 1980). Metacercariae of *Dietziella egregia* have been observed in the renal cavity of *P. canaliculata* from Argentina (M.C. Digiani & M. Ostrowski de Núñez, unpublished). Although the life cycle of this digenean is not fully known, the white-faced ibis (*Plegadis chihi*) could be the definitive host. Encysted metacercariae of *Edietziana malacophilum*, an intestinal parasite of the snail kite, *Rostrhamus sociabilis*, have been found in the hepatopancreas of *Pomacea paludosa* in Cuba (Pérez Vigueras, 1944). Other metacercariae were found in *Pomacea canaliculata* (Keawjam *et al*., 1993) in Southeast Asia (Table 2). Nasir & Díaz (1968a, b) described new species of Echinostomatidae in *Pomacea glauca*, i.e. *Echinochasmus zubedakhaname* (parasitizing a small passerine bird, *Fluvinicola pica*) and *Stephanoprora heteroglandula*. The snail also carried many cercariae of different morphological types (Table 2).

Diseases associated with apple snails

Dermatitis

Schistosome dermatitis occurs when a person becomes the accidental host of cercariae of non-human schistosome trematodes. The cercariae penetrate the skin where they soon die, causing a hypersensitive reaction of the skin (Hoeffler, 1974). Leedom & Short (1981) reported dermatitis caused by furcocercous cercariae from *Pomacea paludosa* in Florida (United States).

Echinostomiasis

This is a disease caused by infections by flukes of the family Echinostomatidae (echinostomes) via oral intake of undercooked infected snails and clams. Twenty species belonging to eight genera of this family are known to infect humans worldwide (Chai, 2009). The main clinical symptoms involve diarrhoea, abdominal pain, anaemia and eosinophilia (Mehlhorn, 2008). In cases with heavy loads of echinostomes mortality is caused by intestinal perforation or marked malnutrition and anaemia. *Echinostoma ilocanum* was discovered in the Philippines at the beginning of the twentieth century. Bonne *et al*. (1953) found it infecting humans in Malaysia, with *Pila scutata* as one of the first intermediate hosts, among other snails. Human infections with *E. ilocanum* were later reported in Indonesia, China, Thailand, India and Cambodia. The main cause of these infections was the consumption of raw or undercooked flesh of *Pila scutata* (Sohn *et al*., 2011). [Note that the name *Pila luzonica* as used by Sohn *et al*. (2011) is now considered a junior synonym of *Pila conica*, and *Pila conica* as used by Bonne *et al*. (1953) is considered a junior synonym of *Pila scutata*; see Cowie (2015)].

Gnathostomiasis

This is an unusual human infection by larvae of the nematode *Gnathostoma spinigerum*, a parasite of felines and canids. It is acquired by eating raw or undercooked flesh of infected intermediate hosts (fish, amphibians, reptiles, birds). The larvae in the intermediate hosts enter human tissue and may migrate through many tissues causing intermittent subcutaneous swellings and (often) intestinal nodules, and an inflammatory reaction associated with production of many eosinophils. The larvae are especially destructive when they die in the brain or eye. *Pomacea canaliculata* and *Pila ampullacea* are suitable paratenic hosts for third stage larvae of *G. spinigerum* (Komalamisra *et al*., 2009).

Angiostrongyliasis

Angiostrongyliasis is caused by two species of nematodes of the genus *Angiostrongylus* (Secernentea, Metastrongyloidea). *Angiostrongylus cantonensis* causes eosinophilic meningitis and meningoencephalitis, and the disease is sometimes referred to as neuroangiostrongyliasis, while *A. costaricensis* causes abdominal angiostrongyliasis, a gastrointestinal syndrome (Mehlhorn, 2008; Murphy & Johnson, 2013).

Angiostrongylus cantonensis was discovered by Chen (1935) in the pulmonary arteries and hearts of domestic rats in China, and therefore became known as the rat lungworm. It is endemic in South Asia, the Pacific islands, Australia and the Caribbean islands. Its life cycle involves rats as definitive hosts, snails and slugs as intermediate hosts, and various other animals, including crustaceans (prawns and land crabs), land planarians, frogs and monitor lizards, as paratenic (transfer or transport) hosts (Cowie, 2013b). Humans acquire *A. cantonensis* by eating raw or undercooked intermediate or paratenic hosts that contain the infective third larval stage of the worm, or inadvertently by eating vegetables contaminated with infected snails. When infective larvae are ingested, they penetrate the intestinal wall and reach the circulatory system, and finally reach the brain where the third stage larvae develop to a sub-adult stage. These worms are not able to leave the brain and they generally die. The immune reaction causes inflammation, and the meninges and cerebral vessels are infiltrated with lymphocytes, plasma cells and eosinophils (Wang *et al*., 2008). Also, physical brain lesions, and even in the spinal cord, are caused especially by the movements of live worms (Chotmongkol & Sawanyawisuth, 2002; Cowie, 2013a). The larvae can also move to the eyes and cause ocular angiostrongyliasis, resulting in visual disturbance (Sawanyawisuth *et al*., 2006).

The first human case of angiostrongyliasis was reported in Taiwan in 1945 (Beaver & Rosen, 1964). Since then, several outbreaks of the disease have been reported in the Pacific islands and other endemic regions. In Taiwan many cases of angiostrongyliasis have been reported, mainly in children, with *Achatina fulica* and *Pomacea canaliculata* the most frequent intermediate hosts (Tseng *et al*., 2011). *Angiostrongylus cantonensis* has been reported from 13 provinces in China, where the spread of the invasive species *P. canaliculata* would facilitate expansion of the disease (Lv *et al*., 2011). Unfortunately, *P. canaliculata* is very susceptible to *A. cantonensis* and has become an important intermediate host in these regions (Wang *et al*., 2008).

Several *Pila* spp. (*P. scutata*, *P. gracilis*, *P. virescens* and *P. ampullacea*) have been found infected with *A. cantonensis* in Malaysia and Thailand (Harinasuta *et al*., 1965). These species are used as a food resource and thus could cause human infection. *Pomacea paludosa* infected by *A. cantonensis* was found in Cuba (Aguiar *et al*., 1981). *Pomacea paludosa* was also reported to be infected in the Hawaiian Islands (Wallace & Rosen, 1969a, b) but this may have been a misidentification as this species is not known to have ever been present in the wild in the Hawaiian Islands (Cowie *et al*., 2007). However, other non-native apple snails are present in Hawaii and are used for human consumption and as aquarium pets (Cowie *et al*., 2007), and *Pomacea canaliculata* is reported to be infected (Kim *et al*., 2014). *Angiostrongylus cantonensis* has recently been found in *Pomacea maculata* introduced in the southeastern United States (Louisiana). This introduced snail is currently spreading rapidly across this region, triggering concerns about establishment of the parasite (Teem *et al*., 2013).

The first cases of human infection by *Angiostrongylus costaricensis* were diagnosed mainly in children. They are characterized by the formation of granulomas with heavy eosinophilic infiltration in the abdominal cavity (Morera & Céspedes, 1971). Rodents are the final hosts. The larvae leave the intestine in the faeces. Intermediate hosts (slugs or snails) feed on these larvae, which develop into the infectious third stage larvae. Humans are accidentally infected when in contact with the molluscs. The adult worms are localized in the mesenteric arteries of the definitive host, where inflammatory reactions are common. Many of the arterioles containing adults become thrombosed after the worms die, causing intestinal obstruction (Mehlhorn, 2008). Abdominal angiostrongyliasis has been recorded from the southern United States to northern Argentina. Up to 500 human cases are reported annually in Costa Rica. In Brasil, cases have been reported mainly in the southern states (Thiengo *et al*., 2013).

The main intermediate hosts of *A. costaricensis* are slugs. However, apple snails are potential hosts. *Pomacea flagellata* was introduced to Costa Rica for human consumption and has been successfully infected experimentally (Briceño Lobo, 1986).

Salmonellosis

Various outbreaks of food-borne illnesses such as salmonellosis have been associated with snail consumption. Salmonellosis is a disease caused by the bacterium *Salmonella*. Most people have diarrhoea, fever and stomach pains. Consumption of apple snails, known locally as *kuhol*, as a fish and meat substitute has become popular in the Philippines. The main concern about this culinary culture is undercooking the snails. *Salmonella* serotype *typhimurium* populations survive at 60 °C, while the sharpest drop in the counts of these bacteria is observed at 90 °C (Gabriel & Ubana, 2007).

Bartlett & Trust (1976) isolated different serotypes of *Salmonella* and other potential pathogens in apple snails from aquaria in North America. Their study revealed a formerly unreported zoonotic reservoir of salmonellae. There is a reason to believe that this association of salmonellae with apple snails could explain some cases of human salmonellosis, as other aquarium species have already been shown to contribute towards many cases of this infectious disease (Bartlett & Trust, 1976).

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References

Aguiar, H., Gispert, J., Dumenigo, B., Perera, G. & Galvez, M. 1981. *Angiostrongylus cantonensis*. Hospederos intermediarios en dos provincias habaneras. *Revista Cubana de Medicina Tropical* 33: 173-177.

Bartlett, K.H. & Trust, T.J. 1976. Isolation of salmonellae and other potential pathogens from the freshwater aquarium snail *Ampullaria*. *Applied and Environmental Microbiology* 31: 635-639.

Bathia, B.L. 1936. *The Fauna of British India Including Ceylon and Burma. Protozoa: Ciliophora*. Taylor and Francis, London.

Beaver, P.C. & Rosen L. 1964. Memorandum on the first report of *Angiostrongylus* in Man, by Nomura and Lin, 1945. *American Journal of Tropical Medicine and Hygiene* 13: 589-590.

Bonne, C., Bras, G. & Joe, L.K. 1953. Five echinostomes found in Man in the Malayan Archipelago. *American Journal of Digestive Diseases* 20: 12-16.

Briceño Lobo, A.C. 1986. Infección experimental de *Pomacea flagellata* (Gastropoda: Ampullariidae) con *Angiostrongylus costaricensis* (Nematoda: Angiostrongylidae). Thesis. Universidad de Costa Rica, San José.

Buckland-Nicks, J., Chisholm, S.A. & Gibson, G. 2013. The living community inside the common periwinkle, *Littorina littorea. Canadian Journal of Zoology* 91: 293-301.

Castro-Vazquez, A., Albrecht, E., Vega, I., Koch, E. & Gamarra-Luques C. 2002. Pigmented corpuscles in the midgut gland of *Pomacea canaliculata* and other neotropical apple-snails (Prosobranchia, Ampullariidae): a possible symbiotic association. *Biocell* 26: 101-109.

Cazzaniga, N.J. 1988. *Hyalinella vahiriae* (Ectoprocta) en la Provincia de San Juan. *Revista de la Asociación de Ciencias Naturales del Litoral* 19: 205-208.

Chai, J.Y. 2009. Echinostomes in humans. In: *The Biology of Echinostomes* (Fried, B. & Toledo, R., ed.), p 147-183. Springer, New York.

Chen, H.T. 1935. Un nouveau nématode pulmonaire, *Pulmonema cantonensis* n. g., n. sp., des rats de Canton. *Annales de Parasitologie Humaine et Comparé* 13: 312-370.

Chotmongkol, V. & Sawanyawisuth, K. 2002. Clinical manifestations and outcome of patients with severe eosinophilic meningoencephalitis presumably caused by *Angiostrongylus cantonensis*. *Southeast Asian Journal of Tropical Medicine and Public Health* 33: 231-34.

Cowie, R.H. 2013a. Biology, systematics, life cycle, and distribution of *Angiostrongylus cantonensis*, the cause of rat lungworm disease. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 6-9.

Cowie, R.H. 2013b. Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 70-74.

Cowie, R.H. 2015. The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa* 3940(1): 1-92.

Cowie, R.H., Hayes, K.A., Tran, C.T. & Levin, P. 2007. Distribution of the invasive apple snail *Pomacea canaliculata* (Lamarck) in the Hawaiian Islands (Gastropoda: Ampullariidae). *Bishop Museum Occasional Papers* 96: 48-51.

Damborenea, M.C. 1992. Espécies de *Temnocephala* (Platyhelminthes, Temnocephalidae) de crustaceos y moluscos de la Argentina. *Iheringia, Série Zoologia* 72: 3-21.

Damborenea, M.C. 1996. Patrones de distribución y abundancia de *Temnocephala iheringi* (Platyhelminthes, Temnocephalidae) en una población de *Pomacea canaliculata* (Mollusca Ampullariidae). *Gayana, Zoología* 60: 1-12.

Damborenea, M.C. 1998. Distribution patterns of temnocephalids commensal with Crustacea and Mollusca from Argentina. *Hydrobiologia* 383: 269-274.

Damborenea, C. & Brusa, F. 2008. A new species of *Temnocephala* (Platyhelminthes, Temnocephalida) commensal of *Pomella megastoma* (Mollusca Ampullariidae) from Misiones, Argentina. *Revista Mexicana de Biodiversidad* 79: 1S-7S.

Damborenea, C. & Cannon, L.R.G. 2001. On Neotropical *Temnocephala* (Platyhelminthes). *Journal of Natural History* 35: 1103-1118.

Damborenea, M.C. & Gullo, B.S. 1996. Hirudíneos asociados a la cavidad paleal de *Pomacea canaliculata* (Lamarck 1822) (Gastropoda: Ampullariidae) del balneario Bagliardi, Río de la Plata, Argentina. *Neotropica* 42: 97-101.

Damborenea, M.C., César, I.I. & Armendariz, L. 1997. Especies de *Temnocephala* (Platyhelminthes, Temnocephalidae) de la Isla Martín García, Buenos Aires, Argentina. *Neotropica* 43 (109-110): 123-124.

Damborenea, C., Brusa, F. & Paola, A. 2006. Variation in worm assemblages associated with *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) in sites near the Río de la Plata estuary, Argentina. *Biocell* 30(3): 457-468.

Darrigran, G. & Damborenea, C. 2005. A bioinvasion history in South America. *Limnoperna fortunei* (Dunker, 1857), the golden mussel. *American Malacological Bulletin* 20: 105-112.

De-Carli, B.P., Rotundo, M.M., Paschoal, L.R.P., Andrade, D.P., Cavallaris, D.C., Oceguera-Figueroa, A. & Alonso, D.D. 2014. First record of the association between the leech *Helobdella triserialis* (Hirudinea, Glossiphoniidae) and two species of *Pomacea* (Gastropoda, Ampullariidae) in Brazil. *Pan-American Journal of Aquatic Sciences* 9(2): 136-140.

Dioni, W. 1967. *Temnocephalas uruguayas* II. Descripción de *Temnocephala talicei* n.sp. y otras notas sobre *Temnocephala axenos*. *Physis B* 26(73): 477-484.

Di Persia, D.H. & Radici de Cura, M.S. 1973. Algunas consideraciones acerca de los organismos epibiontes desarrollados sobre Ampullariidae. *Physis B* 32: 309-319.

Dias, R.J.P., D'Ávila, S. & D'Agosto, M. 2006. First record of epibionts Peritrichids and Suctorians (Protozoa, Ciliophora) on *Pomacea lineata* (Spix, 1827). *Brazilian Archives of Biology and Technology* 49(5): 807-812.

Dias, R.J.P., D'Ávila, S., Wieloch, A.H. & D'Agosto, M. 2008. Protozoan ciliate epibionts on the freshwater apple snail *Pomacea figulina* (Spix, 1827) (Gastropoda, Ampullariidae) in an urban stream of south-east Brazil. *Journal of Natural History* 42(19-20): 1409-1420.

Dias, R.J.P., Cabral, A.F., Siqueira-Castro, I.C.V., Silva-Neto, I.D. & D'Agosto, M. 2010. Morphometric study of a Brazilian strain of *Carchesium polypinum* (Ciliophora: Peritrichia) attached to *Pomacea figulina* (Mollusca: Gastropoda), with notes on a high infestation. *Zoologia* 27(3): 483-488.

Gabriel, A. & Ubana. N.A. 2007. Decimal reduction times of *Salmonella* Typhimurium in *guinataang kuhol*: An indigenous Filipino dish. *Food science and Technology* 40(6): 1108-1111.

Gamarra-Luques, C., Vega, I.A., Koch, E. & Castro-Vázquez, A. 2004. Intrahost distribution and transmission of a new species of cyclopoid copepod endosymbiotic to a freshwater snail*, Pomacea canaliculata* (Caenogastropoda, Ampullariidae) from Argentina. *Biocell* 28: 155-164.

Garcés, A.C., Puerta, L., Tabares, Y., Lenis, C. & Velásquez, L.E. 2013. *Temnocephala colombiensis* n. sp. (Platyhelminthes: Temnocephalidae) from Antioquia, Colombia. *Revista Mexicana de Biodiversidad* 84: 1090-1099.

Gascón, A. 1975. Dos ciliados del género *Parasicuophora* parásitos de *Pomacea canaliculata* (Lamarck). *Revista de Biología del Uruguay* 3(2): 111-125.

Godoy M.S., Castro-Vasquez, A. & Vega, I.A. 2013. Endosymbiotic and host proteases in the digestive tract of the invasive snail *Pomacea canaliculata*: diversity, origin and characterization. *PLoS ONE* 8(6): e66689.

Gorni, G.R. & Alves, R.G. 2006. Naididae (Annelida, Oligochaeta) associated with *Pomacea bridgesii* (Reeve) (Gastropoda, Ampullaridae). *Revista Brasileira de Zoologia* 23 (4): 1059-1061.

Hamann, M.I. 1992. *Catadiscus pomaceae* sp. n. (Trematoda, Paramphistomatidae) from *Pomacea canaliculata* (Lamarck, 1801) (Prosobranchia, Ampullariidae). *Memórias do Instituto Oswaldo Cruz* 87(1): 9-14.

Harinasuta, C.P., Setasubun, P. & Radomyca, P. 1965. Observations on *Angiostrongylus cantonensis* in rats and mollusks in Thailand. *Journal of the Medical Association of Thailand* 48: 158172.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Ho, J.S. & Thatcher, V.E. 1989. A new family of cyclopoid copepods (Ozmanidae) parasitic in the hemocoel of a snail from the Brazilian Amazon. *Journal of Natural*

History 23: 903-911.

Hoeffler, D.F. 1974. Cercarial dermatitis, its etiology, epidemiology, and clinical aspects. *Archives of Environmental Health* 29(4): 225-229.

Hyman, L.H. 1955. Miscellaneous marine and terrestrial flatworms from South America. *American Museum Novitates* 1742: 1-33.

Keawjam, R.S., Poonswad, P., Upatham, E.S. & Banpavichit, S. 1993. Natural parasitic infections of the golden apple snail, *Pomacea canaliculata*. *Southeast Asian Journal Tropical Medical Public Health* 24: 170-177.

Kim, J.R., Hayes, K.A., Yeung, N.W. & Cowie, R.H. 2014. Diverse gastropod hosts of *Angiostrongylus cantonensis*, the rat lungworm, globally and with a focus on the Hawaiian Islands. *PLoS ONE* 9(5): e94969.

Koch, E., Vega, I.A., Albrecht, E.A., Gamarra-Luques, C. & Castro-Vazquez, A. 2003. Evidence for direct mother-offspring transmission of a possible symbiont inhabiting the midgut gland (MGG) of *Pomacea canaliculata*. *Biocell* 27: 24.

Koch, E., Vega, I.A., Albrecht, E.A., Ortega, H.H. & Castro-Vazquez, A. 2006. A light and electron microcopic study of pigmented corpuscles in the midgut gland and feces of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Veliger* 48: 45-52.

Komalamisra, C., Nuamtanong, S. & Dekumyoy, P. 2009. *Pila ampullacea* and *Pomacea canaliculata*, as new paratenic hosts of *Gnathostoma spinigerum*. *Southeast Asian Journal of Tropical Medicine and Public Health* 40(2): 243-246.

Leedom, W.S. & Short, R.B. 1981. *Ceracria pomaceae* sp. n., a dermatitis-producing schistosome cercaria from *Pomacea paludosa*, the Florida apple snail. *Journal of Parasitology* 67(2): 257-261.

Lim, B.L., Ow-Yang, C.K. & Lie, K.J. 1965. Natural infection of *A. cantonensis* in Malaysian rodents and intermediate hosts and preliminary observations on acquired resistance. *American Journal of Tropical Medicine and Hygiene* 14: 610-617.

Lv S., Zhang, Y., Liu, H.X., Hu, L., Yang, K., Steinmann, P., Chen, Z., Wang, L.Y., Utzinger, J. & Zhou, X.N. 2009. Invasive snails and an emerging infectious disease: results from the first national survey on *Angiostrongylus cantonensis* in China. *PLoS Neglected Tropical Diseases* 3(2): 1-8.

Lv, S., Zhang, Y., Steinmann, P., Yang, G.J., Yang, K. Zhou, X.N. & Utzinger, J. 2011. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biology* 56: 717-734.

Machado, V.L.L. & Sampaio, F.A. 1980. Desenvolvimiento de *Echinostoma* *parcespinosum* Lutz, 1924 em *Pomacea lineata* (Spix, 1827). *Naturalia* 5: 23-29.

Martín, P.R., Estebenet, A.L. & Burela, S. 2005. Factors affecting the distribution and abundance of the commensal *Temnocephala iheringi* (Platyhelminthes: Temnocephalidae) among the southernmost populations of the apple snail *Pomacea canaliculata* (Mollusca: Ampullariidae). *Hydrobiologia* 545(1): 45-53.

Martorelli, S.R. 1987. Estudios parasitológicos en biotopos lénticos de la República Argentina. IV. El ciclo biológico de *Echinostoma parcespinosum* Lutz, 1924 (Digenea) parásito de *Rallus maculatus maculatus* y *Rallus sanguinolentus sanguinolentus* (Aves: Rallidae). *Revista del Museo de La Plata, Nueva Serie, Zoología* 14: 48-59.

Mehlhorn, H. 2008. *Encyclopedia of Parasitology, Third Edition*. Springer, Berlin, Heidelberg, New York.

Morera, P. & Céspedes, R. 1971. *Angiostrongylus costaricensis* n. sp. (Nematoda: Metastrongyloidea), a new lungworm occurring in man in Costa Rica. *Revista de Biología Tropical* 18(1, 2): 173-185.

Murphy, G.S. & Johnson, S. 2013. Clinical aspects of eosinophilic meningitis and meningoencephalitis caused by Angiostrongylus cantonensis, the rat lungworm. *Hawai'i Journal of Medicine and Public Health* 72(6), Supplement 2: 35-40.

Nasir, P. 1965. Studies on freshwater larval trematodes. Part 1. A new species of a microcotylous xiphidiocercaria, *Cercaria cumanensis*, from Venezuela. *Proceedings of the Helminthological Society of Washington* 32(1): 103-105.

Nasir, P. 1971. Freshwater larval trematodes. XXVIII. Three new species of cercariae. *Proceedings of the Helminthological Society of Washington* 38(2): 206-210.

Nasir, P. &. Díaz, M.T. 1968a. Studies on freshwater larval trematodes. Part XVI. Five new species of cercariae from Venezuela. *Proceedings of the Helminthological Society of Washington* 35(1): 67-74.

Nasir, P. & Díaz, M. T. 1968b. Studies on freshwater larval trematodes XVIII. The life cycle of *Echinochasmus zubedakhaname* sp. n. *Zeitschrift fur Parasitenkunde* 30: 126-133.

Nasir, P. & Díaz, M.T. 1973. Freshwater larval trematodes. XXXI. Two new species of cercariae. *Proceedings of the Helminthological Society of Washington* 40(1): 69-73.

Nasir, P. & Silva, J. 1972. Freshwater larval trematodes. XXIX. Life cycle of *Guaicaipuria parapseudoconcilia* sp. n. *Proceedings of the Helminthological Society of Washington* 39(1): 142-146.

Nasir, P., Díaz, M.T., Salazar, L.J.H. & Guevara, S. 1969. Studies on freshwater larval trematodes. XXI. Two new species of macrocercous cercariae. *Proceedings of the Helminthological Society of Washington* 36(1): 102-106.

Ostrowski de Nuñez, M. 1978. Fauna de agua dulce de la República Argentina VII. cercarias de la familia Schistosomatidae (Trematoda, Digenea). *Revista Museo Argentino de Ciencias Naturales Bernardino Rivadavia* 2(3): 65-76.

Ostrowski de Nuñez, M. 1979. Ungewöhnliche Xiphidiocercariae aus *Ampullaria canaliculata* nebst Bemerkungen über *Travtrema stenocotyle*. *Angewandte Parasitologie* 20: 46-52.

Panyagupta, S. 1965. Eosinophilic meningoencephalitis in Thailand: summary of nine cases and observations on *Angiostrongylus cantonensis* as a causative agent and *Pila ampullacea* as a new intermediate host. *American Journal of Tropical Medicine and Hygiene* 14: 370-374.

Pereira, C. & Cuocolo, R. 1941. Estudos sobre "Temnocephalidae Monticelli, 1899", com estabelecimento de dois novos géneros australianos e descrição de duas novas espécies neotrópicas. *Arquivos do Instituto Biológico* 12 (9): 101-127

Pérez Vigueras, I. 1944. Trematodes de las superfamilia Echinostomatoidea, con descripción de siete especies nuevas de Cuba. *Revista Universidad de La Habana* 55-57: 221-234.

Pinto, H.A., Cantanhede, S.P.D., Thiengo, S.C., de Melo A.L. & Fernandez, M.A. 2015. The apple snail *Pomacea maculata* (Caenogastropoda: Ampullariidae) as the intermediate host of *Stomylotrema gratiosus* (Trematoda: Stomylotrematidae) in Brazil: the first report of a mollusc host of a stomylotrematid trematode. *Journal of Parasitology* 101(2): 134-139.

Ponce de León, R. 1979. Especies americanas de Temnocephalidae Behnam (Platyhelmintha), I. Descripción de *Temnocephala rochensis* n.sp. de la cámara paleal de *Pomacea canaliculata* (Lamarck). *Revista de Biología del Uruguay* 7(1): 39-48.

Ponce de León, R. 1989. Description of *Temnocephala haswelli* n.sp. (Platyhelminthes) from the mantle cavity of *Pomacea canaliculata* (Lamarck). *Journal of Parasitology* 75(4): 524-526.

Ringuelet, R. 1945. Hirudíneos del Museo de La Plata. *Revista del Museo de La Plata (Nueva Serie) Zoología* 4: 95-137.

Ringuelet, R. 1949. Notas sobre hirudíneos neotropicales. VI. Presencia del género *Glossiphonia* en la Argentina y otras adiciones al conocimiento de la hirudinofauna de los países del Plata. *Notas del Museo de La Plata* 14: 141-159.

Ringuelet, R. 1985. Annulata Hirudinea. In: *Fauna de Agua Dulce de la República*

Argentina (de Castellanos Z.J.A., ed.), p. 1-321. Fundación para la Educación, la Ciencia y la Cultura, Buenos Aires.

Rosso de Ferradás, B. & Fernández, H.R. 2005. Elenco y biogeografía de los ácaros acuáticos (Acari, Parasitengona, Hydrachnidia) de sudamérica. *Graellsia* 61(2): 181-224.

Sawanyawisuth, K., Kitthaweesin, K., Limpawattana, P., Intapan, P.M., Tiamkao, S., Jitpimolmard, S. & Chotmongkol, V. 2006. Intraocular angiostrongyliasis: clinical findings, treatments and outcomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101: 497-501.

Seixas, S.A., Amato, J.F.R. & Amato, S.B. 2010a. First report of *Temnocephala haswelli* (Platyhelminthes: Temnocephalida) in *Pomacea canaliculata* (Mollusca: Ampullariidae) from Brazil: description update based on specimen from the state of Rio Grande do Sul, Brazil. *Zoologia* 27: 455-464.

Seixas, S.A., Amato, J.F.R. & Amato, S.B. 2010b. Redescription of *Temnocephala iheringi* (Platyhelminthes: Temnocephalida) based on specimens from *Pomacea canaliculata* (Mollusca: Ampullariidae) of the state of Rio Grande do Sul, Brazil: the possible type host and type locality. *Zoologia* 27: 245-257.

Seixas, S.A., Amato, J.F.R. & Amato, S.B. 2010c. First report of *Temnocephala rochensis* (Platyhelminthes: Temnocephalida) from *Pomacea canaliculata* (Mollusca: Ampullariidae) outside Uruguay - description update based on specimens from the state of Rio Grande do Sul, Brazil. *Zoologia* 27: 820-828.

Sohn, W.M., Kim, H.J., Yong, T.S., Eom, K.S., Jeong, H.G., Kim, J.K., Kang, A.R., Kim, M.R., Park, J.M., Ji, S.H., Sinuon, M., Socheat, D. & Chai, J.Y. 2011. *Echinostoma ilocanum* infection in Oddar Meanchey Province, Cambodia. *Korean Journal of Parasitology* 49(2): 187-190.

Teem, J.L., Qvarnstrom, Y., Bishop, H.S., Da Silva, A.J., Carter, J., White-McLean, J. & Smith, T. 2013. The occurrence of the rat lungworm, *Angiostrongylus cantonensis*, in nonindigenous snails in the Gulf of Mexico Region of the United States. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 11-14.

Thiengo, S.C., Simões, R.O., Fernandez, M.A. & Maldonado, A. 2013. *Angiostrongylus cantonensis* and rat lungworm disease in Brazil. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 18-22.

Tseng, Y.T., Tsai, H.C., Sy, C.L., Lee, S.J. S., Wann, Y.H., Wang, Y.H., Chen, J.K.,Wu, K.S. & Chen, Y.S. 2011. Clinical manifestations of eosinophilic meningitis caused by *Angiostrongylus cantonensis*: 18 years' experience in a medical center in southern Taiwan. *Journal of Microbiology, Immunology and Infection* 44: 382-389.

Uribe, C. 1925. Notes of two Venezuelan Xiphidiocercariae. *Journal of Parasitology* 11(3): 125-136.

Utz, L.R.P. 2007. First record of *Epistylis plicatilis* (Ciliophora: Peritrichia) attached to *Pomacea canaliculata* (Mollusca: Gastropoda) in southern Brazil. *Zootaxa* 1454: 49-57.

Vega, I.A., Gamarra-Luques, C., Koch, E., Bussmann, L.E. & Castro-Vazquez, A. 2005. A study of corpuscular DNA and midgut gland occupancy by putative symbiotic elements in *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Symbiosis* 39: 37-45.

Vega, I.A., Damborenea, M.C., Gamarra-Luques, C., Koch, E., Cueto, J.A. & Castro-Vazquez, A. 2006. Facultative and obligate symbiotic associations of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Biocell* 30: 367-375.

Vega, I.A., Arribere, M.A., Almonacid, A.V., Ribeiro Guevara, S. & Castro-Vazquez, A. 2012. Apple snails and their endosymbionts bioconcentrate heavy metals and uranium from contaminated drinking water. *Environmental Science and Pollution Research* 19: 3307-3316.

Wallace, G. & Rosen, L. 1969a. Experimental infection of Pacific Island mollusks with *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene* 18(1): 13-19.

Wallace, G. & Rosen, L. 1969b. Studies on eosinophilic meningitis V. Molluscan hosts on Pacific Islands. *American Journal of Tropical Medicine and Hygiene* 18(1): 13-19.

Wang, Q.P., Lai, D.H., Zhu, X.Q., Chen, X.G. & Lun, Z.R. 2008. Human angiostrongyliasis. *The Lancet* 8: 621-630.

Woodruff, D.S. & Upatham, E.S. 1993. Snail-transmitted diseases of medical and veterinary importance in Thailand and the Mekong valley. *Journal of Medical and Applied Malacology* 4: 1-12.

Zeliff, C.C. 1933. A new protozoan from the larva of the beetle *Osmoderma scabra*. *Proceedings of the United States National Museum* 82(2964): 1-3, pls. 1, 2.

Apple snail perivitellins, multifunctional egg proteins

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Abstract

Egg reserves of most gastropods are accumulated surrounding the fertilised oocyte as a perivitelline fluid (PVF). Its proteins, named perivitellins, play a central role in reproduction and development, though there is little information on their structural-functional features. Studies of mollusc perivitellins are limited to *Pomacea.* A proteomic study of the eggs of *P. canaliculata* identified over 59 proteins in the PVF, most of which are of unknown function, and have not been isolated and characterised. Information on molecular structure of the most abundant perivitellins of *P. canaliculata* have shown that they possess other functions besides being storage proteins, most remarkably in defence against predation and abiotic factors. They are a cocktail containing at least neurotoxic, antinutritive and antidigestive perivitellins, with others that may provide the eggs with a bright and conspicuous colour (aposematic signal). This review compiles the current knowledge of *Pomacea* perivitellins with emphasis on the novel physiological roles they play in the reproductive biology of these gastropods that have evolved the ability to lay their eggs above the water.

Additional keywords: Ampullariidae, egg defences, Mollusca, *Pomacea*, predation, protein structure and function

Introduction

During vitellogenesis the main components of the egg vitellus (lipids, proteins, carbohydrates) are synthesised either outside or inside the ovary and incorporated into primary oocytes to serve mainly as energetic and structural sources for development. In invertebrates, the major egg-yolk proteins are usually associated with lipids and/or carbohydrates, forming glycolipoproteins, complex water-soluble particles called vitellins or lipovitellins (Wallace *et al*., 1967). Most gastropods, however, contain a limited amount of vitellus. Instead, egg reserves are accumulated surrounding the fertilised oocyte as a perivitelline fluid (PVF) (Jong-Brink *et al*., 1983). Therefore, proteins present in the PVF have been termed perivitellins. In spite of the central role vitellins and perivitellins play in reproduction and development, there is little information on their structural features in invertebrates. Studies in molluscs are limited to the perivitellins of *Pomacea*, mostly those of *P. canaliculata*, but also of *P. scalaris* and *P. maculata* (Fig. 1). A proteomic study of the eggs of *P. canaliculata* identified over 59 proteins in the PVF of this species (Table 1), most of which are of unknown function and have not been isolated and characterised (Sun *et al*., 2012).

Fig. 1. The colour of egg clutches of *Pomacea* species is provided by pigmented perivitellins, which have only been studied in the three species depicted here (perivitellin names in brackets). The conspicuous and bright colour is presumably an aposematic or warning signal. (Photos: H. Heras and M.Y. Pasquevich)

This review compiles the current knowledge of *Pomacea* perivitellins with emphasis on their structure and the novel physiological roles they play in the reproductive biology of these freshwater gastropods that have the unusual strategy of laying their eggs above water.

Structure and functions

The perivitellins of **Pomacea canaliculata**

Structural aspects

BIOCHEMICAL COMPOSITION Although the partial sequences of all the proteins present in the eggs of this species have been reported (Sun *et al*., 2012), detailed biochemical, structural and functional information is only available for two perivitellins, named ovorubin (PcOvo) (Cheesman, 1958) and perivitellin-2 (PcPV2). They are large particles composed of a small number of subunits (oligomeric proteins). PcOvo is a 300 kDa multimer of a combination of multiple copies of three different ~30 kDa subunits, and PcPV2 is a 400 kDa octamer of four heterodimers (Frassa *et al*., 2010). PcOvo and PcPV2 particles occur in large quantities in the PVF of *P. canaliculata* and are the most abundant perivitellins (57.0 % and 10 % of egg total protein, respectively) (Garín *et al*., 1996; Heras & Pollero, 2002; Dreon *et al*., 2006). They form glyco-lipoproteic complexes with variable amounts of sugars attached.

All subunits of PcOvo are highly glycosylated (17.8 % w/w carbohydrates) giving rise to glycoforms; that is, different copies of a polypeptide bear different amounts and types of oligosaccharides (Fig. 2A, C). PcPV2 contains only 2.5 % w/w carbohydrates. Mannose is the major monosaccharide in both perivitellins, but sialic acid and fucose are also present, as in other mollusc glycoproteins (Dreon *et al*., 2004a; Ituarte *et al*., 2010). The different glycosylation patterns of the two major perivitellins of *P. canaliculata* probably allow the differential uptake and protein targeting of these molecules observed during apple snail embryogenesis (Heras *et al*., 1998).

These perivitellin complexes contain low but physiologically relevant amounts of lipids. PcOvo and PcPV2 lipid moieties are mainly composed of typical membrane lipids. There is also a complex fraction called PcPV3, which contains a third of all egg lipids, mainly neutral lipids and phospholipids (Garin *et al*., 1996).

Table 1. Proteome of the PVF of *Pomacea canaliculata*. Proteins that have been biochemically characterized are grouped by their sequence similarity and within groups according to their abundance (highest to lowest) in the PVF.

Protein name[a]	Unigene /GenBank	Tissue of origin
PcOvo carotenoprotein subunits and related proteins		
PcOvo1	SSH9 / JQ818217	Albumen gland
novel protein	SSH2 / JQ818214	Albumen gland
novel protein	SSH20	Albumen gland
PcOvo2	SSH4 / JQ818215	Albumen gland
PcOvo3	SSH8 / JQ818216	Albumen gland
novel protein	SSH95 / JQ818222	Albumen gland
novel protein	SSH122	Albumen gland
ovomucoid / egg protease inhibitor	SSH140	Albumen gland
novel protein	SSH3	Albumen gland
PcPV2 neurotoxin subunits		
PcPV2 membrane attack complex and perforin (MACPF)	SSH208 / JX155861	Albumen gland
PcPV2 tachylectin-like protein	SSH115 / JX155862	Albumen gland
tachylectin-related protein	SSH218	Albumen gland
Other proteins		
cell adhesion protein	TSA:Pc109422	Extra-glandular
thioester-containing protein	TSA:Pc66440	Extra-glandular
thioester-containing protein	TSA:Pc111579	Extra-glandular
apoptosis-inducing factor	TSA:Pc123838 / JQ818223	Extra-glandular
thioester-containing protein	TSA:Pc108510	Extra-glandular
C1q domain containing protein	SSH36 / JQ818219	Albumen gland
alpha-2-macroglobulin-like protein	TSA:Pc379	Extra-glandular
novel protein	TSA:Pc54251	Extra-glandular
transferrin	TSA:Pc99555	Extra-glandular
melanotransferrin	TSA:Pc119627	Extra-glandular
melanotransferrin	TSA:Pc124918	Extra-glandular
transferrin	TSA:Pc101904	Extra-glandular
novel protein	TSA:Pc75576	Extra-glandular
novel protein	TSA:Pc46253	Extra-glandular
scavenger receptor cysteine-rich protein	TSA:Pc52282	Extra-glandular
neurotrypsin-like	SSH25	Albumen gland
FG-GAP repeat protein	TSA:Pc94087	Extra-glandular
melanotransferrin-like	TSA:Pc49292	Extra-glandular
novel protein	TSA:Pc47034	Extra-glandular
tachylectin-like protein	TSA:Pc52841	Extra-glandular
novel protein	TSA:Pc59410	Extra-glandular
novel protein	TSA:Pc65629	Extra-glandular
novel protein	SSH111	Albumen gland
novel protein	SSH6	Albumen gland
Niemann-Pick disease type C2-like (cholesterol-binding glycoprotein)	TSA:Pc29496	Extra-glandular
thioester-containing protein	TSA:Pc112854	Extra-glandular
cysteine rich transmembrane BMP regulator	TSA:Pc53884	Extra-glandular
ubiquitin	TSA:Pc86328	Extra-glandular
15-hydroxyprostaglandin dehydrogenase	TSA:Pc117840	Extra-glandular
transmembrane protease serine	TSA:Pc119142	Extra-glandular
alpha-2-macroglobulin	TSA:Pc71049	Extra-glandular
telomeric repeat-binding factor 2-interacting protein	TSA:Pc87491	Extra-glandular
beta actin	TSA:Pc17674	Extra-glandular
scavenger receptor cysteine-rich protein	SSH42	Albumen gland
peptidoglycan recognition protein S1L [SF]	SSH14 / JQ818218	Albumen gland
novel protein	SSH94	Albumen gland
copper/zinc superoxide dismutase (SOD)	TSA:Pc101185	Extra-glandular
kunitz-like protease inhibitor	SSH51 / JQ818220	Albumen gland
Gap-Pol polyprotein-like	TSA:Pc100845	Extra-glandular
chitotriosidase (chitinase)	TSA:Pc120886	Extra-glandular
selenium-dependent glutathione peroxidase (GPx)	TSA:Pc114721	Extra-glandular
novel protein	TSA:Pc94566	Extra-glandular
aldehyde dehydrogenase	TSA:Pc106229	Extra-glandular
calcium-binding protein	SSH86 / JQ818221	Albumen gland
aldehyde oxidase	TSA:Pc120959	Extra-glandular
lysosome-associated membrane glycoprotein 1	TSA:Pc122290	Extra-glandular
novel protein	TSA:Pc34343	Extra-glandular

[a] Proteins were annotated by BLAST against the NCBI Non-redundant Protein Database. A protein with no homologue in this database is listed as a novel protein. Modified from Sun *et al*. (2012).

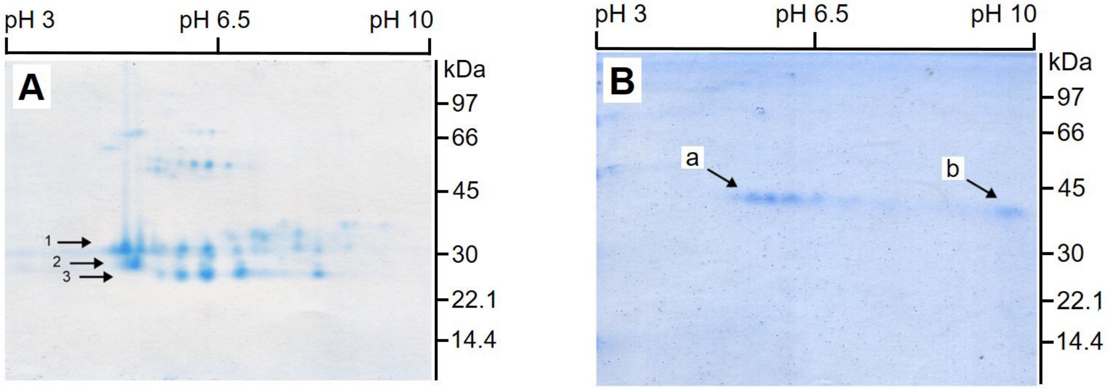

Fig. 2. Two-dimensional electrophoretic analysis of PcOvo egg perivitellins highlighting the variety provided by the attachment of carbohydrates to subunits and the presence of several isoelectric point (pI) isoforms. A: PcOvo subunits: pI isoforms of the 35 kDa subunit (1), pI isoforms of the 32 kDa subunit (2), pI isoforms of the 28 kDa subunit (3). B: chemically deglycosylated PcOvo – acidic pI spots (a), basic pI spots (b). Data from Ituarte *et al*. (2010).

The PcOvo and PcPV3 fractions are pigmented with the carotenoid astaxanthin (Dreon *et al*., 2004b). Astaxanthin is frequently observed in invertebrate carotenoproteins and its non-covalent binding to PcOvo is strong and specific (Dreon *et al*., 2007). The presence of pigmented perivitellins (carotenoproteins) that provide eggs with their conspicuous colour seems to be an acquisition of most aerial egg-laying ampullariids (Heras *et al*., 2007; Hayes *et al*., 2009).

STRUCTURE AND STABILITY In spite of being essential for understanding protein function, knowledge of the structure of gastropod perivitellins is mostly limited to those of *Pomacea canaliculata*.

The cDNA sequences coding for the three PcOvo subunits show no similarity to any known sequence, though they are related among themselves (Table 1) (Dreon *et al*., 2002, 2003; Sun *et al*., 2012). However, the cDNA sequences of the PcPV2 subunits show that the small subunit has homology with tachylectins (sugar binding proteins), while the large subunit has high identity with a family of pore forming proteins known as membrane attack complex and perforin (MACPF) (Table 1). Both types of proteins are involved in the immune system in other animals.

Studies of the shape of native PcOvo and PcPV2 by small angle X-ray scattering (SAXS) showed that they are globular proteins with an anisometric shape of 130 x 63 Å and 130 x 44 Å, respectively (Dreon *et al*., 2008; Frassa *et al*., 2010).

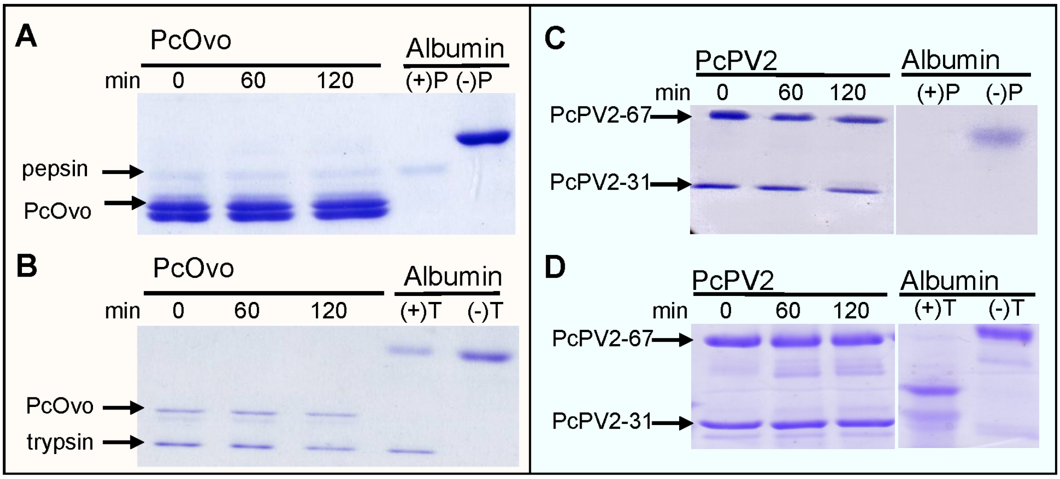

Fig. 3. *In vitro* digestibility of PcPV2 and PcOvo analysed by SDS-PAGGE. A, C: gastric digestion – lanes 1-3, 0, 60 and 120 min incubation, lanes 4 and 5 positive and negative controls, respectively. B, D: duodenal digestion – lanes 1-3, 0, 60 and 120 min incubation; lanes 4 and 5, positive and negative controls, respectively. Positive controls were albumin with enzyme, negative controls were albumin without enzyme. PcPV2-67 is the 67 KDa subunit, PcPV2-31 is the 31 KDa subunit. Data from Dreon *et al*. (2010, 2013).

The removal of astaxanthin does not affect the thermal or chemical structural stability of PcOvo, suggesting that it is not essential for protein stability, in contrast to most other vertebrate and invertebrate carotenoproteins (Dreon *et al*., 2007).

PcOvo and PcPV2 are stable over a wide pH range. PcOvo shows no structural perturbations between pH 4.5 and pH 12.0, while PcPV2 is not altered between pH 4.0 and pH 10.0, a remarkably wide range for oligomeric proteins (Dreon *et al*., 2008, 2013). PcOvo is highly stable thermally up to 100 °C (Dreon *et al*., 2007), while PcPV2 is stable up to 60 °C (Frassa *et al*., 2010). Both PcOvo and PcPV2 are highly resistant to the combined action of pepsin and trypsin proteases (Dreon *et al*., 2010, 2013) (Fig. 3).

Functions

SOURCE OF NUTRIENTS The major perivitellins of *P. canaliculata* are storage proteins that provide energetic and structural precursors for the developing embryo (Garín *et al*., 1996; Heras *et al*., 1998). In particular, PcOvo carries and stabilises astaxanthin within the PVF. Astaxanthin is a potent antioxidant that could be readily incorporated into the cytoplasmic membranes of the embryo for protection (Dreon *et al*., 2004b) when PcOvo is taken up by the embryos at later development stages (Heras *et al*., 1998).

DEFENCE AGAINST PREDATION In contrast with most eggs, which are intensely predated because of their high nutritional value, the eggs of *P. canaliculata* have only one reported predator, the fire ant *Solenopsis geminata* (see Yusa, 2001) (Fig. 4). This lack of predation is due to a suite of defences that are currently under active research. In fact, *P. canaliculata* has developed some fascinating mechanisms at the biochemical level, in which perivitellins play a central role. At present, of the 59 proteins of the PVF proteome, three proteinaceous components of the egg defences have been identified: pigmented perivitellins responsible for the warning colour of the clutches, a neurotoxin and an antinutritive/antidigestive perivitellin. Fig. 5 summarizes the role of the studied perivitellins in embryo defence.

The conspicuous reddish-pink colour of the eggs of *P. canaliculata* is provided by the pigmented perivitellins PcOvo and PcPV3, and presumably advertises to visually hunting predators the presence of egg defences, that is, it is an aposematic signal (Heras *et al*., 2007).

PcPV2 is a potent neurotoxin that damages the spinal cord of mice, causing death within 30 hours. PcPV2 induces neuronal apoptosis and alterations in calcium homeostasis and glycan expression in the dorsal horn of the spinal cord, which may play a role in the neurological disorders it induces (Heras *et al*., 2008; Fernández *et al*., 2011). *Pomacea canaliculata* is the first animal known to have a proteinaceous egg neurotoxin. If orally administered to rats at sublethal concentrations, this unusual neurotoxin, combining a lectin (presumably a delivery subunit) and a pore forming subunit (presumably the toxic moiety), is able to reach the intestine in a biologically active conformation, binding to glycocalix of enterocytes and eventually reaching general circulation (Dreon *et al*., 2013). But PcPV2 is rather slow-acting and it seems unlikely that it could by itself account for the presence of only one predator worldwide.

In studying the structure of PcOvo, another line of defence was found. The high stability of this proteinase inhibitor under a wide range of pH and its resistance to pepsin and trypsin digestion makes it possible, as for PcPV2, to also reach the predator's intestine in a fully active form. PcOvo decreases rat growth when administered orally, probably by combining the inhibition of trypsin activity (antidigestive role) and the resistance of the protein to digestion by gut enzymes (antinutritive), thereby limiting the predator's capacity to digest egg nutrients (Dreon *et al*., 2010) (Fig. 6). *Pomacea canaliculata* is the first animal known to have antinutritive and antidigestive compounds in its eggs.

Fig. 4. The fire ant *Solenopsis geminata* is the only reported predator of *Pomacea canaliculata* eggs. (Photo: Y. Yusa, with permission)

OTHER FUNCTIONS The successful strategy of laying eggs above the water exposes them to a variety of selective challenges imposed by stressful environmental conditions such as solar radiation and desiccation that may affect embryonic development and survival of offspring (Przeslawski 2004; Przeslawski *et al*., 2004). PcOvo exhibits functions that help the eggs cope with these harsh conditions, in addition to the roles it plays in nutrient storage and defence summarised above. For instance, the saccharide moiety of PcOvo, together with the high content of the polysaccharide galactogen in the PVF (Heras *et al*., 1998), prevents egg desiccation.

In addition, the pigmented perivitellins not only play a role as antioxidant carriers and warning signal molecules, they may also protect embryos against solar radiation by acting as filters (Dreon *et al*., 2004b). Another perivitellin that probably complements the antioxidant role of PcOvo

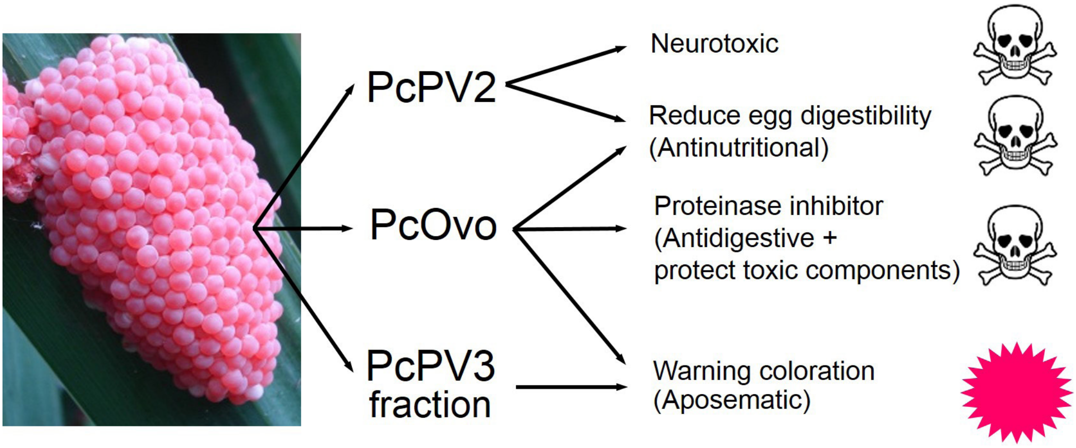

Fig. 5. Components of the biochemical defence system against predation of *Pomacea canaliculata* eggs.

is a copper-zinc superoxide dismutase, a well known enzyme of the antioxidant defence system in almost all eukaryotic cells (Table 1).

The proteome of PVF also contains several perivitellins possibly related to defence against pathogens, such as a C1q domain-containing protein, a peptidoglycan recognition protein and a chitotriosidase (Table 1). However, although PcOvo had long been assumed to defend the eggs against microbial infections (Norden, 1972), as other egg proteinase inhibitors do (Christeller, 2005), recent studies have found no bactericidal activity of either PcOvo or the whole cytosol against common bacteria like *Escherichia coli* and *Salmonella typhimurium* (Dreon *et al*., 2010).

More than half (34) of the 59 proteins found in the PVF are of unknown function (Sun *et al*., 2012), which highlights the need to perform a comprehensive functional characterization through biochemical studies.

Synthesis

The synthesis of most perivitellins occurs in the albumen gland, an accessory gland of the female reproductive tract that is conspicuously reddish-pink in *P. canaliculata* and *P. maculata* (Fig. 7A). Albumen gland dry weight is mainly represented by ash, mostly calcium carbonate, the inorganic component of the egg capsule. Calcium, stored by the labyrinth cells (Fig. 7) is largely transferred to the eggs, providing

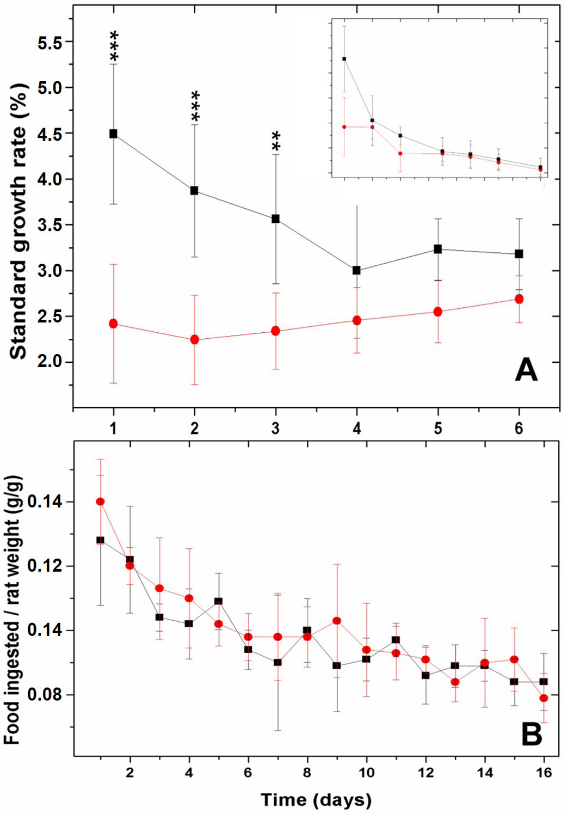

Fig. 6. Effect of diets supplemented with PcOvo on the standard growth rate and food consumption of Wistar rats. A: standard growth rate during the first six days; inset: standard growth rate during 16 days showing rat adaptation to protease inhibitor (axes as in main graph); control (black square), treated (red circle); values represent the mean ± 1 SD (n = 12); *** p<0.001, ** p<0.01. B: food ingestion during a 16-day experiment showing that the decrease in growth rate is not due to a reduction in food intake. Data from Dreon *et al*. (2010).

an additional defence in the calcareous egg shell and later in the shell of hatchlings. Proteins are the main biochemical component, followed by lipids and carbohydrates (Cadierno, Dreon & Heras, unpublished).

Although extra-glandular synthesis of several components of the PVF was inferred using a suppressive subtractive hybridisation (SSH) cDNA library approach (Sun *et al.*, 2012), the synthesis of most perivitellins including the major components, PcOvo and PcPV2-like precursors, occurs only in the albumen secretory cells of the parenchymal mass of the albumen gland with no circulating perivitellin precursors in the haemolymph (Fig. 6) (Dreon *et al.*, 2002, 2003), in agreement with the perivitellogenic mechanism of these snails (Jong-Brink *et al.*, 1983). Ultrastructural studies describing the parenchymal mass of the albumen gland identified two major cell types, the albumen secretory cells, involved in the synthesis of perivitellins and galactogen, and the labyrinth cells, involved in the storage and delivery of the large calcium levels of the PVF (Fig. 7B-D). Immunoelectron microscopy showed that the albumen secretory cells are the only parenchymal cells involved in the synthesis of PcOvo and PcPV2 and that both proteins are packed into the same secretory granules (Fig. 7C, D) (Catalán *et al.*, 2006).

The albumen gland cytosolic fraction has a lethal effect after intraperitoneal administration in mice (Cadierno, Dreon & Heras, unpublished). The toxicity of the organ extract may be due to the presence of an active conformation of the neurotoxin PcPV2. Moreover, mice showed similar symptoms to those given purified PcPV2. This would explain the behaviour of predators, such as the snail kite (*Rostrhamus sociabilis*) and rats (*Rattus norvegicus*), which invariably discard this gland when feeding on adult female *Pomacea* spp. (Snyder & Kale, 1983; Sykes, 1987; Yusa *et al.*, 2000).

The perivitellins of **Pomacea scalaris** *and* **P. maculata**

The PVF of *P. maculata* resembles that of *P. canaliculata*, with two major perivitellins called PmPV1 (a carotenoprotein) and PmPV2, and a group of low molecular weight proteins called, collectively, the PmPV3 fraction (Pasquevich, Dreon & Heras, unpublished). In contrast, *P. scalaris* eggs contain two major perivitellins of which only the most abundant has been studied; this protein, named scalarin (PsSC), is a carotenoid-binding glycoprotein (Ituarte *et al.*, 2008).

The carotenoproteins of both species show several biochemical and structural similarities with PcOvo. These similarities are probably related to similarities in the reproductive strategy, since all *Pomacea* species lay eggs above the water line.

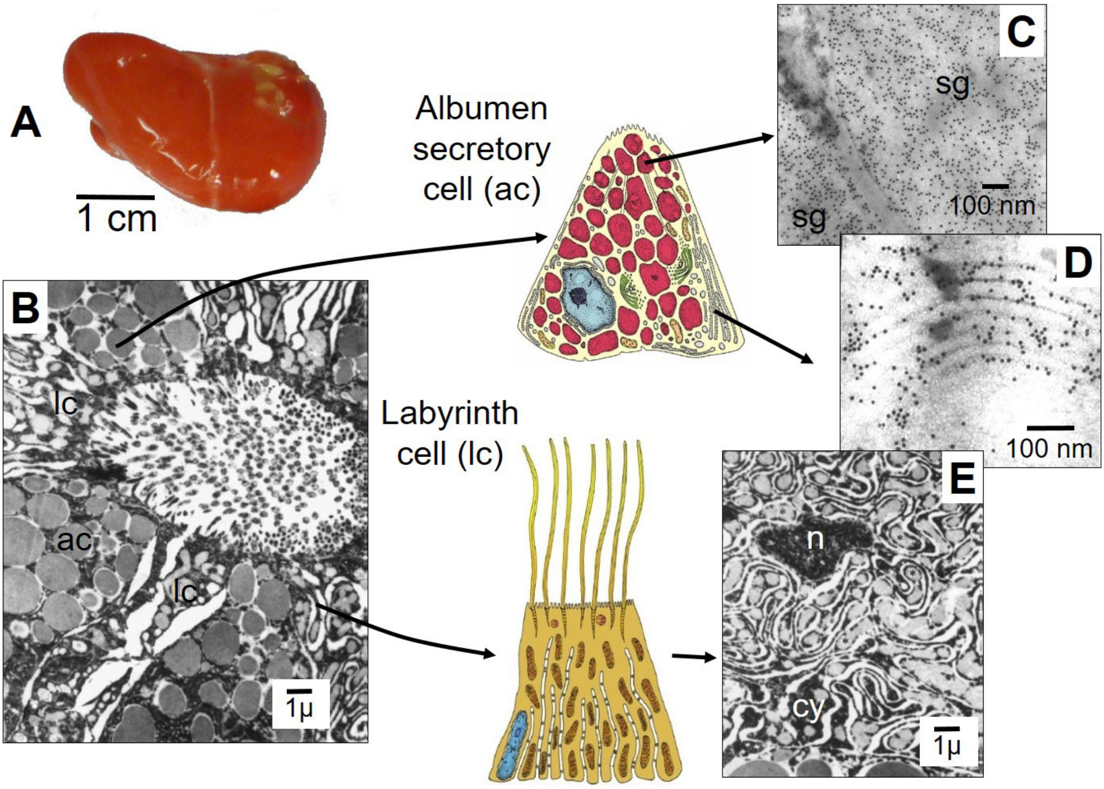

Fig. 7. Synthesis and storage of perivitellins in the albumen gland. A: the albumen gland has a bright and conspicuous colour given by pigmented perivitellins. B: acinus of the parenchymal mass formed by albumen secretory cells (ac) interspersed with labyrinth cells (lc). C: immunocytochemistry of an albumen secretory cell at the electron-microscope level – secretory granules (sg) clearly showing they store large amounts of PcOvo particles, as revealed by an antibody against PcOvo (dots). D: PcOvo is synthesized in the albumen secretory cell, as revealed by its presence in the rough endoplasmic reticulum of an albumen secretory cell immunolabelled with an antibody against PcOvo. E: Labyrinth cells provide the large amount of calcium found in the eggs; note the electron-dense calcium deposits that infiltrate the whole cytoplasmic matrix (cy) and the completely calcified nucleus (n); asterisks indicate mitochondria. From Catalan *et al*. (2006) and Dreon (2003). (Albumen gland photo: M.P. Cadierno)

Structure

Both the perivitellins of *P. maculata* and *P. scalaris*, PmPV1 and PsSC, respectively, are high molecular weight glycoproteins, composed of multiple subunits.

PmPV1 is the most abundant perivitellin in *P. maculata*, representing more than 50 % of the PVF protein, and is the principal perivitellin providing the reddish-pink colour to the eggs. It is a high molecular weight glycolipo-carotenoprotein (294 kDa) composed of five different isoelectric point (pI) isoform subunits each with mass ~30 kDa. It is highly glycosylated (13 % w/w), while the lipid component is less than 1 %.

PsSC has a molecular weight of 380 kDa, and is also composed of subunits with masses around 30 kDa, and with pIs between pH 5.0 and pH 9.0. The carbohydrate content is higher than that of PcOvo and PmPV1, 21 % w/w, and has interesting compositional features, like the presence of xylose and sialic acid. As in PcOvo, these attached sugars generate glycoforms; the multiple subunits normally observed by electrophoresis are due to the presence of glycans attached to a few different polypeptides (Figs. 2B, D) (Ituarte *et al*., 2010).

The main cofactors of both PmPV1 and PsSC are carotenoids, which give these proteins a characteristic colour. The carotenoid fraction of PmPV1 is composed, as in PcOvo, of non-esterified astaxanthin and astaxanthin monoesters and diesters. PsSC carotenoids are also mostly composed of free astaxanthin, but instead of astaxanthin esters, an unidentified carotenoid was present. These cofactors are not essential for quaternary structure stabilisation in either PmPV1 or PsSC; this is another similarity with PcOvo, and an important difference from other invertebrate carotenoproteins. PsSC also carries phosphate groups attached to serine residues; these groups may represent a phosphorous reserve for the embryo, as has been reported for other egg proteins (Ituarte *et al*., 2010).

Only N-terminal sequences are known for the PsSC subunits and they are very similar to the N-terminal sequences of PcOvo. No matches with other known proteins were found, probably because of the lack of gastropod egg-protein sequences in the databases (Ituarte *et al*., 2012).

Regarding structural characteristics, PsSC is a moderately thermally stable protein, showing no important structural alterations up to 60 °C and fully unfolding only at 90 °C. It is also stable over a wide pH range (pH 2.0 to pH 10.0), denaturing only in extremely alkaline conditions (pH 12.5). PsSC is resistant to sequential enzymatic proteolysis by pepsin and trypsin (Ituarte *et al*., 2012). These structural features of PsSC are also characteristic of PcOvo, and mark a difference between these pigmented perivitellins from *Pomacea* and other invertebrate carotenoproteins (Zagalsky *et al*., 1990).

Functions

Apart from its obvious role in providing nutritive molecules to the embryo, other functional aspects of PmPV1 have not yet been studied. As in PcOvo, astaxanthin cofactors are extremely labile in solution, but become protected from degradation when bound to PsSC; thus the perivitellin may be acting as a carotenoid carrier (Ituarte *et al.*, 2008). In this way pigmented perivitellins play a dual role giving pigmentation to the egg and later supplying the embryo with antioxidant molecules (Ituarte *et al.*, 2008).

In addition, PsSC showed the capacity to agglutinate red blood cells from different species, especially rabbits and humans (A and B groups). Haemagglutination is due to the capacity of the protein to recognise and bind plasma membrane carbohydrates; specificity was high towards glucosamine, galactosamine and N-acetyl galactosamine. Although, as mentioned, PsSC is structurally stable in a wide pH and temperature range, lectin activity is more sensitive: the protein was active only between pH 4.0 and pH 8.0 and at temperatures below 60 °C (Ituarte *et al.*, 2012). The presence of as yet unidentified factors that agglutinate rabbit and human erythrocytes has been reported in egg extracts of other ampullariid snails, namely *P. canaliculata* and *Pila ovata*, and in the albumen gland extracts of *Pomacea urceus* (Uhlenbruck *et al.*, 1973; Baldo & Uhlenbruck, 1974). Given the structural stability of PsSC over a wide pH range, especially at acidic pH values, and its protease resistance, it seems plausible that this perivitellin may be involved in an antinutritive defence of the eggs, like PcOvo, and as has been reported for plant lectins (Peumans & Van Damme, 1995); this remains to be explored.

The studies on PsSC and PmPV1 have shown that *Pomacea* species with aerial egg laying strategies have perivitellins with similar structural characteristics that are very different from other invertebrate carotenoproteins. Studies on perivitellins of other ampullariid species, particularly those that lay their eggs under water, will reveal if the acquisition of these peculiar proteins is exclusive to aerial eggs.

Ecological and evolutionary implications

The shift to aerial oviposition was probably a key event in ampullariid evolution, since the derived taxa are the most speciose and widely distributed (Hayes *et al.*, 2009). Adults have developed noteworthy anatomical and physiological adaptations to leave the water during oviposition (Hayes *et al.*, 2015). Similarly, the evolutionary selective pressure exerted on apple snail eggs by predators and by the harsh environment probably led to the acquisition of new features in their storage proteins. Data suggest that they have been co-opted into new functions, notably in embryo defences against predation. It appears that at the biochemical level, these adaptations involve a novel group of perivitellins, which in addition to being storage proteins, are multifunctional complexes constituting a suite of very efficient defences against predation and harsh environmental conditions. The presence of proteinase inhibitors/storage proteins that limit the nutritional quality of eggs as a means of defence has not been reported in the animal kingdom, but it is similar to plant defences against herbivory.

Among predator avoidance tactics, conspicuous colour advertises antipredator defence across many taxa. In this regard, *P. canaliculata* is unusual in that PcOvo provides not only the warning signal molecule but also participates in the biochemical defence. This is the only defence model reported so far that involves no trade-offs between conspicuousness and noxiousness by encoding into the same molecule both the aposematic warning signal and an antinutritive/antidigestive defence. In addition, this makes synthesis even more cost-effective because females do not need to ingest toxic prey to endow their eggs with chemical defences. Furthermore, as well as performing these multiple defence roles, PcOvo is a storage protein that is consumed at a later time by developing embryos and hatchlings (Heras *et al*., 1998). On the whole, *P. canaliculata* egg defences appear to be a solution to allocation costs, opening new perspectives on the study of aposematism and mimicry.

The perivitellin PcPV2 is also unusual among animal toxins, as a lectin-pore-forming combination has not been reported in other species, providing the first evidence of a neurotoxic lectin in animals, and a novel function for ancient and widely distributed proteins (Dreon *et al*., 2013). In fact, the combination of two unrelated immune polypeptides resulted in a novel protein with neurotoxic properties, a feature differing from the roles classically ascribed to either animal lectins (Vasta & Ahmed, 2008) or perforins (Rosado *et al*., 2008). The combination of a lectin united with a toxic subunit by a disulphide bridge has only been reported in plant defences against herbivory, for example in ricin seeds (type II ribosome-inactivating protein) and in the bacterial attack neurotoxin of *Chlostridium botulinum* ("BoTox") (Fig. 8). Comparative analyses of the evolutionary origin of the PcPV2 subunits indicate that both chains evolved separately (Dreon *et al*., 2013).

It is worth recalling that eggs and seeds are static targets and, therefore, particularly vulnerable. In this regard, it is interesting that apple snail eggs and plant seeds may both have developed (passive) biochemical defence systems to protect their embryos as an adaptation to predation, including the preferential accumulation of toxic lectins (Peumans & Van Damme, 1995). When considering the evolution of defences, it is important to remember that something effective against one set of predators may be ineffectual against others. *Pomacea canaliculata* eggs are an exception because there is only one confirmed predator worldwide. Is seems that multiple egg defences acting simultaneously would impair the acquisition of nutrients and be toxic to the predator, rendering *P. canaliculata* eggs unusually well defended.

Considering that conspicuously coloured aerial eggs are very frequent across the Ampullariidae, biochemical defences similar to those of *P. canaliculata* are probably more widespread, though more comparative work is needed to test this hypothesis.

Apple snail eggs provide an exceptional model to study the evolution of biochemical and physiological adaptations, which may have profound implications for addressing

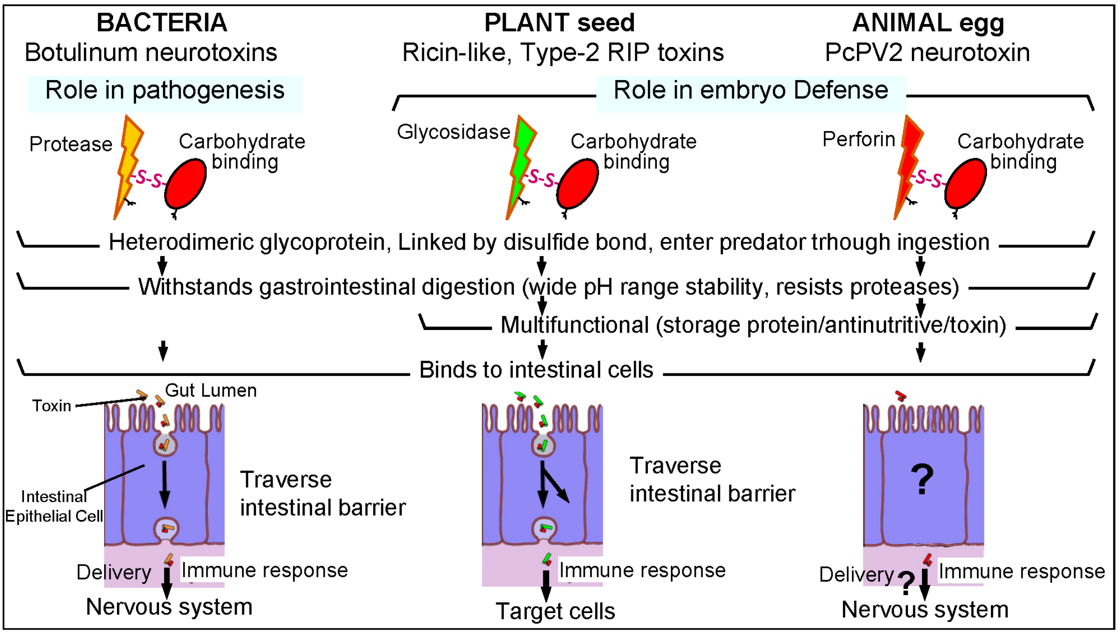

Fig. 8. Similarities and differences in structure and function of the apple snail PcPV2 neurotoxin and related dichain toxic lectins from bacteria and plants. The PcPV2 toxin is composed of a combination unique in animals: a pore-forming protein (MACPF) strongly attached by disulfide bonds to a sugar-binding protein (lectin). The structures of these so called AB toxins have only been observed in the botulinum neurotoxin and in ricin-like plant seed toxins. These three toxins also share a similar mode of entry into the predator body after ingestion, as they all withstand the harsh gastrointestinal environment, bind to intestinal cells and traverse the intestinal barrier to enter general circulation. Also, similar to seed toxins, PcPV2 is not only a defensive protein but also a storage protein with antinutritive properties. Question marks indicate unknown steps. Data from Dreon *et al.* (2013).

References

Baldo, B.A. & Uhlenbruck, G. 1974. Studies on the agglutinin specificities and blood group O(H)-like activities in extracts from the molluscs *Pomacea paludosa* and *Pomacea urceus*. *Vox Sanguinis* 27: 67-80.

Catalán, M., Dreon, M.S., Heras, H., Pollero, R.J., Fernández, S.N. & Winik, B. 2006. Pallial oviduct of *Pomacea canaliculata* (Gastropoda): ultrastructural studies of the parenchymal cellular types involved in the metabolism of perivitellins. *Cell and Tissue Research* 324: 523-533.

Cheesman, D.F. 1958. Ovorubin, a chromoprotein from the eggs of the gastropod mollusc *Pomacea canaliculata*. *Proceedings of the Royal Society of London, B Biological Sciences* 149: 571-587.

Christeller, J.T. 2005. Evolutionary mechanisms acting on proteinase inhibitor variability. *FEBS Journal* 272: 5710-5722.

Dreon, M.S. 2003. *Origin and Characterization of Gastropod Lipovitellins*. PhD thesis. FCE Universidad Nacional de La Plata, Argentina.

Dreon, M.S., Lavarías, S., Garín, C.F., Heras, H. & Pollero, R.J. 2002. Synthesis, distribution, and levels of an egg lipoprotein from the apple snail *Pomacea canaliculata* (Mollusca: Gastropoda). *Journal of Experimental Zoology* 292: 323-330.

Dreon, M.S., Heras, H. & Pollero, R.J. 2003. Metabolism of ovorubin, the major egg lipoprotein from the apple snail. *Molecular and Cellular Biochemistry* 243: 9-14.

Dreon, M.S., Heras, H. & Pollero, R.J. 2004a. Characterization of the major egg glycolipoproteins from the perivitellin fluid of the apple snail *Pomacea canaliculata*. *Molecular Reproduction and Development* 68: 359-364.

Dreon, M.S., Schinella, G., Heras, H. & Pollero, R.J. 2004b. Antioxidant defense system in the apple snail eggs, the role of ovorubin. *Archives of Biochemistry and Biophysics* 422: 1-8.

Dreon, M.S., Heras, H. & Pollero, R.J. 2006. Biochemical composition, tissue origin and functional properties of egg perivitellins from *Pomacea canaliculata*. *Biocell* 30: 359-365.

Dreon, M.S., Ceolín, M. & Heras, H. 2007. Astaxanthin binding and structural stability of the apple snail carotenoprotein ovorubin. *Archives of Biochemistry and Biophysics* 460: 107-112.

Dreon, M.S., Ituarte, S., Ceolín, M. & Heras, H. 2008. Global shape and pH stability of ovorubin, an oligomeric protein from the eggs of *Pomacea canaliculata*. *FEBS Journal* 275: 4530.

Dreon, M.S., Ituarte, S. & Heras, H. 2010. The role of the proteinase inhibitor ovorubin in apple snail eggs resembles plant embryo defense against predation. *PLoS One* 5: e15059.

Dreon, M.S., Frassa, M.V., Ceolin, M., Ituarte, S., Qiu, J.W., Sun, J., Fernandez, P.E. & Heras, H. 2013. Novel animal defenses against predation: a snail egg neurotoxin combining lectin and pore-forming chains that resembles plant defense and bacteria attack toxins. *PLoS One* 8: e63782.

Fernández, P.E., Frassa, M.V., Gimeno, E., Dreon, M.S. & Heras, H. 2011. Changes in carbohydrate expression in the cervical spinal cord of mice intoxicated with perivitellin PV2 from *Pomacea canaliculata*. In: *Poisoning by Plants, Mycotoxins and Related Toxins* (Riet-Correa, F., Pfister, J., Schild, A.L. & Wierenga, T., ed.), p. 482-498. CABI, Wallingford, UK.

Frassa, M.V., Ceolín, M., Dreon, M.S. & Heras, H. 2010. Structure and stability of the neurotoxin PV2 from the eggs of the apple snail *Pomacea canaliculata*. *Biochimica et Biophysica Acta* 1804: 1492-1499.

Garín, C.F., Heras, H. & Pollero, R.J. 1996. Lipoproteins of the egg perivitellin fluid of *Pomacea canaliculata* snails (Mollusca: Gastropoda). *Journal of Experimental Zoology* 276: 307-314.

Hayes, K.A., Cowie, R.H., Jørgensen, A., Schultheiß, R., Albrecht, C. & Thiengo, S.C. 2009. Molluscan models in evolutionary biology: apple snails (Gastropoda: Ampullariidae) as a system for addressing fundamental questions. *American Malacological Bulletin* 27: 47-58.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Heras, H. & Pollero, R.J. 2002. Lipoproteins from plasma and perivitellin fluid of the apple snail *Pomacea canaliculata*. *Biocell* 26: 111-118.

Heras, H., Garín, C.F. & Pollero, R.J. 1998. Biochemical composition and energy sources during embryo development and in early juveniles of the snail *Pomacea canaliculata* (Mollusca: Gastropoda). *Journal of Experimental Zoology* 280: 375-383.

Heras, H., Dreon, M.S., Ituarte, S. & Pollero, R.J. 2007. Egg carotenoproteins in neotropical Ampullariidae (Gastropoda: Arquitaenioglossa). *Comparative Biochemistry and Physiology C* 146: 158-167.

Heras, H., Frassa, M.V., Fernández, P.E., Galosi, C.M., Gimeno, E.J. & Dreon, M.S. 2008. First egg protein with a neurotoxic effect on mice. *Toxicon* 52: 481-488.

Ituarte, S., Dreon, M.S., Pollero, R.J. & Heras, H. 2008. Isolation and partial characterization of a new lipo-glyco-carotenoprotein from *Pomacea scalaris* (Gastropoda: Ampullariidae). *Molecular Reproduction and Development* 75: 1441-1448.

Ituarte, S., Dreon, M.S., Pasquevich, M.Y., Fernández, P.E. & Heras, H. 2010. Carbohydrates and glycoforms of the major egg perivitellins from *Pomacea* apple snails (Architaenioglossa: Ampullariidae). *Comparative Biochemistry and Physiology B* 157: 66-72.

Ituarte, S., Dreon, M.S., Ceolin, M. & Heras, H. 2012 Agglutinating activity and structural characterization of scalarin, the major egg protein of the snail *Pomacea scalaris* (d'Orbigny, 1832). *PLoS ONE* 7: e50115.

Jong-Brink, M., Boer, H.H. & Joosse, J. 1983. Mollusca. In: *Reproductive Biology of Invertebrates* (Adiyodi, K.G. & Adiyodi, R.G., ed.), p. 297-355. John Wiley & Sons, New York.

Norden, D.A. 1972. The inhibition of trypsin and some other proteases by ovorubin, a protein from the eggs of *Pomacea canaliculata*. *Comparative Biochemistry and Physiology B* 42: 569-576.

Peumans, W.J. & Van Damme, E.J.M. 1995. Lectins as plant defense proteins. *Plant Physiology* 109: 347-352.

Przeslawski, R. 2004. A review of the effects of environmental stress on embryonic development within intertidal gastropod egg masses. *Molluscan Research* 24: 43-63.

Przeslawski, R., Davis, A.R. & Benkendorff, K. 2004. Effects of ultraviolet radiation and visible light on the development of encapsulated molluscan embryos. *Marine Ecology Progress Series* 268: 151-160.

Rosado, C.J., Kondos, S., Bull, T.E., Kuiper, M.J., Law, R.H., Buckle, A.M., Voskoboinik, I., Bird, P.I., Trapani, J.A., Whisstock, J.C. & Dunstone, M.A. 2008. The MACPF/CDC family of pore-forming toxins. *Cellular Microbiology* 10: 1765-1774.

Snyder, N.F.R. & Kale, H.W. 1983. Mollusk predation by snail kites in Colombia. *The Auk* 100: 93-97.

Sun, J., Zhang, H., Wang, H., Heras, H., Dreon, M.S., Ituarte, S., Ravasi, T., Qian, P.Y. & Qiu, J.W. 2012. First proteome of the egg perivitelline fluid of a freshwater gastropod with aerial oviposition. *Journal of Proteome Research* 11: 4240-4248.

Sykes, P.W., Jr. 1987. The feeding habits of the snail kite in Florida, USA. *Colonial Waterbirds* 10: 84-92.

Uhlenbruck, G., Steinhausen, G. & Cheesman, D.F. 1973. An incomplete anti B agglutinin in the eggs of the prosobranch snail *Pila ovata*. *Experientia* 29: 1139-1140.

Vasta, G.R. & Ahmed, H. 2008. *Animal Lectins: a Functional View*. CRC Press, Boca Raton, Florida.

Wallace, R.A., Walker, S.L. & Hauschka, P.V. 1967. Crustacean lipovitellin: isolation and characterization of the major high-density lipoprotein from eggs of decapods. *Biochemistry* 6: 1582-1590.

Yusa, Y. 2001. Predation on eggs of the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) by the fire ant *Solenopsis geminata*. *Journal of Molluscan Studies* 67: 275-279.

Yusa, Y., Sugiura, N. & Ichinose, K. 2000. Predation on the apple snail, *Pomacea canaliculata* (Ampullariidae), by the Norway rat, *Rattus norvegicus*, in the field. *Veliger* 43: 349-353.

Zagalsky, P.F., Eliopoulos, E.E. & Findlay, J.B. 1990. The architecture of invertebrate carotenoproteins. *Comparative Biochemistry and Physiology B* 97: 1-18.

Response to abiotic stress in *Pomacea canaliculata* with emphasis on cold tolerance

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Abstract

Pomacea apple snails have invaded temperate East Asia as well as tropical Southeast Asia. Cold winter temperatures undoubtedly limit the distribution of *P. canaliculata* in temperate regions. Snowfall might also prevent hibernation. However, *P. canaliculata* enhances its cold tolerance before the onset of winter. Winter snails were approximately nine times more cold-tolerant than summer snails, based on the time to 50 % mortality at 0 ^{o}C. Gradually decreasing temperatures in autumn and increasingly dry conditions are the environmental cues for enhancement. Physiologically, cold-tolerant snails in winter accumulate glycerol in their bodies, while glycogen levels decrease. Cold tolerance is linked with desiccation tolerance: cold tolerant snails survive longer after desiccation exposure than cold intolerant snails. After winter, warm temperatures together with irrigation prior to rice planting remove the cold tolerance of overwintered snails in temperate paddy fields. When paddy fields are drained before harvest, the snails bury themselves into the soil or move under rice straw. This behaviour is effective for avoiding cold and desiccation. It is apparent that *P. canaliculata* cannot colonize temperate paddy fields without physiological enhancement of cold tolerance together with behavioural avoidance. *Pomacea canaliculata* is more tolerate of cold and desiccation than is the other important invasive apple snail, *P. maculata*, such that *P. canaliculata* may be more adapted to ephemeral Asian paddy fields.

Additional keywords: Ampullariidae, desiccation, hibernation, invasive species, *Pomacea maculata*, Mollusca, salinity, temperature

Introduction

Pomacea canaliculata and *P. maculata* (junior synonym *P. insularum*) are widely distributed invasive species originating in South America. The former is listed as among 100 of the world's worst invasive species (Lowe *et al*., 2000). Abiotic environmental stresses (e.g. climate) are major factors determining success of their colonization of new regions. For tropical and subtropical species, cold weather in winter undoubtedly limits colonization and range expansion in temperate regions. Although the natural distribution of *P. canaliculata* extends further south (to cooler regions) than any other South American *Pomacea* species (Seuffert *et al*., 2010), expansion of its range in east Asia has been restricted by cold winter weather (Ito, 2002; Yoshida *et al*., 2009, 2013). Here, we focus primarily on cold tolerance in *P. canaliculata*, the mechanism of which has been well studied in Japan (Wada & Matsukura, 2007, 2011; Matsukura & Wada, 2007; Matsukura *et al*., 2008, 2009a, b), but also touch on studies dealing with other abiotic stresses.

Seasonal adaptation to cold in *Pomacea canaliculata*

Pomacea canaliculata was introduced to Japan in the early 1980s (Mochida, 1991; Wada, 2004). Snails in paddy fields in temperate Japan increase their cold tolerance before the onset of winter (Fig. 1; Wada & Matsukura, 2007; Matsukura & Wada, 2007).

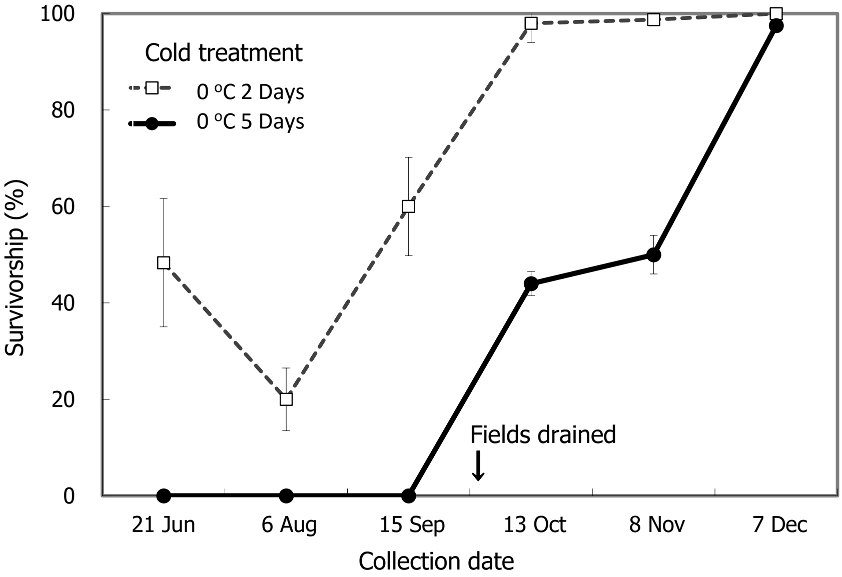

Fig. 1. Seasonal fluctuation of cold tolerance (survival when exposed to low temperatures) in *P. canaliculata* juveniles collected from Japanese paddy fields (submerged and drained) at different times of year.

This increase is not great, compared to other examples of cold adaptation in many insects and some molluscs (Lee, 1991; Ansart & Vernon, 2003), but is nonetheless important. Assessing survival at 0 °C for five days is a good approach to detect changes in cold tolerance. No snails collected from submerged paddy fields in summer survived this treatment, while most snails overwintering in drained fields survived. Snails overwintering in waterways also enhance cold tolerance in winter. Winter snails were approximately nine times more cold-tolerant than summer snails, based on the time (survival days) to 50 % mortality at 0 °C (Wada & Matsukura, 2007). Generally, juveniles of intermediate size (10-20 mm) are more cold tolerant than smaller juveniles (< 10 mm) or adults (Syobu *et al*., 2001; Wada & Matsukura, 2007).

Not only snails from temperate Japan but also snails from the tropical Philippines that had never experienced cold temperatures through many generations over the three decades since their introduction responded to cold acclimation by enhancing cold tolerance (Wada & Matsukura, 2011). Since snails collected from northern Argentina showed the same response (Yoshida *et al*., 2014), adaptation to cold temperatures in *P. canaliculata* is a trait originating in snails in South America rather than a trait evolved after introduction into temperate Asia (Seuffert *et al*., 2010; Wada & Matsukura, 2011).

Mechanism of cold tolerance

Gradually decreasing temperatures in autumn is the main environmental cue to enhance cold tolerance (Matsukura & Wada, 2007). Dry conditions (depriving the snails of water) enhances cold tolerance to some extent even at 25 °C. Photoperiod shows no clear effect on cold tolerance.

After winter, temperature and water availability lead to decreasing cold tolerance of overwintered snails (Matsukura *et al*., 2009b). Under aquatic conditions, cold tolerance broke down within four days at 25 °C and eight days at 20 °C. However, snails held at 15 °C retained their cold tolerance more than 64 days. Snails kept under dry conditions maintained their cold tolerance for at least 64 days even at 25 °C. These results indicate that warm temperatures together with irrigation before rice planting lead to loss of cold tolerance of overwintered snails in these temperate paddy fields.

Physiologically, cold-tolerant snails in winter accumulate glycerol and possibly glucose in their bodies, while glycogen concentration decreases (Matsukura *et al*., 2008). Among low molecular-weight compounds besides glycerol and glucose, increases in glutamine and carnosine and a decrease in phenylalanine were observed. It is generally considered that in insects and other organisms concentrations of low molecular-weight compounds such as polyols and sugars increase with advancement of cold tolerance in winter (Lee, 1991; Storey & Storey, 1997). In molluscs, such increases have been reported in only a few instances: the intertidal snail *Melampus bidentatus*, the intertidal bivalve *Mytilus edulis* (Loomis, 1985; Loomis *et al*., 1988) and the land snail *Helix pomatia* (Nowakowska *et al*., 2006). In general, low molecular-weight compounds decrease supercooling points, acting as cryoprotectants (Lee, 1991; Storey & Storey, 1997). Other possible functions related to enhancement of cold hardiness are to prevent protein denaturation by hydrogen-bonding, to prevent membrane damage by inhibition of lipid phase transition (Michaud *et al*., 2008; Sjursen & Somme, 2000) and to reduce cuticular water loss by binding water (Williams & Lee, 2008). But no difference in supercooling points was observed in *P. canaliculata* between snails with and without cold tolerance (Matsukura *et al*., 2009a), possibly because the glycerol concentration in cold tolerant *P. canaliculata* is much lower than in many insects (Lee, 1991; Matsukura *et al*., 2008). *Pomacea canaliculata* cannot tolerate freezing (Matsukura *et al*., 2009a). But this fact seems ecologically unimportant because death occurred experimentally and in fields at much milder temperatures (0-10 ^{o}C) than that at which the snails freeze (ca. -7 ^{o}C) (Wada & Matsukura, 2007; Matsukura & Wada, 2007; Matsukura *et al*., 2009a). Even in other organisms, mechanisms of lethal injury by chilling (mild low temperatures) in the absence of freezing have been rarely understood as compared to freezing tolerance. Thus, the actual function of glycerol in *P. canaliculata* is unknown.

At the histological level, the mantle is the most susceptible among various organs examined to sustain injury from cold temperatures (Matsukura *et al*., 2009a). In cold-tolerant snails, decrease of glycogen and increase of glucose in the kidney, increase of glucose in the mantle and increase of glycerol in foot muscle and digestive glands were prominent.

Hibernation in new Asian habitats and the significance of enhancing cold tolerance

Pomacea canaliculata has invaded both temperate East Asia and tropical Southeast Asia. Its main habitat in these regions is ephemeral paddy fields, where submerged and dry conditions cycle with rice cultivation and intervening fallow periods. In more temperate regions the snails hibernate during winter, adopting at least two tactics. One is the physiological enhancement of cold tolerance described above. The other is behavioural avoidance of cold and desiccating conditions. When paddy fields are drained before harvest, snails bury into the soil or after harvest move under rice straw. Temperatures under straw are much milder than at the exposed soil surface. In an experiment carried out in Kyushu (southern Japan), temperatures under straw were almost always above 0 °C, when soil surface temperatures frequently went down below -5 °C (Fig. 2; Wada & Matsukura, 2007). However, many snails fail to bury and these snails, even with physiological cold tolerance, are killed during winter (Kiyota & Sogawa, 1996). Adult snails often cannot bury entirely because of their large size. Due to this imperfect behaviour together with size dependent cold tolerance and the higher possibility of shell breakage caused by tillage, there are very few adult survivors after winter in temperate paddy fields. Therefore, the great majority of snails that appear at the first irrigation of the fields are juveniles of intermediate size (10-22 mm shell height) (Wada *et al*., 2004). Mortalities during winter are usually very high (85 %, Watanabe *et al*., 2000; 92 %, Wada & Matsukura, 2007; 91-99 %, Yoshida *et al*., 2009), indicating that *P. canaliculata* could not colonize these temperate paddy fields without physiological cold enhancement combined with behavioural avoidance.

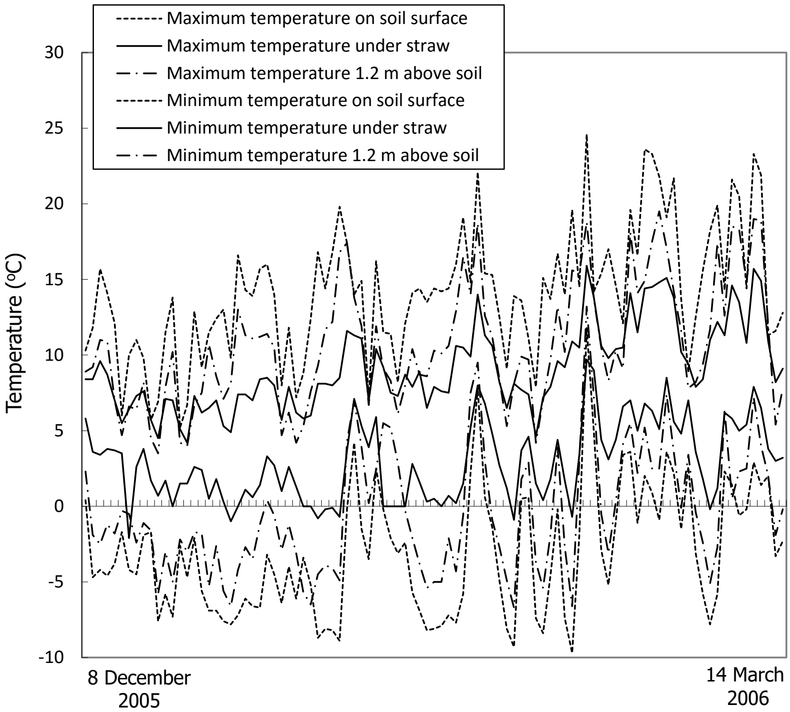

Fig. 2. Daily maximum and minimum temperatures measured at three sites in paddy fields (on the soil surface, under rice straw and 1.2 m above soil surface) during the 2005-2006 winter in Kyushu, Japan.

Influence of temperature on growth and activity

In *P. canaliculata* growth and activity including feeding and crawling are entirely suppressed below 10 ^{o}C (Kaneshima *et al*., 1987; Seuffert *et al*., 2010). Juvenile developmental time (1/juvenile period) increases linearly between 15 and 30 ^{o}C, while growth is reduced at 35 ^{o}C compared to 30 ^{o}C (Kaneshima *et al*., 1987). Activity also declines above 30 ^{o}C and the snails fall into heat coma at 36.2 ^{o}C (Seuffert *et al*., 2010). The lower temperature thresholds and cumulative heat requirement for embryonic development ranged between 16 and 18 ^{o}C and between 89 and 134 day-degrees, respectively (Seuffert *et al*., 2012). At 0 ^{o}C 50 % mortality occurred in 1.3 days for summer snails and 11.9 days for winter snails (Wada & Matsukura, 2007). At -3 ^{o}C about 50 % of snails died in 2 days. The supercooling point of the snails was between -6.6 and -7.1 ^{o}C (Matsukura *et al*., 2009a). Nonetheless, incubation for long periods at temperatures of 0-10 ^{o}C is fatal (Matsukura & Wada, 2008, Wada unpublished data) and thus such temperatures are ecologically much more important than the value of the supercooling point. Furthermore, the cumulative number of day-degrees below 10 ^{o}C (CNDDb10) during winter is sometimes used to assess overwintering success (Ozawa & Akino, 1988; Yoshida *et al*., 2009). Heat shock proteins, which may work to reduce temperature stress, have been studied by Zheng *et al*. (2012) and Giraud-Billoud *et al*. (2013). Although the literature is limited, lethal temperatures in other ampullariids were summarized by Cowie (2002).

Some recent implications for hibernation and distributions

In temperate Asia cold weather limits *P. canaliculata* distribution (Ito, 2002; Yoshida *et al*., 2009, 2013). However, mere mean winter temperatures or CNDDb10 do not always explain actual distributions. In central-southern Japan *P. canaliculata* inhabits paddy fields in the areas facing the Seto Inland Sea (Sanyo and southern Kinki Districts; Fig. 3) where the mean temperatures in the coldest months generally ranges from 2 to 6 ^{o}C (Fig. 4). The areas with the same winter mean temperatures are widely distributed in the regions facing the Sea of Japan (Sanin and Hokuriku Districts; Fig. 4). However, *P. canaliculata* does not occur in these areas except at a location (Togo town) having hot-springs (Fig. 3; Wada, 2015). In addition, several examples of the disappearance of *P. canaliculata* following colonization have been recorded in Sanin and Hokuriku. There

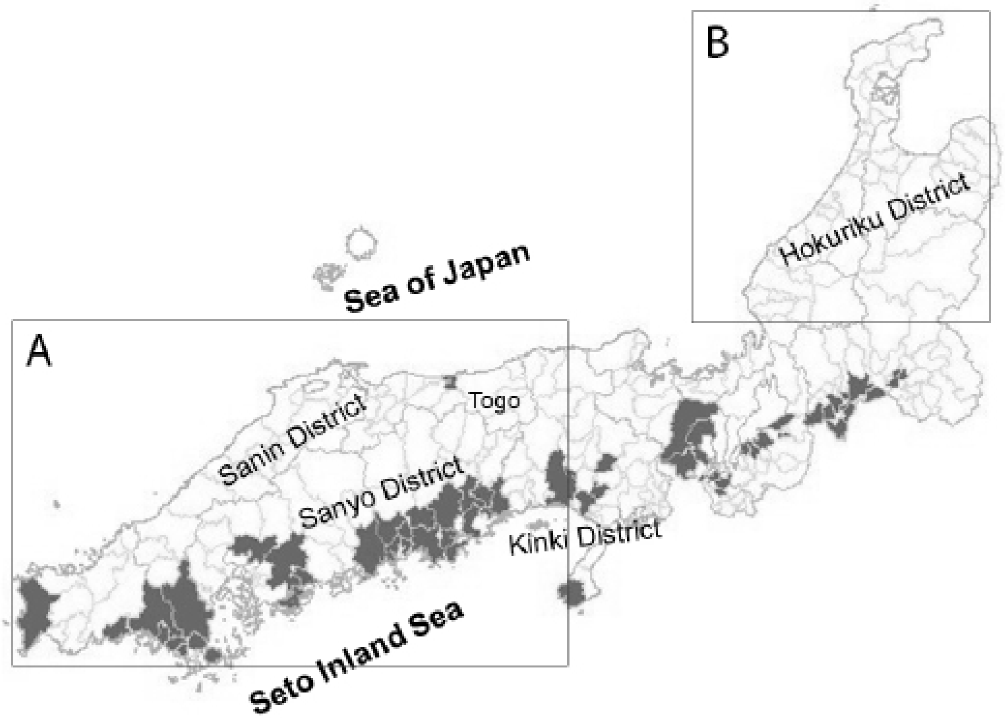

Fig. 3. County-based distribution of *P. canaliculata* in selected prefectures in central-southern Japan. The counties coloured dark are those where *P. canaliculata* was found in paddy fields. Areas A and B correspond to the areas on the map in Fig. 4. The distribution map was drawn using data from surveys conducted by Prefectural Plant Protection Offices mainly in 2011 (modified from Wada, 2015).

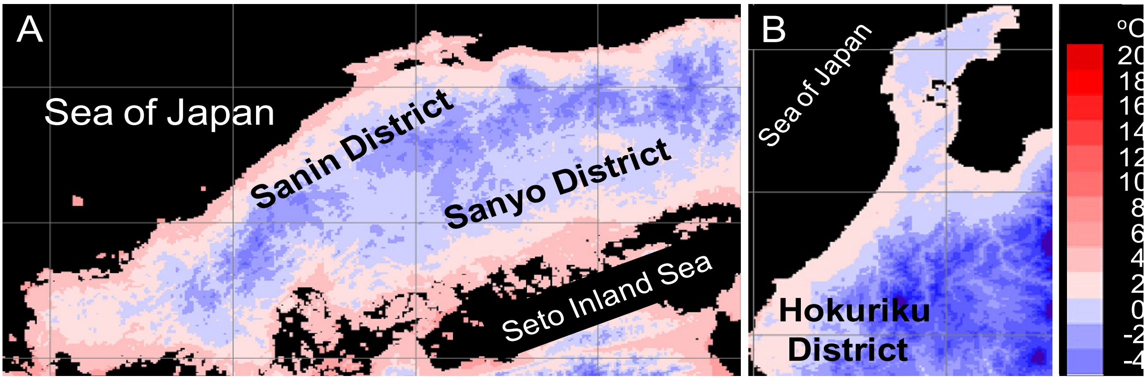

Fig. 4. Mean temperatures in the coldest month (January) in central-southern Japan: A, Sanin and Sanyo Districts; B, Hokuriku District. The map was drawn using the software Mesh 2012 (Japan Meteorological Business Support Center, Chiyoda-Ku, Tokyo) (modified from Wada, 2015).

is a marked difference in climate during winter between the two areas facing the Sea of Japan and the Seto Inland Sea: Sanin and Hokuriku have a snowy, rainy and cloudy climate, while sunny days with large diurnal temperature ranges often continue in Sanyo and southern Kinki into winter. Snow cover keeps the soil surface at approximately 0 ^{o}C for long periods (Fukui, 1977). Continuous low temperatures close to zero are critical for the snails. All hibernating snails died experimentally at 0 ^{o}C for 20 days (Wada & Matsukura, 2007). In addition, snails under conditions of small diurnal temperature fluctuations died after far fewer CNDDb10 than snails under highly fluctuating temperature conditions (Wada, unpublished data). Thus, a climate with frequent snowfall and temperatures with small diurnal range are suspected to prevent snails from overwintering in Sanin and Hokuriku even though the winter mean temperatures seems to be appropriate for overwintering.

Occurrence of hybrids between *P. canaliculata* and *P. maculata* was demonstrated based on analysis of the nuclear EF1*α* DNA marker by Matsukura *et al*. (2013). *Pomacea canaliculata* is more cold tolerant than *P. maculata* (Yoshida *et al*., 2013; Matsukura *et al*., 2016), and therefore *P. canaliculata* occurs in both tropical and temperate Southeast and East Asia, whereas *Pomacea maculata* is confined to tropical and subtropical regions. Hybrids, however, inhabit both tropical and temperate regions but do not reach the northern parts of the *P. canaliculata* distribution. Hybrids produced experimentally through back-crossing of F1 to *P. canaliculata*, exhibited intermediate cold tolerance between the two species. This phenomenon helps to explain the current distribution of the three types of *Pomacea* snails in East and Southeast Asia. It is possible that *P. maculata* genes expanded northwards through hybridization (Matsukura *et al*., 2016).

Response to other abiotic stresses

Desiccation

Many ampullariids bury themselves and enter aestivation when their habitats dry up (Cowie, 2002). Desiccation tolerance therefore seems important for the snails' ability to survive such conditions. Field collected *P. canaliculata* survived 11 months in dry soil in a laboratory experiment (Yusa *et al*., 2006a). In dry conditions but with occasional moistening (a shower two or three times a month) they survived up to 29 months. Juveniles of intermediate size (ca. 15 mm) were more tolerant than smaller juveniles (ca. 8 mm) and adults (ca. 30 mm). These results are relevant to the time it will take for total snail mortality to be achieved following adoption of an upland, non-irrigated crop, as a control tactic (Wada *et al*., 2004).

Cold-acclimated laboratory-reared juveniles (ca. 11 mm) exhibited ca. 20 % survival 17 weeks after the start of a desiccation treatment at 25 °C and 25-55 % relative humidity (Wada & Matsukura, 2011). Moreover, linkage of cold tolerance and desiccation tolerance is found in the progenies of both temperate and tropical snails: cold-tolerant snails survive longer after desiccation exposure than cold intolerant snails. There are often similarities in mechanisms underlying adaptation to cold temperatures and desiccation; notably, accumulation of metabolites including polyols and sugars, adjustments of water content and modification of habitat preferences (Ring & Danks, 1994; Michaud *et al*., 2008). *Pomacea canaliculata* was more tolerate to desiccation than *P. maculata*, suggesting that *P. canaliculata* is more adapted to ephemeral Asian paddy fields (Yoshida *et al*., 2014).

Survival of *Pomacea paludosa* in relation to seasonal desiccation in Florida wetlands has been investigated and relevant aspects of water management have been discussed by Darby *et al*. (2003, 2008).

Salinity

Although *P. canaliculata* does not occur in salty and very alkaline environments and inhabits shallow quite turbid sites with a low $Na^+/(K^++Mg^{++})$ ratio in its native Argentina (Martín *et al*., 2001), the snails seems to be somewhat tolerant of salinity. Kijima *et al*. (1988) investigated survival of small juveniles in a range of salinities. Although tolerance was different among strains, some mortality occurred in 0.2 % salt water and the majority were dead in 0.4 % (but mortality was very low for one strain). Cowie (2002) suggested *P. canaliculata* was sufficiently tolerant of sea water to survive long enough to spread to nearby river systems. But in northern Peninsular Malaysia, sea water irrigation of paddy fields was successfully adopted to eradicate newly invaded snails (Yahaya *et al*., 2006).

Conclusions

Pomacea canaliculata accumulates glycerol in its body and enhances cold tolerance before the onset of winter. When paddy fields are drained, the snails bury themselves in the soil or move under rice straw, where they hibernate in a much buffered environment. The capability of physiologically increasing cold tolerance together with behavioural avoidance of cold and desiccation are major factors enabling this species to expand its range into temperate East Asia. *Pomacea canaliculata* is more tolerate to cold and desiccation than *P. maculata*, which may indicate that *P. canaliculata* is better adapted to ephemeral Asian paddy fields, the major habitat of these invasive apple snails. A major constraint on spread and abundance in tropical regions seems to be drought, but there are still few studies dealing with desiccation stress. Biotic factors are also important even in the newly invaded habitats. Numerous predators of *P. canaliculata* are known in Japan and some may effectively regulate local populations (Yusa *et al*., 2006b; Yamanishi *et al*., 2012). Biotic factors as well as abiotic factors should be considered when success or failure in colonization by invasive organisms is discussed.

References

Ansart, A. & Vernon, P. 2003. Cold hardiness in molluscs. *Acta Oecologica* 24: 95-102.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192, CAB International, Wallingford, UK.

Darby, P.C., Valentine-Darby, P.L. & Percival, H.F. 2003. Dry season survival in a Florida apple snail (*Pomacea paludosa* Say) population. *Malacologia* 45: 179-184.

Darby, P.C., R.E. Bennetts & H.F. Percival. 2008. Dry down impacts on apple snail (*Pomacea paludosa*) demography: implications for wetland water management. *Wetlands* 28: 204-214.

Fukui, E. 1977. *Developments in Atmospheric Science 8: Climate of Japan*. Kodansha, Tokyo, 317 p.

Giraud-Billoud, M., Vega, I.A., Tosi, M.E.R., Abud, M.A., Calderón, M.L. & Castro-Vazquez, A. 2013. Antioxidant and molecular chaperone defences during estivation and arousal in the South American apple snail *Pomacea canaliculata. Journal of Experimental Biology* 216: 614-622.

Ito, K. 2002. Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan. *Applied Entomology and Zoology* 37: 655-661.

Kaneshima, M., Yamauchi, S. & Kurozumi, T. 1987. Effect of rearing temperature and density upon the growth of apple snail, *Pomacea canaliculata* (Lamarck). *Proceedings of the Association for Plant Protection of Kyushu* 33: 110-112. [in Japanese]

Kijima, A., Umeda, H. & Fujio, Y. 1988. Strain difference in apple snail under saline water conditions. *Suisan Ikushu* [*Fish Genetics and Breeding Science*] 13: 35-38. [in Japanese]

Kiyota, H. & Sogawa, K. 1996. Ecology and management of the apple snail in Kyushu, Japan. In: *Proceeding of the international workshop on pest management strategies in Asian monsoon agroecosystems* (Hokyou, N. & Norton, G., ed.), p. 187-195. Kyushu National Agricultural Experiment Station, Kumamoto.

Lee, R.E., Jr. 1991. Principles of insect low temperature tolerance. In: *Insects at Low Temperature* (Lee, R.E., Jr. & Denlinger, D.L., ed.), p. 131-148. Chapman & Hall, New York.

Loomis, S.H. 1985. Seasonal changes in the freezing tolerance of the intertidal pulmonate gastropod *Melampus bidentatus* Say. *Canadian Journal of Zoology* 63: 211-228.

Loomis, S.H., Carpenter, J.F. & Crowe, J.H. 1988. Identification of strombine and taurine as cryoprotectants in the intertidal bivalve *Mytilus edulis*. *Biochimica et Biophysica Acta* 943: 113-118.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species*. IUCN Invasive Species Specialist Group, Auckland. 12 p.

Martín, P.R., Estebenet, A.L. & Cazzaniga, N.J. 2001. Factors affecting the distribution of *Pomacea canaliculata* (Gastropoda: Ampullariidae) along its southernmost natural limit. *Malacologia* 43: 13-23.

Matsukura, K. & Wada, T. 2007. Environmental factors affecting the increase of cold hardiness in the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Applied Entomology and Zoology* 42: 533-539.

Matsukura, K., Tsumuki, H., Izumi, Y. & Wada, T. 2008. Changes in chemical components in the freshwater apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in relation to the development of its cold hardiness. *Cryobiology* 56: 131-137.

Matsukura, K., Tsumuki, H., Izumi, Y. & Wada, T. 2009a. Physiological response to low temperature in the freshwater apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Journal of Experimental Biology* 212: 2558-2563.

Matsukura, K., Tsumuki, H., Izumi, Y. & Wada, T. 2009b. Temperature and water availability affect decrease of cold hardiness in the apple snail, *Pomacea canaliculata*. *Malacologia* 51: 263-269.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading

East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Matsukura K, Izumi, Y., Yoshida, K. & Wada, T. 2016. Cold tolerance of invasive freshwater snails, *Pomacea canaliculata*, *P. maculata*, and their hybrids helps explain their different distributions. *Freshwater Biology* 61: 80-87.

Michaud, M.R., Benoit, J.B., Lopes-Martinez, G., Elnitsky, M.A., Lee, R.E., Jr. Denlinger, D.L. 2008. Metabolomics reveals unique and shared metabolic changes in response to heat shock, freezing and desiccation in the Antarctic midge, *Belgica antarctica*. *Journal of Insect Physiology* 54: 645-655.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-62.

Nowakowska, A., Caputa, M. & Rogalska, J. 2006. Seasonal changes in cryoprotectants concentrations in *Helix pomatia* snails. *Journal of Physiology and Pharmacology* 57 Supplement 8: 93-105.

Ozawa, A. & Makino, T. 1988. Overwintering of the apple snail, *Pomacea canaliculata* (Lamarck), in Shizuoka prefecture. *Bulletin of Shizuoka Agricultural Experiment Station* 33: 65-77. [in Japanese]

Ring, R.A. & Danks, H.V. 1994. Desiccation and cryoprotection - overlapping adaptations. *Cryoletters* 15: 181-190.

Seuffert, M.E., Burela S. & Martín, P.R. 2010. Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *Journal of Thermal Biology* 35: 77-84.

Seuffert, M.E., Saveanu, L. & Martín, P.R. 2012. Threshold temperatures and degree-day estimates for embyonic development of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Malacologia* 55: 209-217. Sjursen, H. & Somme, L. 2000. Seasonal changes in tolerance to cold and desiccation in *Phauloppia* sp. (Acari, Oribatida) from Finse, Norway. *Journal of Insect Physiology* 40: 1387-1396.

Storey, Y.K. & Storey, J. 1997. To freeze or not to freeze. *The Biochemist* 19: 8-13.

Syobu, S., Mikuriya, H., Yamaguchi, J., Matsuzaki, M., Zen, S. & Wada, T. 2001. Estimating the overwintering mortality of the apple snail, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) in a paddy field of southern Japan using temperature data. *Japanese Journal of Applied Entomology and Zoology* 45: 203−207. [in Japanese with English summary]

Wada, T. 2004. Strategies for controlling the apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) in Japanese direct-sown paddy fields. *Japan Agricultural Research Quarterly* 38: 75-80.

Wada T. 2015. Distribution and chemical control of the apple snail, *Pomacea canaliculata*, in Japanese paddy fields. *Shokubutsu Boeki* 69: 155-159 (in Japanese).

Wada, T. & Matsukura, K. 2007. Seasonal changes in cold hardiness of the invasive freshwater apple snail, *Pomacea canaliculata* (Lamarck). *Malacologia* 49: 383-392.

Wada, T. & Matsukura, K. 2011. Linkage of cold hardiness with desiccation tolerance in the invasive freshwater apple snail, *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Journal of Molluscan Studies* 77: 149-153.

Wada, T., Ichinose, K., Yusa, Y. & Sugiura, N. 2004. Decrease in density of the apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) in paddy fields after crop rotation with soybean, and its population growth during the crop season. *Applied Entomology and Zoology* 39: 367-372.

Watanabe, T., Tanaka, K., Higuchi, H., Miyamoto, K., Kiyonaga, T., Kiyota, H., Suzuki, Y. & Wada, T. 2000. Emergence of the apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), after irrigation in a paddy. *Applied Entomology and Zoology* 35: 75-79.

Williams, J.B. & Lee, R.E., Jr. 2008. Differences in cold tolerance, desiccation resistance, and cryoprotectant production between three populations of *Eurosta solidaginis* collected from different latitudes. *Journal of Comparative Physiology B* 178: 365-375.

Yahaya, H., Nordin, M., Hisham, M.N.M. & Sivapragasam, A. 2006. Golden apple snail in Malaysia. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 215-230. Philippine Rice Research Institute, Nueva Ecija.

Yamanishi, Y., Yoshida, K., Fujimori, N. & Yusa, Y. 2012. Predator-driven biotic resistance and propagule pressure regulate the invasive apple snail *Pomacea canaliculata* in Japan. *Biological Invasions* 14: 1343-1352.

Yoshida, K., Hoshikawa, K., Wada, T. & Yusa, Y. 2009. Life cycle of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) inhabiting Japanese paddy fields. *Applied Entomology and Zoology* 44: 465-474.

Yoshida K., Hoshikawa, K., Wada, T. & Yusa, Y. 2013. Patterns of density dependence in growth, reproduction and survival in the invasive freshwater snail *Pomacea canaliculata* in Japanese rice fields. *Freshwater Biology* 58: 2065-2073

Yoshida K., Matsukura, K., Cazzaniga, N.J. & Wada, T. 2014. Tolerance to low temperature and desiccation in two invasive apple snails, *Pomacea canaliculata* and *P. maculata* (Caenogastropoda: Ampullariidae), collected in their original distribution area (northern and central Argentina). *Journal of Molluscan Studies* 80: 62-66.

Yusa, Y., Wada, T. & Takahashi, S. 2006a. Effects of dormant duration, body size, self-burial and water condition on the long-term survival of the apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Applied Entomology and Zoology* 41: 627-632.

Yusa, Y., Sugiura, N. & Wada, T. 2006b. Predatory potential of freshwater animals on an invasive agricultural pest, the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) in southern Japan. *Biological Invasions* 8: 137-147.

Zheng, G., Dong, S., Hou, Y., Yang, K. & Yu, X. 2012. Molecular characteristics of HSC70 gene and its expression in the golden apple snails, *Pomacea canaliculata* (Mollusca: Gastropoda). *Aquaculture* 358-359: 41-49.

Accumulation of copper and other elements by the apple snail *Pomacea canaliculata*

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Abstract

Heavy metal pollution is now prevalent in almost all aquatic ecosystems and will eventually affect human health. There is, then, a need to monitor the presence of these heavy metals. Studies have shown that *Pomacea canaliculata* is a potential biomonitor of heavy metals in freshwater ecosystems because of its ability to bioaccumulate a wide array of elements and because it is a better accumulator than some of the other organisms considered. Studies of bioaccumulation by *P. canaliculata* are reviewed.

Additional keywords: Ampullariidae, *Filopaludina martensi*, fresh water, heavy metals, *Ipomoea aquatica*, Mollusca, *Potamogeton crispus*, sediment

Introduction

The increasing accumulation of heavy metals in the environment, especially in aquatic ecosystems, needs monitoring and this calls for a monitoring tool. Several studies have been done to assess the apple snail *Pomacea canaliculata* as a metal biomonitor in freshwater ecosystems. *Pomacea canaliculata* is an agricultural pest that has continued to spread despite numerous attempts to eliminate it or prevent its spread. Since it cannot be eliminated, is found in almost any freshwater ecosystem in many countries, is big enough to provide sufficient material (soft tissue) for analyses and because it is easy to handle, easy to collect, easy to culture, long lived, numerous, sedentary and can survive for a long time without food, it has the potential to be used widely as a heavy metal biomonitor. This contribution reviews studies of *Pomacea canaliculata* that have been done to assess its bioaccumulation of heavy metals.

Review of studies of bioaccumulation by *Pomacea*

A preliminary study was undertaken on the accumulation of heavy metals by *Pomacea canaliculata* and a bivalve (possibly *Corbicula fluminea*) in the Naga City River, Philippines (Peña, unpublished). Sediment, bivalve and apple snail samples were taken from the most contaminated part of the river. The river bed is a recipient of road run off, domestic sewage and effluents from markets and other commercial and industrial establishments of the city. The first samples were taken in May 1996 and analysed for heavy metal content using flame atomic absorption spectrophotometry (Table 1). Cadmium (Cd) in the sediment was below the detection limit of the instrument but accumulated by the snail and the bivalve from the sediment. Chromium (Cr) was not detected in snail and bivalve samples and lead (Pb) was not detected in the bivalve. Snail soft tissue contained much higher values of copper (Cu) and zinc (Zn) than the sediment. The concentrations of cobalt (Co), iron (Fe), manganese (Mn), nickel (Ni) and Pb in the snail samples were much lower than in the sediment. The metal concentrations in the bivalve were much lower than in the sediment except for Cu and Cd, and all metal concentrations were much lower than those in the snail. Thus, Cd, Cu and Zn are

Table 1. Heavy metal concentrations in sediment, water, apple snails and bivalves in the Naga City River, Philippines, 1996 and 2004.

	Heavy metal concentration mg/kg					
	Sediment		**Water**	**Snail**		**Bivalve**
	1996	2004	2004	1996	2004	1996
Cadmium (Cd)	*	*	*	0.798	*	0.304
Cobalt (Co)	47.7	–	–	14.5	–	1.83
Chromium (Cr)	60.8	–	–	*	–	*
Copper (Cu)	33.2	141	*	373	1050	40.0
Iron (Fe)	114,000	–	–	9460	–	1080
Lead (Pb)	54.7	–	–	7.04		*
Manganese (Mn)	1360	–	–	520	–	85.7
Nickel (Ni)	29.2	–	–	9.48	–	5.67
Zinc (Zn)	55.6	250	0.028	1450	580	0.669

accumulated by the snail from the river sediment better than the other metals, and the snail is a better accumulator of heavy metals than the bivalve.

As a follow up, samples of water, sediment and apple snails were taken from the same river and the same site in September 2004. They were analysed for the presence of Cd, Cu and Zn, the metals better accumulated by the snails in the 1996 samples (Table 1). The concentrations of Cu and Cd in the water were below the detection limit of the instrument. Cd was also not detected in the sediment and snails. The concentration of Zn in the water was far below that in the sediment but the concentration in the snail tissue was more than twice that in the sediment. Cu concentration in the snails was seven times higher than that in the sediment. The concentrations of Cu and Zn were much higher in 2004 than 1996 in the sediment and snails but the proportional difference between the levels in the snails and the sediment for these elements was higher in 1996 (Table 1). These results confirm that the metal concentration in sediment is much higher than that in the water and that Cu and Zn are bioaccumulated by the snails. The results also show that there is a continued supply of Cu and Zn into the river but that the accumulation pattern of the snails differed between 1996 and 2004. There could be changes in the conditions of the river that caused the lower uptake of the metal by the snails but the level in the snails is still higher than that in the sediment. These preliminary results indicated that *Pomacea canaliculata* could be used as a biomonitor especially for Cd, Cu and Zn. Although other metals were detected in the snails, it is a particularly strong net accumulator of Cd, Cu and Zn.

Following the preliminary study a further study was undertaken focussing on accumulation of Cu by *Pomacea canaliculata* (Peña & Pocsidio, 2008). Cu and Zn are essential elements, micronutrients, but become toxic above certain threshold levels;

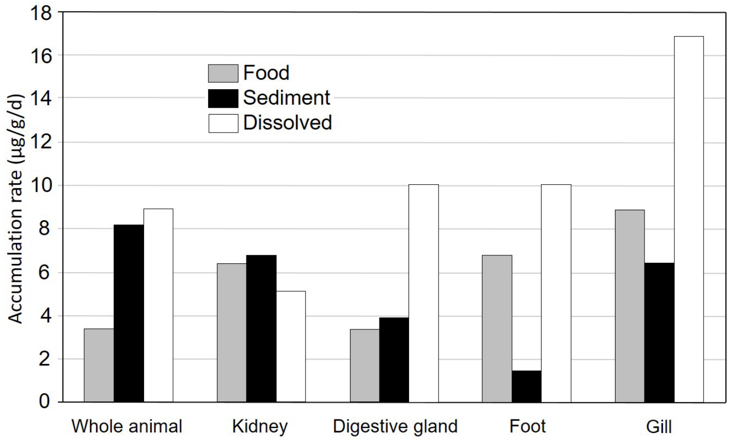

Fig. 1. Rate of copper accumulation above basal level from food (previously soaked in 67.5 µg/L Cu solution), sediment (from the Naga City River) and from water (Merck titrisol Cu (1000 mg/L) added to a final concentration of 67.5 µg/L) by the different organs of *Pomacea canaliculata* (from Peña & Pocsidio, 2008).

however, Cu is much more toxic than Zn. Three-month old apple snails were exposed for seven days to five Cu concentrations: 20, 30, 45, 67.5 and 101.25 µg/L. The experiment was done under natural photoperiod (13:11 h light:dark) at pH 6.0-6.5, 7-8 ppm dissolved oxygen and temperature of 28-32^o C. Behavioural responses (closing of operculum, respiratory pumping, mucus secretion) were only seen at 67.5 and 101.25 µg Cu/L. Cu was accumulated by the snails at all concentrations, with the control having the lowest levels (15.47 ± 3.29 µg/g dry weight) and those exposed to 45 µg/L having the highest levels (90.98 ± 15.21). Levels in the 45 and 67.5 µg/L treatments were significantly greater than in the control at the 5% significance level (ANOVA followed by Tukey's multiple comparison test). The reduction of net accumulation at high copper concentrations could be explained by behavioural responses of the snails. At 67.5 µg/L, if they could still remain upright, they still opened the operculum but showed increased respiratory pumping and produced more mucus, which might reduce net copper accumulation. At 101.25 µg/L the snails never opened the operculum until they became flaccid, signalling the lethality of the solution, although copper was still accumulated in their tissues despite the closed operculum. When exposed to sediment from the Naga City River (Table 1), the snails had an accumulation rate of 8.97 µg Cu/g/day and an assimilation efficiency of 89%, indicating a high capacity to accumulate and assimilate copper from food and the surrounding environment in just a seven-day exposure. Whole soft tissue copper accumulation from dissolved Cu, sediment and food differed significantly from those of the controls. Cu uptake from the sediment and dissolved Cu do not differ significantly but they are significantly higher ($p < 0.05$) than that from the food (Fig. 1). There were no significant differences ($p > 0.05$) in total Cu accumulation (from dissolved Cu, sediment and food) among the snails' organs (kidney, digestive system, foot, gills), though the highest value of accumulated Cu was in the gill (Fig. 1). Gills absorbed by far the most dissolved Cu from the water (Fig. 1). The gill is the route of exposure for dissolved Cu. *Pomacea canaliculata* is thus a good Cu accumulator at sublethal concentrations and could be used as a biomarker at high concentrations.

Dummee *et al*. (2012) studied accumulation of heavy metals by a species of *Pomacea* in Thailand (although identified as *Pomacea canaliculata*, it could have been *P. maculata*, as both are present in Thailand and many authors have not distinguished them). They studied seasonal changes in the concentrations of Cu, Mn, Fe, Zn, Pb and Cd in water, sediment, *Pomacea* and the aquatic plant *Ipomoea aquatica* in three tributaries of Beung Boraphet reservoir, Nakhon Sawan province, central Thailand. Fe, Cu, Mn and Zn were detected in all sediment samples (both in wet and dry seasons) using a flame atomic absorption spectrophotometer. Cd and Pb were below detection limits. Metal concentrations in sediment were in the order Fe > Mn > Cu > Zn. Fe, Mn, Cu and Zn were found in *Pomacea* tissues and the Mn concentration was higher than that in the sediment, especially in the wet season (33 times higher). The bioconcentration factors (BCF) in snails were in the order Mn > Zn > Cu > Fe and in the plant were Mn > Zn > Fe > Cu. Both *Pomacea* and *Ipomoea aquatica* have more Mn in their tissues than in the sediment. Mn levels were positively correlated between sediment and snails ($R = 0.959$), sediment and plants ($R = 0.891$) and snails and plants ($R = 0.793$). Positive correlations were also found between Zn ($R = 0.926$) and Cu ($R = 0.910$) concentrations in snails and those in the sediment (Pearson correlation, $P < 0.01$ in all cases). At one station Mn and Zn concentrations were higher in the dry season while Cu concentration was higher in the wet season. Fe did not show significant seasonal variation. In a laboratory experiment in which three-month old snails were exposed to contaminated sediment for two months, Fe and Mn concentrations were significantly higher in the digestive gland ($P < 0.05$) than in other organs studied but there were no significant differences in Cu and Zn levels among organs ($P > 0.05$).

Mn accumulation by *Pomacea* in the study of Dumee *et al*. (2012) is not in agreement with the result of Peña (2004) who found Mn levels in the snails to be lower than in the sediment. However, both studies showed that Cu accumulated more in digestive gland than in foot muscle and that there was no statistically significant difference in Cu concentrations among the organs studied. They also both showed that Fe concentration in snail tissue was lower than in the sediment and that Zn concentration in the snails was higher than the Cu concentration (in Peña’s 1996 study but not her 2004 study).

Kruatrachue *et al*. (2011) investigated accumulation of heavy metals by a species of *Pomacea* they identified as *P. canaliculata* (this also could have been *P. maculata*) in the Mae Klong River (Samut Songkhram Province), which is one of the largest and most important rivers in Thailand. Sediments were taken from the river and its tributaries and analysed for Cd, Cr, Cu, Fe, Pb, Ni and Zn content using mass spectrometry. A laboratory experiment exposed the snails to these sediments for three months in water at 25 ± 2 °C, pH 6.8-7.3 and dissolved O_2 7.5-7.8 mg/L. Heavy metals in digestive gland, digestive tract and gill were determined using a graphite atomic absorption spectrometer for Cd, Cr, Pb and Ni and a flame atomic absorption spectrophotometer for Cu, Fe and Zn. Concentrations in sediments were ranked as follows: Fe > Zn > Pb > Cr > Cu > Ni > Cd. The highest concentrations did not exceed the probable-effects levels of the US Environmental Protection Agency (1997). No snails died after three months exposure.

Tissue-specific analyses showed that Zn, Cr and Fe were stored mainly in the digestive gland. Levels of Cu did not differ significantly among organs (digestive tract, digestive gland, gill), as reported by Peña & Pocsidio (2008) and Dummee *et al*. (2012), although the gill showed the highest concentration, as also reported by Peña & Pocsidio (2008), and Fe levels being higher in the digestive gland agrees with the results of Dummee *et al*. (2012). Levels of some Cu and Zn were much greater in snails than in sediments (19 and 39 times respectively), as also found in Peña's 2004 study, above, indicating that they were more available to snails than the other metals in these studies, and exceeded the standard limits for consumption (20 µg/g for Cu, 100 µg/g for Zn; Ministry of Public Health, Thailand) and may therefore be of concern.

Sumritdee (2007) also studied metal accumulation by *Pomacea* in the Mae Klong River tributaries, providing data on levels in the sediment, snails from the tributaries and snails exposed experimentally to the sediment for 3 months (Table 2). Snails from the river exhibited the highest accumulation of Ni, Fe, Zn and Cd in the digestive gland, while kidney had the highest accumulation of Cu, Pb and Cr. Experimental snails had the highest levels of Cr, Zn and Fe in the digestive gland, the highest level of Ni in the gills, of Cu in the kidney and of Pb in the digestive tract. Cd was found in gill, digestive gland and digestive tract. That the highest level of Cu was found in the kidney, both in the wild and experimental snails, agrees with the result of Peña & Pocsidio (2008; Fig. 1). Kruatrachue *et al*. (2011) did not analyse kidney and found the Cu level to be highest in

Table 2. Heavy metal concentrations in sediment and apple snails from the Mae Klong River tributaries, Thailand (range of values), and apple snails exposed experimentally to the river sediment (mean values) (from Sumritdee, 2007).

	Heavy metal concentration mg/kg							
	Sediment	**Snails from river**			**Experimental snails**			
		Digestive gland	Kidney	Gill	Digestive gland	Digestive tract	Kidney	Gill
Cadmium (Cd)	0.02-0.83	0.01-0.33	0.00-0.13	0.00-0.03	0.01	<0.005	0.01	0.01
Chromium (Cr)	2.90-14.3	0.02-0.80	0.00-6.25	0.00-0.05	1.15	<0.005	<0.005	<0.005
Copper (Cu)	1.72-57.5	6.00-176	0.00-781	35.3-224	44.87	70.93	96.49	94.27
Iron (Fe)	2,466-11,906	2,315-12,417	0.00-1,795	0.00-533	2,439	1,101	1,235	345
Lead (Pb)	3.53-18.9	0.05-3.86	0.00-55.8	0.00-36.2	0.09	0.15	<0.005	0.01
Nickel (Ni)	1.42-11.6	0.12-0.88	0.00-0.22	0.02-1.54	0.08	0.08	0.00	0.18
Zinc (Zn)	7.23-271	171-2,800	19.8-75.6	19.5-54.4	1657	1,657	10.57	<0.005

the gill. Both Kruatrachue *et al*. (2011) and Sumritdee (2007), in the experimental snails, found higher concentrations of Fe, Zn and Cr in the digestive gland and Ni in the gills. However, the snails from the wild in the study of Sumritdee (2007) concentrated more Ni and Cd as well as Fe and Zn, but less Cr in the digestive gland. The highest level of Fe being in the digestive gland (Sumritdee 2007) agrees with the study of Dummee *et al*. (2012).

A study in Argentina (Vega *et al*. 2012) on the differential ability of apple snail tissues, endosymbionts and eggs to bioaccumulate Zn, Cr, Fe, as well as antimony (Sb), arsenic (As), barium (Ba), bromine (Br), mercury (Hg), selenium (Se) and uranium (U) exposed *Pomacea canaliculata* from a cultured strain to drinking water and reconstituted water. The highest bioconcentration factors (BCF), in decreasing order, were: in the midgut gland, Ba, Zn, Se, As, U, Br and Hg; in the kidney, Ba, Br and Hg; and in the foot Ba, Hg, Br and Se, with the low levels of Fe and Zn in the foot agreeing with the results of Dummee *et al*. (2012). Calcified tissues (uterus containing eggs, shell) and eggs showed low BCFs except for Ba. Both C corpuscles (putative endosymbionts) and midgut gland tissue showed statistically higher BCFs than K corpuscles (the alternate form of the endosymbionts) for Ba, Fe, U, Br and Sb. The concentration of most of the elements was significantly lower in tissues and endosymbionts obtained from snails cultured in reconstituted water than in drinking water. Snails cultured in reconstituted water and then exposed to Hg, As and U (at the maximum contaminant level allowed by the US Environmental Protection Agency) also accumulated high levels in the midgut gland, endosymbionts and kidney. Vega *et al*. (2012) suggested that the midgut gland (and the symbionts therein), kidney and foot may be useful bioindicators of Hg, As and U pollution in freshwater bodies and that the unrestricted use of ampullariid snails as human and animal food must be considered with caution.

Ruangareerat (2004) undertook experiments on juvenile *Pomacea* sp. in Thailand to assess the bioaccumulation of Hg, Cd and Cu and the possibility of using *Pomacea* sp. as a biomonitor for heavy metal contaminants in freshwater resources. Snails were exposed to a range of concentrations of each of Hg, Cd and Cu for 96 h. Uptake was measured in the whole soft body by spectrophotometry. Hg, Cd and Cu were not detected in the controls, in either the water or the snails. In all treatments, the concentration of Hg, Cd and Cu in the snail tissue differed significantly ($P \leq 0.05$) from the control, and the concentration of the three metals in the snail tissue was higher than in the water to which they were exposed. The higher the metal concentration in the water, the higher it was in the soft tissue. The bioconcentration factors were 7000-10,830 for Hg, 1000-1253 for Cd and 989-4543 for Cu.

Pomacea sp. have also been used in a bioaccumulation study of radionuclides and heavy metals from lakes formed from abandoned mines in Kampung Gajah, Perak, Malaysia (Redwan, 2008). Element concentrations in snail muscle tissue were determined via short and long radiation at Nuclear Malaysia using the nuclear activation analysis method. The short radiation detected vanadium (V, 3.45 ppm), aluminium (Al, 127,367 ppm), manganese (Mn, 174.5 ppm), chlorine (Cl, 0.205 ppm), potassium (K, 5920 ppm) and sodium (Na, 2665 ppm); the long radiation, detected uranium (U, 1.73 ppm), bromine (Br, 0.616 ppm), arsenic (As, 31.23 ppm), ytterbium (Yb, 179.0 ppm), thorium (Th, 2351 ppm), cesium (Cs, 14.08 ppm), scandium (Sc, 1.28 ppm), iron (Fe, 2795 ppm) and samarium (Sm, 228.8 ppm). This study demonstrated the presence of these elements, including radioactive ones, in the snails, and therefore the possibility of their bioaccumulation by the snails.

Another study, in the Niger Delta, Nigeria, on several freshwater snails identified as *Pomacea canaliculata*, *Pomacea bridgesii* and *Lanistes libycus* (the first two species are almost certainly incorrectly identified, as neither has been reported from West Africa previously; it is more likely that they were species of *Pila*; R.H. Cowie, pers. comm.) has been undertaken to investigate heavy metals in the edible portion of the snails (Adebayo-Tayo *et al*., 2011). Concentrations determined using atomic absorption spectrophotometry in the species identified as *Pomacea canaliculata* were: Fe, 21.3-28.6 mg/kg; Zn, 83.2-86.3 mg/kg; Cu, 12.6-15.7 mg/kg; Mg, 0.262-0.293 mg/kg; Mn, 58.6-71.8 mg/kg; Pb, 0.07-0.11 mg/kg; As, 0.04-0.15 mg/kg. Comparing the mean metal accumulation among the species, the species identified as *P. canaliculata* had the highest uptake of Fe, that identified as *P. bridgesii* the highest of Cu, Pb and As, and *L. libycus* the highest of Mg and Mn, although the values did not differ much among them. Concentrations were high except for Pb and As but the majority were within the standard limits prescribed by the Commission of the European Community and FAO. Adebayo-Tayo *et al*. (2011) suggested that these concentrations in the snails could be related to industrial and other human activities in the area, and that the presence of heavy metals, combined with the microbial status of the snails may mean that they are not totally safe for human consumption and may pose a serious health hazard to consumers.

Another study in Thailand was undertaken in Beung Jode reservoir in Ban Gudnamsai, Nampong district, Khon Kaen province (Neeratanaphan & Phalaraksh, 2008). This reservoir receives a considerable amount of effluent from a paper mill factory before flowing into the Pong River. Nongbualamphu and Khon Kaen provinces use water in this river for daily use and agriculture. Concentrations of heavy metals in the water, sediments and snails were determined using an optical emission spectrometer in three consecutive seasons: rainy, cold-dry and hot-dry. The mean sediment concentrations were in the order Zn > Cu > Pb > Cd > Hg in the rainy season, while in cold and hot seasons they were in the order Zn > Pb > Cu >. Zn concentration was highest in the cold season, followed by the hot season and then by the rainy season. Mean concentration of Pb was highest in the hot season followed by the cold and rainy seasons. The concentrations of Cu were higher in the rainy season compared with the hot and cold seasons while Cd was only detected in the rainy season. Hg could not be detected in the sediment but was detected in the snails in the rainy season. Two snail species were analyzed: *Filopaludina martensi* (Viviparidae) and a species identified as *Pomacea canaliculata* (which may be incorrect, see above). The concentrations of the metals in snails were in the order Zn > Cu > Pb in all three seasons. Heavy metal, especially Zn, concentrations in the snails were higher during the rainy season than in the dry seasons. In the rainy season, mean concentrations of Hg and Cd were 0.21 and 0.32 mg/kg respectively, while in the dry seasons neither could be detected. Zn concentration in *Filopaludina martensi* was higher than in *Pomacea canaliculata* but there were no differences between the species for the other metals. The concentrations of metals were higher in the snails than in the sediment, indicating that the snails, which were exposed to effluent from the paper mill, accumulate heavy metals in their tissues.

Losussachan (2006), also in Thailand, used atomic absorption spectrophotometry to assess heavy metal contamination seasonally in the Phi Lok canal system, Phraek Nam Daeng district, Samut Songkhram province, assessing accumulation of Cd, Cu, Fe, Pb and Zn in the water, sediment, *Pomacea* sp. (identified as *P. canaliculata*) and fish. Heavy metal levels were in the order sediment > snail > fish > water and were highest near pig farms and lowest at fish and shrimp raising ponds, but varied according to the particular heavy metal, habitat, feeding behaviour and the animals' desorption mechanism. In no case did heavy metals exceed values for human consumption designated by various health organizations. Seasonally, heavy metal concentrations in water were in the order cold > rainy > hot season, whereas those in sediment and snails were in the order rainy > cold > hot. This seasonality in the snails agrees with the findings of Neeratanaphan & Phalaraksh (2008) but is contrary to those of Peña (2004), who recorded higher Cu and Zn concentrations during the rainy season (September) than the hot season (May). Factors other than season may affect accumulation.

Finally, in southern China Deng *et al*. (2008) assessed *Potamogeton crispus* and a species of *Pomacea* (probably *P. canaliculata*) as potential biomonitors for metal contamination in the sediment. Samples (leaves of pondweed, snails and sediment) were collected in March 2004 from eight sites in Fankou Stream, which receives the wetland-treated effluent from Fankou Pb/Zn mine, and analyzed spectrometrically. There were statistically significant correlations between the concentrations of Pb and Zn in pondweed tissue and sediment. Cu concentration was highest at the site closest to the mine and lowest at the site farthest from the mine, but this trend for Cu was not as obvious as for the other metals, suggesting Cu accumulation by the pondweed was less dependent on Cu level in the sediment. Cd in the pondweed at the site farthest from the mine was much higher than in uncontaminated macrophytes. There was no significant correlation between metals in the snail tissue and those in the sediment, nor between the snails and the pondweed. Much higher levels of Cd, Cu, Pb and Zn were associated with the viscera than the head-foot. This suggests that *P. crispus* possesses several attributes of a biomonitor and its tissue concentrations of Cd, Pb and Zn may reflect the levels of sedimentary contamination by these metals, but that *P. canaliculata*, although it may accumulate metals to high levels, can only serve as an indicator of metal contamination and not of its level because tissue metal concentrations did not correlate with those of the sediment nor of the pondweed.

Conclusion

The studies summarised here show that species of *Pomacea* have potential as metal biomonitors over a wide geographical range. *Pomacea* bioaccumulates a wide range of elements: essential elements and heavy metals as well as radionuclide/radioactive elements. It is a strong (exceeded environmental concentrations) accumulator of Cu, Zn, Cd, Mn, Pb, Fe and Hg (this last at least in the rainy season). The snails accumulate Cu in proportion to the concentrations to which they are exposed, even for a short time (7 days) at sublethal concentrations. However, some results were inconsistent with this, reporting that although the snails accumulate metals to high levels and can serve as indicators of metal contamination, their tissue metal concentrations did not always correlate with those of the sediments or macrophytes.

Thus, the pondweed *Potamogeton crispus* was considered better than *P. canaliculata* as a potential biomonitor because its metal concentrations correlated with those of the sediment (Deng *et al*., 2008). Another aquatic plant, *Ipomea aquatica*, exhibited similar bioconcentration regarding Mn (Dummee *et al*., 2012). *Pomacea* was a better metal accumulator than an unidentified bivalve (Peña, 2004) and fish (Losussachan, 2006). Bioaccumulation varied among species of ampullariid snails but the differences were not great (Adebayo-Tayo *et al*., 2011), and there was little difference between another snail, *Filopaludina martensi*, and *Pomacea* other than that the former exhibited higher Zn concentrations (Neeratanaphan & Phalaraksh, 2008). While some studies analysed the entire snail soft tissue, others used digestive gland, digestive tract, kidney, gill and foot, all of which exhibited bioaccumulation, but to varying degrees.

Bioavailability of heavy metals depends on many factors but for a broad scale assessment *Pomacea* has potential as a biomonitor of heavy metal contamination in freshwater ecosystems.

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References

Adebayo-Tayo, B.K., Onilude, A.A. & Etuk, F.I. 2011. Studies on microbiological, proximate mineral and heavy metal composition of freshwater snails from Niger Delta Creek in Nigeria. *Assumption University Journal of Technology* 14: 290-298.

Deng, P.Y., Shu, W.S., Lan, C.Y. & Liu, W. 2008. Metal contamination in the sediment, pondweed, and snails of a stream receiving effluent from a lead/zinc mine in southern China. *Bulletin of Environmental Contamination and Toxicology* 81: 69-74.

Dummee, V., Kruatrachue, M., Trinachartvanit, W., Tanhan, P., Pokethitiyook, P. & Damrongphol, P. 2012. Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet reservoir, Thailand. *Ecotoxicology and Environmental Safety* 86: 204-212.

Kruatrachue, M., Sumritdee, C., Pokethitiyook, P. & Singhakaew, S. 2011. Histopathological effects of contaminated sediments on golden apple snail (*Pomacea canaliculata*, Lamarck 1822). *Bulletin of Environmental Contamination and Toxicology* 86: 610-614.

Losussachan, W. 2006. Accumulation and distribution of heavy metals in water, sediment, *Pomacea canaliculata* and some fish species in the Phi Lok canal system, Samut Songkhram province. MSc thesis, Mahidol University, Bangkok.

Neeratanaphan, L. & Phalaraksh, C. 2008. Water quality and heavy metals contamination in sediment and edible mollusks at Beung Jode reservoir, Khon Kaen Province. *Khon Kaen University Research Journal* 13: 197-207.

Peña, S. & Pocsidio, G. 2008. Accumulation of copper by golden apple snail *Pomacea canaliculata* Lamarck. *Philippine Journal of Science* 137: 153-158.

Redwan, R.B.M. 2008. Bioaccumulation of radionuclides and heavy metals by *Pomacea canaliculata* (apple snails) in Kampung Gajah District. BSc (Hons.) thesis, Universiti Teknologi MARA, Shah Alam, Malaysia.

Ruangareerat, S. 2004. Comparative toxicity of heavy metal compounds to golden apple snail *Pomacea* sp. Juvenile. MSc thesis, Kasetsart University, Bangkok.

Sumritdee, C. 2007. Ecotoxicological effects of sediments from Mae Klong river tributaries on golden apple snail (*Pomacea canaliculata*, Lamarck 1822). MSc thesis, Mahidol University, Bangkok.

US Environmental Protection Agency. 1997. The incidence and severity of sediment contamination in surface waters of the United States, vol 1-national sediment quality survey. US Environmental Protection Agency Report 823-R-97-006.

Vega, I.A., Arribére, M.A., Almonacid, A.V., Ribeiro Guevara, S. & Castro-Vazquez, A. 2012. Apple snails and their endosymbionts bioconcentrate heavy metals and uranium from contaminated drinking water. *Environmental Science and Pollution Research* 19: 3307-3316.

Invasive apple snails: ecology and management in Hong Kong

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Abstract

Apple snails in the genus *Pomacea* (Ampullariidae) invaded Hong Kong in the early 1980s. It is unknown how many species have been introduced into Hong Kong.
A recent study has shown that only one species (*Pomacea canaliculata*) is present in the New Territories, yet it is not present in Hong Kong Island nor Lantau Island, the two largest islands with streams and abandoned rice paddies. In the New Territories, it is widely distributed in various freshwater habitats (ponds, drainage channels, semi-aquatic vegetable gardens, abandoned rice paddies, rivers and streams). Elsewhere in Asia, *P. canaliculata* is often a pest of rice, but in Hong Kong where most rice paddies have been abandoned, the first negative impact of *P. canaliculata* was reported by farmers who discovered that the snails devoured semi-aquatic vegetables, especially watercress (*Nasturtium officinale*) and water spinach (*Ipomoea aquatica*). In recent years, it has become clear that *P. canaliculata* could have reduced wetland biodiversity by grazing on macrophytes and by predation on benthic invertebrates, and altered wetland function by releasing nutrients into the water. It also has the potential to out-compete local macroinvertebrates because of its high secondary production. Apple snails in vegetable gardens are controlled by hand-picking, supplemented by application of chemicals, especially lime and tea seed cake. Those in constructed wetlands are controlled by hand-picking. A recent field study showed that black carp could be used to control apple snails in constructed wetlands.

Additional keywords: Ampullariidae, biological control, grazing, macrophytes, Mollusca, nutrient, phytoplankton, *Pomacea*, secondary production

Introduction

With the increasing travel and trade associated with rapid economic globalization in recent decades, there has been a dramatic increase in species introductions (Blumenthal, 2006). Whether an introduced species will establish and become invasive is dependent on both the characteristics of the recipient ecosystem, such as its diversity, resource abundance and frequency and scale of disturbances (Davis *et al*., 2000), as well as the biology of the introduced species, including its dietary flexibility, reproductive potential, growth rate and tolerance to adverse environmental conditions (Kolar & Lodge, 2001). Invasive apple snails possess many characteristics of successful invaders. They exhibit high reproductive potential, fast growth rate, high dietary flexibility and strong resistance to a number of environmental conditions including hypoxia, high temperature and desiccation (Cowie, 2002; Estebenet & Martín, 2002; Boland *et al*., 2008).

Pomacea (Ampullariidae) is a large genus of apple snails with more than 100 described species (Cowie & Thiengo, 2003). They are indigenous to freshwater habitats in South and Central America, with one species native to North America. At least three species have been introduced into Asia since the 1980s (Jhang, 1985; Anderson, 1993; Yusa & Wada, 1999; Joshi *et al*., 2001; Chen *et al*., 2004; Cowie *et al*., 2006; Joshi, 2007; Hayes *et al*., 2008, 2012; Lv *et al*., 2013). *Pomacea canaliculata* was the first species introduced to Asia, through importation of egg clutches to establish snail aquaculture in Taiwan in 1980 (Hamada & Matsumoto, 1985; Cha, 1989). In addition to *P. canaliculata*, *P. maculata* and *P. scalaris* were also introduced into Asia (Cowie *et al*., 2006; Hayes *et al*., 2008, 2012, 2015). *Pomacea canaliculata* and/or *P. maculata* was soon introduced to Japan, China and several countries in Southeast Asia including the Philippines, Vietnam, Cambodia and Thailand, often without distinguishing them or under the assumption that they were *P. canaliculata* (e.g. Hamada & Matsumoto, 1985; Cha, 1989; Mochida, 1991; Joshi & Sebastian, 2006). In southern China, *P. canaliculata* and *P. maculata* exhibit a wide and mosaic distribution (Lv *et al*., 2009, 2013). In Taiwan, *P. canaliculata* is widely distributed, but *P. scalaris* is restricted to the southern part (Lee & Wu, 1996; Wu *et al*., 2011). Due to their rapid growth and high protein content, apple snails were initially widely promoted as human food and as a protein supplement in animal feed. However, apple snail aquaculture farms soon stopped operation because of the toughness of apple snail meat, high processing costs and infection with the nematode *Angiostrongylus cantonensis*, a cause of eosinophilic meningitis (Mochida, 1991; Yu *et al*., 2001; Lv *et al*., 2008). Abandoned snails soon established large populations in various freshwater habitats by active crawling and passive transportation by water flow in drainage systems (Mochida, 1991; Yusa & Wada, 1999; Cowie, 2002). In this paper we review the ecology (distribution, secondary production and impacts on wetland flora and fauna and wetland function) and management of apple snails in the agricultural and non-agricultural areas of Hong Kong.

Distribution and identity of apple snails in Hong Kong

Hong Kong is situated on China's south coast, facing the Pearl River estuary in the west and the South China Sea in the east and south (Fig. 1). The territory is small, 1104 km^2 in area. It consists of Hong Kong Island, Kowloon Peninsula, New Territories and over 200 offshore islands of which the largest is Lantau Island. Apple snails were first reported

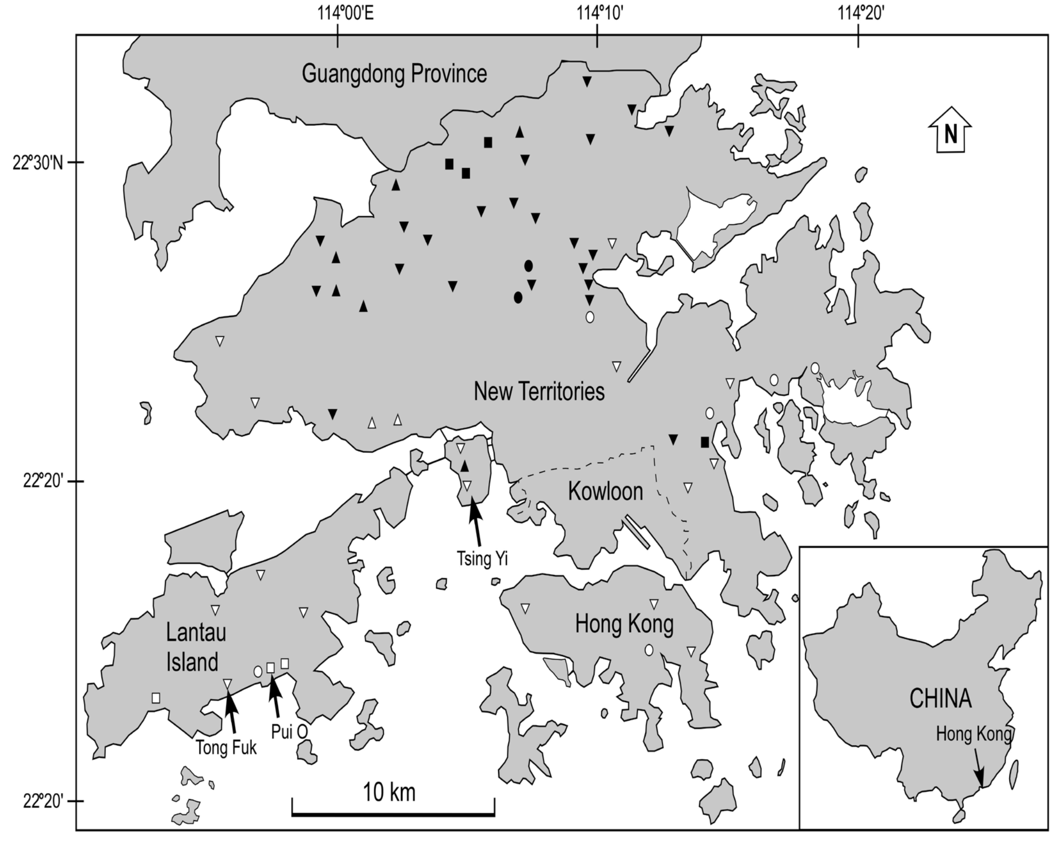

Fig. 1. Distribution of *Pomacea canaliculata* in Hong Kong. Sampling locations/habitat types are shown by different symbols (○ - stream, ▽ - drainage channel, △ - pond, □ - abandoned wet farmland). Filled/open symbols indicate presence/absence of apple snails. Figure is adapted from Kwong *et al*. (2008).

in northern New Territories following a territory-wide survey conducted in 1988 by Yipp *et al*. (1991), who estimated that apple snails were introduced to this agricultural area in many vegetable gardens and abandoned rice paddies in 1980-1983. This estimate was consistent with the results of an earlier territory-wide survey of molluscs conducted in 1980-1981 by Yipp (1983), who did not find apple snails. There is no consensus about the source and invasion pathway for apple snails in Hong Kong. As the first report of apple snails in the agricultural area of the New Territories was very close in timing to the first report of apple snails in Guangdong (Yu *et al*., 2001), and as the agricultural areas of Hong Kong and Shenzhen (southern Guangdong) are connected by a network of streams and drainage channels, it was difficult to determine to which of these two areas apple snails were first introduced.

Within the subsequent two decades of their invasion, apple snails spread to most lowland wetlands in the New Territories including streams, ponds, freshwater marshes, abandoned rice paddies, vegetable gardens and drainage channels. The periodic flooding during the summer rainy season must have helped apple snail dispersal in the lowland wetlands, which are connected through streams and drainage channels. A territory-wide survey of apple snails conducted in 2006 (Kwong *et al*., 2008) revealed the wide distribution of apple snails in the New Territories. The sites inhabited by apple snails typically had high levels of phosphate and alkalinity, but apple snails were also occasionally found in streams where nutrient and alkalinity levels were low. Geographical isolation and a lack of agricultural activities may have halted the spread of apple snails to other areas with apparently suitable habitats in Hong Kong. Specifically, although the water quality of sites visited (i.e. abandoned rice paddies) on Lantau Island were apparently suitable for apple snails, they had not been colonized, probably because of a lack of physical connection with the New Territories. However, the building of a bridge connecting the New Territories and Lantau Island in recent years has greatly enhanced traffic, with development of ecotourism, and there is concern that apple snails will be introduced to Lantau Island during replanting of abandoned rice paddies with agricultural crops, on which apple snail eggs may be transported inadvertently.

There has been some confusion regarding the identity of apple snails introduced into Hong Kong (Kwong *et al*., 2008). Cha (1989) and Yipp *et al*. (1991) identified two species of apple snails as *Ampullarius levior* and *A. gigas* (*Ampullarius* is not a valid name, the correct genus name for these species is *Pomacea*). Although both species were recorded in the northern New Territories, they showed some differences in their distributions (Cha, 1989). Yipp *et al*. (1991) concluded that *P. gigas* preferred habitats with bottom sediment of coarser grain, while *P. levior* was a dominant grazer in finer grain sediments. Lam (1994) identified the apple snails collected from a stream in the New Territories as *P. levior*, whereas Dudgeon & Corlett (2004) identified the species of apple snail widely distributed in Hong Kong as *P. lineata*. To determine more rigorously the identity of apple snails in Hong Kong, selected snails collected from different locations (Kwong *et al*., 2008) showing the greatest morphological variation with respect to size, shell colour and shell shape were sent to Robert Cowie and Kenneth Hayes at the University of Hawaii, who sequenced a segment of the COI gene from 12 individuals and showed that all the snails were *P. canaliculata* (K. Hayes, pers. comm.). Therefore *P. canaliculata* is probably the only species of *Pomacea* currently present in Hong Kong. Based on comparison of shell morphology, the records of *P. levior* by Cha (1989) and Yipp *et al*. (1991) and of *P. levior* by Lam (1994) were probably *P. canaliculata*. It is possible that the records of *P. gigas* by Cha (1989) and Yipp *et al*. (1991) were *P. maculata* (*P. gigas* is now considered a junior synonym of *P. maculata*), which can reach a larger maximum size than *P. canaliculata* (Hayes *et al*., 2012). *Pomacea maculata* has established in some areas of southern China (Lv *et al*., 2013), but if this is the correct identification of the snails recorded in Hong Kong as *P. gigas*, then this species may have become locally extinct in Hong Kong. It is also possible that these snails were simply large individuals of *P. canaliculata* and that *P. maculata* has never been present in Hong Kong.

Secondary production of apple snails

Secondary production is an integration of life-history traits and reflects resource use (Benke & Huryn, 2007), thereby indicating the role a species plays in energy flow and material transfer in an ecosystem (Hall *et al*., 2006). Determining secondary production of apple snails could help elucidate their ecological roles in the recipient ecosystems and offer a good indicator of their resource use and potential impacts on local fauna that may compete for the same food source. To this end, 1-year monthly surveys of *P. canaliculata* populations were conducted in four wetlands representing the diverse wetland habitats in Hong Kong (abandoned paddy, oxbow pond, drainage channel, river meander) (Kwong *et al*., 2010). The mean density of *P. canaliculata* ranged from 26 to 43 individuals m^{-2}

and secondary production (ash-free dry mass [AFDM]) varied from 165.9 to 233.3 g m^{-2} y^{-1} among the four sites. There were considerable seasonal differences in secondary production, with relatively low values during the cool dry northeast monsoon, and high values throughout much (7-10 months) of the year, especially during the warm, wet summer months. Data from the four apple snail populations were compared with published secondary production estimates for macroinvertebrates in Hong Kong, as well as those for freshwater gastropods around the world (Kwong *et al*., 2010) and showed that apple snail annual production was >10 times greater than production estimates for other benthic macroinvertebrates in Hong Kong (0.004-15 g AFDM m^{-2} y^{-1}, n = 29). Also, annual production estimates for three of the four apple snail populations (i.e. > 230 g AFDM m^{-2} y^{-1}) were greater than published estimates for any other freshwater snails (0.002-194 g AFDM m^{-2} y^{-1}, n = 33), regardless of climatic regime or habitat type. The high production by *P. canaliculata* in Hong Kong was probably facilitated by the tropical climate (annual mean temperature ~24 °C), which permits rapid snail growth and reproduction, as well as the dietary flexibility of *P. canaliculata* and a lack of predators to effectively control its populations. The high secondary production of *P. canaliculata* compared to other macrobenthic invertebrates in Hong Kong may mean that it can monopolize food resources and compete strongly for macrophytes with other primary consumers.

Grazing on macrophytes including semi-aquatic vegetables

Although *P. canaliculata* is considered “harmless and useless” in South America, its native range (Cazzaniga, 2006), it has become a major pest of rice in Asia and taro in Hawaii because of its voracious appetite for these semi-aquatic crops (Halwart, 1994; Naylor, 1996; Cowie, 2002; Joshi & Sebastian, 2006). Laboratory experiments have shown that *P. canaliculata* also feeds on various wild macrophytes in Argentina (Estebenet, 1995) and Hawaii (Lach *et al*., 2000). In general, *P. canaliculata* exhibited a clear preference for certain macrophytes, and its growth rates were high when fed with the preferred macrophytes. However, it is not known what properties of the macrophytes determined the snails’ preferences. In wetlands of Southeast Asia, Carlsson *et al*. (2004) reported population density of *P. canaliculata* (in fact probably *P. maculata*; R.H. Cowie, pers. comm.) to be negatively correlated with diversity of aquatic macrophytes but positively correlated with nutrient concentrations and phytoplankton biomass. Mesocosm experiments showed that grazing by apple snails caused a reduction in wild macrophyte biomass and an increase in phytoplankton biomass (Carlsson *et al*., 2004; Carlsson & Lacoursière, 2005).

To assess the snails' preference for macrophytes, the physical and chemical basis of food preference and the consequences of such feeding on water quality and phytoplankton in southern China, especially Hong Kong, snails collected from four sites were dissected to examine their gut contents (Kwong *et al*., 2010), and several experiments were conducted in the laboratory (Qiu & Kwong, 2009; Wong *et al*., 2010; Qiu *et al*., 2011) and in the field (Wong *et al*., 2009; Fang *et al*., 2010). The dietary analysis showed that apple snails mainly feed on detritus and macrophytes, despite considerable variation in the composition and cover of aquatic plants among the four sites (Kwong *et al*., 2010). Among the laboratory studies, Qiu & Kwong (2009) examined how fresh leaves from various macrophytes commonly found in Hong Kong influenced feeding rate, growth and reproduction differently. They used five cultivated macrophytes (*Amaranthus gangeticus*, *Apium graveolens dulce*, *Ipomoea aquatica*, *Nasturtium officinale* and *Colocasia esculenta*) and five wild semi-aquatic macrophytes (*Eichhornia crassipes*, *Ludwigia adscendens*, *Murdannia nudiflora*, *Myriophyllum aquaticum* and *Polygonum hydropiper*). The snails showed strong preferences, with daily feeding rates varying from 1.3 % to 22 % of snail body mass among the ten macrophytes. Among both cultivated and wild macrophytes there were species the snails preferred and did not prefer. Feeding rate was negatively correlated with plant phenolic content. Snail growth rate was significantly positively correlated with phosphorus and nitrogen content. And the number of eggs deposited during the experiment was significantly positively correlated with plant sodium, nitrogen and phosphorus contents. Thus in the field, when several species of macrophytes are present, *P. canaliculata* will probably feed selectively and voraciously on the preferred species and thereby change the floral composition. Also, the result that four of the five cultivated macrophytes were in general desirable may partly explain why this species has become a successful invader in wetland agricultural areas in Asia.

To further understand the food preference of *P. canaliculata*, Wong *et al*. (2010) conducted no-choice feeding assays to test the palatability of the fresh leaves of 21 species of freshwater macrophytes commonly found in Hong Kong. They found that snail daily feeding rate on macrophytes varied greatly from 1.1% to 22% of snail body mass, and there was a positive correlation with nitrogen content but a negative correlation with C:N ratio and dry matter content (DMC). No significant correlation was detected between snail feeding rate and plant phenolic content, but the feeding rate on *Myriophyllum aquaticum*, the macrophyte with the highest phenolic content tested, was among the lowest. To determine whether chemical defence was involved in the food preferences of *P. canaliculata*, Wong *et al*. (2010) conducted another set of feeding assays with 15 species that were not palatable as fresh leaves. They reconstituted plant tissues by mixing dry plant powder with agar. The two species with the highest DMC (*Phragmites australis* and *Vallisneria natans*) were fed upon much more as reconstituted plant than as fresh leaves, suggesting that plant physical structure may be important in defence against snail herbivory. For two macrophytes (*Myriophyllum aquaticum* and *Alternanthera philoxeroides*) with moderate nitrogen and phosphorous contents that were consumed very little as either fresh or reconstituted tissues, their extracts were further incorporated into a palatable agar-based food, which for both species greatly reduced snail feeding rate, indicating the presence of chemical defences in these two macrophytes. Overall, these experimental results suggested that the feeding decisions of *P. canaliculata* are determined by structural and chemical plant traits. Macrophytes with high nitrogen content were favoured, whereas those with a high DMC were disliked. Such plant traits could be used in species screening when selecting plants for use in wetland restoration projects and when predicting the impact of invasion by *P. canaliculata*.

Since the spatial distribution of macrophytes is highly heterogeneous in local wetlands, and they exhibit clear seasonality (Dudgeon & Corlett, 2004), fresh macrophyte leaves may not be available throughout the year across different freshwater habitats in Hong Kong. This raises the question of whether and how *P. canaliculata* could use decaying leaf litter, and the consequences of consuming leave litter for snail growth. A study was thus conducted to compare the consumption and growth of *P. canaliculata* fed with fresh and decaying leaves of three macrophyte species with contrasting levels of nutrients (nitrogen) and general defence chemicals (phenolics) (Qiu *et al*., 2011). Decaying leaves were obtained by placing fresh macrophyte leaves in mesh bags in a wetland pond for 8-28 days. Feeding assays using adult snails lasted one day, and a growth and mortality experiment using snails of various sizes lasted one month. The results clearly indicated that the three species had contrasting effects on the consumption and fitness of the apple snails, and nitrogen and phenolics content, rather than plant freshness, determined snail feeding rate, growth and mortality. Snails consumed very little fresh or decaying leaves of *Polygonum barbatum* (knotweed), a species with low nitrogen and medium phenolic contents, resulting in high mortality and no measureable growth. They consumed a lot of fresh leaves and a moderate amount of decaying leaves of *Murdannia nudiflora* (dayflower), a species with low phenolic content and high nitrogen content; mortality was low in both fresh and decaying leaf treatments. In contrast, decaying leaves of *Myriophyllum aquaticum* (parrot feather) with moderate nitrogen and low phenolic contents were consumed more and supported better snail survival and growth than the fresh leaves with high nitrogen and high phenolic contents. These results provided evidence that leaf nutrient and phenolic contents matter more than leaf freshness and that the snails can use both fresh and decaying leaves with moderate levels of nutrients and low levels of phenolics to maintain fitness. This flexible feeding habit may have contributed to the species' success as an invader in many types of freshwater wetlands lacking a constant supply of nutrient-rich fresh leaves.

Predation on freshwater fauna

Although several species of apple snails including *P. canaliculata* are polyphagous (Cowie, 2002), previous studies have emphasized the impact of grazing by *P. canaliculata* on crop production, and on wetland macrophyte biomass, diversity and productivity. Nevertheless, there have been reports of predation by this invasive species on other macrobenthic invertebrates. Specifically, *P. canaliculata* could prey on the eggs, neonates and adults of the planorbid snail *Biomphalaria peregrina* and the neonates of three other snail species (*Austropeplea ollula*, *Physa acuta*, *Melanoides tuberculata*) but not on the neonates of another snail, *Sinotaia quadrata* (Cha, 1989). It will also actively and selectively prey on freshwater bryozoans, including both tubular and globular colonies (Wood *et al*., 2006). However, in these studies the predatory apple snails were not fed prior to or during the experiments and it is not known whether they would prey on macrobenthic invertebrates when alternative food is present. It is also unknown how the predatory snails detected their prey and why some macroinvertebrates are more susceptible to predation than others.

To answer these questions, a laboratory study was conducted to confirm whether *P. canaliculata* would prey on the early stages (i.e. egg masses and/or neonates) and adults of the above five species of snails, common in the freshwater environments of southern China, in the presence of alternative macrophytic food (Kwong *et al*., 2009). Each species of potential prey was exposed to *P. canaliculata* in laboratory aquaria in the presence of macrophytic food for a period of either 24 h for egg masses or 72 h for neonates and adults, after which the prey consumed was determined.

The tested species and life stages differed in their susceptibility to predation by *P. canaliculata*. Eggs and/or neonates of all five species, and adults of the Heterobranchia, (the 'pulmonates' *A. ollula*, *B. straminea* and *P. acuta*), suffered substantial mortality. However, *P. canaliculata* was not able to prey on the adult Caenogastropoda (the operculate species *M. tuberculata* and *S. quadrata*). This pattern of high susceptibility of early developmental stages of the five snails to predation by *P. canaliculata*, and the differential survival of adults of the five snail species highlighted the importance of shell size, hardness and structure in defence against predation. The eggs and neonates are poorly defended because of their fragility. However, among the adults, the heterobranchs have relatively thin and fragile shells and their shell aperture is not covered by an operculum when the animal withdraws into its shell, whereas the adult caenogastropods have a more robust shell, the aperture of which can be covered by an operculum. Observation of the prey searching behaviour showed that *P. canaliculata* was not able to detect its prey until it physically touched it. However, *P. canaliculata* glides quickly over the benthic substrate (17.8 cm min^{-1}; 2.6 to 8.5 times the speed of four common species of snails in Kong Kong; Dudgeon & Lam, 1985), which may permit ample opportunities for direct encounters with its potential prey.

A study conducted in a local stream compared aspects of the life-history of the snail *Radix plicatulus* in two sites, one with high and one with low densities of *P. canaliculata* (Lam, 1994). At the high density site, *R. plicatulus* exhibited delayed reproduction, a longer recruitment period and a larger number of cohorts per year, demonstrating that *P. canaliculata* can affect the life-history characteristics of sympatric, possibly prey, snail species. Data from two mesocosm studies also included treatments to examine the impact of *P. canaliculata* on other molluscs (Wong *et al*., 2009; Ip *et al*., 2014). The two studies are broadly consistent in showing a lower density of macrobenthos in enclosures with *P. canaliculata* than in enclosures without them, although the differences were not significant in the study of Ip (2013), probably because of large variation among replicates and loss of two control replicates, which reduced the power of the statistics.

A recent field manipulative experiment also demonstrated that apple snails could be important predators of amphibian eggs in local agricultural wetlands (Karraker & Dudgeon, 2014). They put apple snails together with the eggs of amphibians and with water spinach (*Ipomoea aquatica*) in local wetlands and found that the snails consumed the eggs of four of the five amphibian species tested. They did not consume the eggs of the frog *Polypedates megacephalus*, presumably because of the protection of the eggs by a dense, foam matrix and suspension on vegetation attached to the side of the test container above the water surface. These experimental findings are consistent with field observations that apple snail densities are high (Kwong *et al*., 2010) and the eggs of ten species of amphibians could hardly be found in the area (Ma, 2012).

Ecosystem level effects

Several mesocosm studies conducted in natural wetlands in Southeast Asia have demonstrated the grazing impact of apple snails on macrophyte diversity and biomass, and on nutrient and chlorophyll a concentrations (Carlsson *et al*., 2004; Carlsson & Lacoursière, 2005). However, given that macrophytes vary widely in biological characteristics such as nutrient demand, growth rate and defence strategy against herbivory (Carpenter & Lodge, 1986), more data from different environmental settings are required before we can generalize the causal relationship between herbivory by apple snails and state shifts in Asian shallow wetlands. A mesocosm study was therefore conducted in a shallow wetland in Hong Kong (Fang *et al*., 2010) to examine how apple snail density might determine the magnitude of snail herbivory, and how macrophytes with different defence strategies (physical or chemical) might affect herbivory, water quality (nutrients) and floral structure (phytoplankton and filamentous algae). *Myriophyllum aquaticum* (parrot feather, with high content of general chemical defence phenolics in its leaves) and *Eichhornia crassipes* (water hyacinth, with high dry matter content in its leaves) were placed in enclosures with 0-8 individuals of *P. canaliculata* for one month (Fig. 2). The two macrophytes were grazed heavily, with higher biomass reduction at higher snail densities. Given that these two species were considered well-

Fig. 2. Experimental setup in a wetland pond testing the effects of apple snail grazing on macrophytes, nutrients, phytoplankton and filamentous algae (transparent acrylic enclosures), and the effects of common carp on apple snails, non-*Pomacea* apple snails, and macrophytes (rectangular mesh enclosures).
(Photo: J.W. Qiu)

defended compared to other macrophytes commonly found in Hong Kong (Fang *et al*., 2010), the results from this field study further illustrated the high potential for *P. canaliculata* to damage wetland plants.

An interesting finding of Fang *et al*. (2010) was that filamentous algae grew substantially in the control (up to 80.3 g m^{-2}, forming pond scum), but not in the treatment with *P. canaliculata*, indicating that the snails might have controlled the growth of filamentous algae. This finding, together with experiments showing that apple snails could feed on the juveniles and eggs of other freshwater snails that are potential grazers (e.g. *Physa acuta*), reflects the probable complex interactions among *P. canaliculata*, local snails and filamentous algae. In contrast to the expectation that snail grazing would increase nutrient concentrations, nitrogen and phosphorous concentrations were low throughout both experiments, and were not correlated with apple snail density, indicating that the sediment must have absorbed a substantial amount of nutrients from the water and used them to support macrophyte growth. There were clear snail density treatment effects on chlorophyll a and phytoplankton composition, but the results differed between the *M. aquaticum* and the *E. crassipes* experiments, again showing that the effects of apple snails on wetland ecosystems cannot always be simply described by the trophic cascade of snail grazing - nutrient release - phytoplankton growth.

Control of apple snail populations

Since their first invasion in the 1980s, apple snails have become a major pest of rice in Asia and taro in Hawaii (Cowie, 2002). Although Hong Kong is one of the most densely populated cities with more than seven million people in an area of 1108 km^2, there are still approximately 51 km^2 of agricultural land. While existing rice paddies (approximately 10 % of agricultural land) are mainly for demonstration of traditional rice cultivation in Yuen Long, small-scale farms still provide 8 % of vegetables for local consumption. Several species of semi-aquatic vegetables, especially *Ipomoea aquatica* (water spinach), *Nasturtium officinale* (watercress), *Nelumbo nucifera* (Indian lotus) and *Sagittaria sagittifolia* (Chinese arrowhead) are commonly cultivated species. Apple snails are widely distributed in these semi-aquatic vegetable gardens and the drainage channels connecting them. In response to the infestation of apple snails in vegetable gardens, local farmers have adopted cultural and chemical control methods. The cultural method involves simple hand picking of apple snail eggs and large individuals throughout the year, especially in the summer when apple snails reach their peak of reproduction and growth. The chemical methods involve application of lime or tea seed cake (residue of seeds in the Camellia family, after oil extraction, which contains saponin) to apple snails between crops (Wang, 2010).

Due to increasing development pressure, many wetlands in the New Territories have been lost to construction for residential buildings and associated roads. As required by the Environmental Impact Assessment Ordinance, compensation wetlands of similar or larger size to those destroyed were created, with various macrophytes used to build up the floral community (Lau, 2004). An example of such wetland loss is the large-scale (220 ha) residential development in Tin Shui Wai for a population of 340,000 (Cha, 2004). As part of the compensation, Hong Kong Wetland Park (60 ha) was created with channels of different depths connecting many ponds separated by weirs for flow and water depth control, and macrophytes were planted to filter intake water and to create habitats of different floral diversity. The invasion of apple snails into these constructed wetlands may have greatly reduced the floral diversity, and their value as habitats for animals, such as birds. Current management activities to control apple snail populations in constructed wetlands in Mai Po Nature Reserve (managed by WWF Hong Kong), Hong Kong Wetland Park (managed by the Agriculture, Fisheries and Conservation Department and by the Development Bureau, HKSARG) and West Rail wetlands in Kam Tin (managed by Mass Transit Railway Corporation Ltd., Hong Kong) mainly involve hand-picking, which can be an effective method, but is labour-intensive and requires repeated effort (Cowie, 2002; AFCD, 2011; Secretariat of the Terrestrial Biodiversity Working Group, 2014).

Biological control is an appealing method in the management of invasive species, but its effectiveness and potential non-target effects should be carefully evaluated before implementation (Cowie, 2002). To explore the utility of biological control for apple snails in constructed wetlands in Hong Kong, two mesocosm studies were conducted (Wong *et al*., 2009; Ip *et al*., 2014). Wong *et al*. (2009) studied the effectiveness and non-target effects of common carp (*Cyprinus carpio*) as a biological control agent against *P. canaliculata* in a 2-month enclosure experiment (Fig. 2). They examined the impact of common carp on nine species of gastropods, including apple snails, and on three species of macrophytes in a constructed wetland in Hong Kong Wetland Park. Common carp completely eliminated apple snail juveniles that were small enough to fit into their months. However, the size of the fish used was constrained by the size of the enclosures, and their ability to grow larger in local ponds may mean that common carp could be an effective biocontrol agent for larger apple snails. However, common carp also caused a significant reduction of plant biomass and of the densities of most non-*Pomacea* gastropods, that is, strong non-target effects on the local wetland flora and fauna.

To further explore fish as a biocontrol agent for apple snails, a mesocosm study was conducted using black carp (*Mylopharyngodon piceus*) (Ip *et al*., 2014). The aim of the study was to determine whether black carp were as effective as common carp as a biocontrol agent for apple snails, while having less impact on macrophytes and other snails. The experimental setup and duration was very similar to that of Wong *et al*. (2009), except for the use of the two fish species. Both common carp and black carp preyed effectively on *P. canaliculata*, removing almost all individuals that were small enough to fit into their mouths. However, while black carp reduced herbivory on macrophytes through reducing apple snail density, common carp reduced apple snail density but this did not result in a lower level of herbivory because it also fed on macrophytes. Non-target mollusc density was reduced by both fish species.

A manipulative field experiment was further conducted in three constructed wetland ponds (surface area 1000 to 2525 m^2, maximum depth 1.1-2.2 m in the wet season and 0.6-1.3 m in the dry season) to examine how black carp might affect apple snails, as well as non-target macroinvertebrates, macrophytes and water quality in more natural settings (Ip, 2013). In one of the ponds there was a natural earthen partition. In the other two ponds, a steel frame with woven nylon mesh was used to create a partition. Each pond was thus divided into one experimental and one control plot (Fig. 3). The partition prevented snails from migrating to the other side and minimized water exchange between control and treatment plot. Four black carp were introduced into the treatment plot in each pond. The experiment lasted one year. Four individuals were recaptured from experimental plot 3, but only 2 individuals were recaptured from each of experimental plots 1 and 2. Black carp recaptured at the end of the experiment had grown substantially in length to 69.4 ± 2.2 cm (mean ± S.D.) and in weight to 3598.3 ± 250.1 g from a length of 0.2 ± 1.4 cm and weight of 235.8 ± 47.5 g at the beginning of the experiment. The fish were remarkably tolerant of poor water quality conditions, surviving through a period of low dissolved oxygen down to 1.2 mg l^{-1} during the winter. They were effective predators of apple snails with shell length below 25 mm. The predatory effect was non-specific and caused significant reduction in populations of at least two species of native snails. No conclusion could be drawn with respect to the effect of the fish on macrophyte biomass and diversity nor on the water quality (nitrogen and phosphorous).

Fig. 3. Three wetland ponds used in a field experiment testing effectiveness and non-target effects when using black carp as a biological control agent against apple snails. (Photo: K.K.L. Ip)

Conclusions

Overall, studies since the 1980s have shown that although only one species of apple snail (*P. canaliculata*) is present in Hong Kong, it has become widespread and caused dramatic changes to lowland agricultural and non-agricultural freshwater ecosystems.

In agricultural areas, it causes economic loss by feeding on semi-aquatic vegetables. In non-agricultural wetlands, it feeds on macrophytes, thus probably triggering changes in algal production. It may also prey on other macroinvertebrates and alter the faunal composition. Control of apple snails in agricultural areas relies on hand-picking and application of molluscicides. In non-agricultural wetlands, apple snails reduce macrophyte diversity in constructed wetlands. Current management of *P. canaliculata* in constructed wetlands mainly relies on hand-picking of adult snails and their egg masses, but bio-control using fish is being explored. Specifically, a one-year field experiment was conducted in three pairs of wetland ponds to test the effectiveness and non-target effects of black carp (Ip, 2013), which showed that black carp is a non-specific bio-control agent for freshwater molluscs. It could only be used in shallow wetland ponds with low biodiversity of macro-invertebrates, where reducing apple snail abundance and maintaining high macrophyte diversity are of management concern. However, current findings were inconclusive regarding whether controlling apple snail population density could indirectly improve water quality in constructed wetlands.

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References

Anderson, B. 1993. The Philippine snail disaster. *The Ecologist* 23: 70-72.

AFCD [Agriculture, Fisheries and Conservation Department]. 2011. *Ecological Monitoring and Habitat Management Programme of Hong Kong Wetland Park*. NCSC paper 03/11. Advisory Council on the Environment, Nature Conservation Subcommittee, Hong Kong.

Benke, A.C. & Huryn, A.D. 2007. Secondary production of macroinvertebrates. In: *Methods in Stream Ecology* (Hauer F.R. & Lamberti, G.A., ed.), p. 691-710. Academic Press, Oxford.

Blumental, D.M. 2006. Interactions between resource availability and enemy release in plant invasion. *Ecology Letters* 9: 887-895.

Boland, B.B., Meerhoff, M., Fosalba, C., Mazzeo, N., Barnes, M.A. & Burks, R.L. 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of apple snails (*Pomacea*). *Journal of Molluscan Studies* 74: 47-54.

Carlsson, N.O.L., Brönmark, C. & Hansson, L.A. 2004. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575-1580.

Carlsson, N.O.L. & Lacoursière, J.O. 2005. Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. *Biological Invasions* 7: 233-241.

Carpenter, S.R. & Lodge, D.M. 1986. Effects of submerged macrophytes on ecosystem process. *Aquatic Botany* 26: 341-370.

Cazzaniga, N.J. 2006. *Pomacea canaliculata*: harmless and useless in its natural realm (Argentina). In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.). p. 37-61. Philippine Rice Research Institute, Nueva Ecija,

Cha, M.W. 1989. *Interspecific Interactions among Freshwater Gastropoda in Hong Kong.* MPhil thesis. Hong Kong Polytechnic University, Hong Kong.

Cha, M.W. 2004. The application of constructed wetlands for water quality improvement in the deep bay catchment of Hong Kong. In: *Wetlands Ecosystems in Asia: Function and Management* (Wong, M.H., ed.), p. 401-412. Elsevier, Amsterdam.

Chen, Y.H., Yang, P.S., Chen, W.T. & Chen, Y.H. 2004. The impact of golden apple snail (GAS) on Taiwan's agricultural and ecological environment: an economic approach. In: *Proceedings, APEC Symposium on the Management of the Golden Apple Snail* (Lai, P.Y., Chang, Y.F. & Cowie, R.H., ed.), p. 35-46. Institute of Science and Technology, National Pingtung University, Pingtung, Taiwan.

Cowie, R.H., 2002. Apple snails as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 144-192. CAB International, Wallingford, UK.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastopoda: Ampullariidae: *Asolene, Felipponea, Marisa, Pomacea, Pomella*): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-23.

Philippine Rice Research Institute, Nueva Ecija.

Davis, M.A., Grime, J.P. & Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88: 528-534.

Dudgeon, D. & Corlett, R. 2004. *Freshwater Wetlands. The Ecology and Biodiversity of Hong Kong*. Friends of the Country Parks & Joint Publishing (HK) Company Ltd., Hong Kong.

Dudgeon, D. & Lam, P.K.S. 1985. Freshwater gastropod foraging strategies: interspecific comparisons. In: *The Malacofauna of Hong Kong and Southern China II* (Morton, B. & Dudgeon, D., ed.), p. 591-600. Hong Kong University Press, Hong Kong.

Estebenet, A.L. 1995. Food and feeding in *Pomacea canaliculata* (Gastropoda: Ampullariidae). *The Veliger* 38: 277-283.

Estebenet, A.L. & Martín, P.R. 2002. *Pomacea canaliculata* (Gastropoda: Ampullaridae): life history traits and their plasticity. *Biocell* 26: 83-89.

Fang, L., Wong, P.K., Lin, L., Lan, C.Y. & Qiu, J.W. 2010. Impact of invasive apple snails in Hong Kong on wetland macrophytes, nutrients, phytoplankton and filamentous algae. *Freshwater Biology* 55: 1191-1204.

Hall, R.O., Jr., Dybdahl, M.F. & VanderLoop, M.C. 2006. Extremely high secondary production of introduced snails in rivers. *Ecological Applications* 16: 1121-1131.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hamada, Y. & Matsumoto, T. 1985. Apple snails in Kumamoto Prefecture. *Kyushu-no-kai* 24: 5-12.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Ip, K.K.L. 2013. *Biological Control of Golden Apple Snails (*Pomacea canaliculata*) in Freshwater Wetland using Black Carp (*Mylopharyngodon piceus*)*. MPhil thesis. Hong Kong Baptist University, Hong Kong.

Ip, K.K.L., Liang, Y., Wu, H.X., Xue, J.Z. & Qiu, J.W. 2014. Biological control of invasive apple snails by two species of carp: effects on non-target species matter. *Biological Control* 71: 16-22.

Jhang, W.C. 1985. Study of golden apple snail from ecological aspect. *Bulletin of Malacology* 11: 43-51.

Joshi, R.C., dela Cruz, M.S., Martin, E.C., Cabigat, J.C., Bahatan, R.G., Abayao, E.H., Chow-Awon, J., Chilagan, N.P. & Cayong, A.B. 2001. Current status of the golden apple snail in the Ifugao Terraces, Philippines. *Journal of Sustainable Agriculture* 18: 71-90.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija.

Joshi, R.C. 2007. Problems with the management of the golden apple snail *Pomacea canaliculata*: an important exotic pest of rice in Asia. In: *Area-wide Control of Insect Pests* (Vreysen, M.J.B., Robinson, A.S. & Hendrichs, J., ed.), p. 257-264. Springer, Heidelberg.

Karraker, N. & Dudgeon, D. 2014. Invasive apple snails (*Pomacea canaliculata*) are predators of amphibians in South China. *Biological Invasions* 16: 1785-1789.

Kolar, C.S. & Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199-204.

Kwong, K.L., Chan, R.K.Y. & Qiu, J.W. 2009. The potential of the invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia* 51: 343-356.

Kwong, K.L., Dudgeon, D., Wong, P.K. & Qiu, J.W. 2010. Secondary production and diet of an invasive snail in freshwater wetlands: implications for resource utilization and competition. *Biological Invasions* 12: 1153-1164.

Kwong, K.L., Wong, P.K., Lau, S.S.S. & Qiu, J.W. 2008. Determinants of the distribution of apple snails in Hong Kong: two decades after their initial invasion. *Malacologia* 50: 293-302.

Lach, L., Britton, D.K., Rundell, R.J. & Cowie, R.H. 2000. Food preference and reproductive plasticity in an invasive freshwater snail. *Biological Invasions* 2: 279-288.

Lam, P.K.S. 1994. Intraspecific life-history variation in *Radix plicatulus* (Gastropoda: Pulmonata: Lymnaeidae). *Journal of Zoology* 232: 435-446.

Lau, S.S.S. 2004. Wetland creation in Hong Kong. In: *Wetlands Ecosystems in Asia: Function and Management* (Wong, M.H., ed.), p. 435-446. Elsevier, Amsterdam.

Lee, Y.C. & Wu, W.L. 1996. Study on *Pomacea canaliculata* (Lamarck, 1819) in Taiwan. *Bulletin of Malacology, Taiwan* 20: 43-50.

Lv, S., Zhang, Y., Liu, H.X., Hu, L., Yang, K., Steinmann, P., Chen, Z., Wang, L.Y., Utzinger, J. & Zhou, X.N. 2009. Invasive snails and an emerging infectious disease: results from the first national survey on *Angiostrongylus cantonensis* in China. *PLOS Neglected Tropical Diseases* 3: e368.

Lv, S., Zhang, Y., Liu, H.X., Hu, L., Liu, Q., Wei, F.R., Guo, Y.H., Steinmann, P., Hu, W., Zhou, X.N. & Utzinger, J. 2013. Phylogenetic evidence for multiple and secondary introductions of invasive snails: *Pomacea* species in the People's Republic of China. *Diversity and Distributions* 19: 147-156.

Lv, S., Zhang, Y., Steinmann, P. & Zhou, X.N. 2008. Emerging angiostrongyliasis in mainland China. *Emerging Infectious Diseases* 14: 161-164.

Ma, C.Y. 2012. *Assessment of the Effects of Agricultural Practices on Amphibian Populations in Long Valley Wetlands, Hong Kong*. MPhil thesis. University of Hong Kong, Hong Kong.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica Supplement* 3: 51-62.

Naylor, R. 1996. Invasion in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Qiu, J.W., Chan, M.T., Kwong, K.L. & Sun, J. 2011. Consumption, survival and growth in the invasive freshwater snail *Pomacea canaliculata*: does food freshness matter? *Journal of Molluscan Studies* 77: 189-195.

Qiu, J.W. & Kwong, K.L. 2009. Effects of macrophytes on feeding and life history traits of the invasive apple snail *Pomacea canaliculata*. *Freshwater Biology* 54: 1720-1730.

Secretariat of the Terrestrial Biodiversity Working Group. 2014. *Invasive Alien Species. TBWG Information Note 7*. Terrestrial Biodiversity Working Group. Biodiversity Strategy and Action Plan. Agriculture, Fisheries and Conservation Department, Hong Kong.

Wang, C.L. 2010. Seed powder of *Camellia oleifera*. In: *Nonsynthetic Resources for Pest Management. Special Publication No. 42* (Wang, C.L., Yu J.Z., Lu C.T., Lin, F.C. & Shih H.T., ed.), p. 64-65. Taiwan Agricultural Research Institute, Taichung City.

Wong, P.K., Kwong, K.L. & Qiu, J.W. 2009. Complex interactions among fish, snails and macrophytes: implications for biological control of an invasive snail. *Biological Invasions* 11: 2223-2232.

Wong, P.K., Liang, Y., Liu, N.Y. & Qiu, J.W. 2010. Palatability of macrophytes to the invasion of apple snails *Pomacea canaliculata*: differential effects of multiple plant traits. *Freshwater Biology* 55: 2023-2031.

Wood, T.S., Anurakpongsatorn, P., Chaichana,R., Mahujchariyawong, J. & Satapanajaru, T., 2006. Heavy predation on freshwater bryozoans by the golden apple snail, *Pomacea canaliculata* Lamarck, 1822 (Ampullariidae). *The Natural History Journal of Chulalongkorn University* 6: 31-36.

Wu, J.Y., Wu, Y.T., Li, M.C., Chiu, Y.W., Liu, M.Y. & Liu, L.L. 2011. Reproduction and juvenile growth of the invasive apple snails *Pomacea canaliculata* and *P. scalaris* (Gastropoda: Ampullariidae) in Taiwan. *Zoological Studies* 50: 61-68.

Yipp, M.W., 1983. *The Ecology of* Biomphalaria straminea *(Dunker, 1848) (Gastropoda: Pulmonata) Introduced into Hong Kong*. PhD thesis. Department of Zoology, University of Hong Kong, Hong Kong.

Yipp, M.W., Cha, M.W. & Liang, X.Y., 1991. A preliminary impact assessment of the introduction of two species of *Ampullaria* (Gastropoda: Ampullariidae) into Hong Kong. In: *Proceedings of the Tenth International Malacology Congress* (Meier-Brook, C., ed.), p. 393-397. Tübingen.

Yu, X.P., Wada, T., Li, Z.F., Lu, Z.X., Sun, L.P., Zhu, Y.H., Chen, J.M., Zhen, X.S. & Xu, H.X., 2001. Occurrence of golden apple snail, *Pomacea canaliculata* (Lamarck), in paddy fields and its management. *Acta Agriculturae Zhejiangensis* 13: 247-252.

Yusa, Y. & Wada, T., 1999. Impact of the introduction of apple snails and their control in Japan. Naga, *The ICLARM Quarterly* 22: 9-13.

COUNTRY-SPECIFIC REPORTS

Invasive apple snails in Malaysia

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Abstract

South American apple snails, *Pomacea* spp., classified as quarantine pests in Malaysia, were first detected in Malaysia in 1991. However, it took almost 10 years before they developed into one of the major pests of rice in the country. Since then, they have spread to almost all the rice areas in Malaysia. Since their detection, continuous control, containment and eradication programmes and research activities have been conducted by various government agricultural agencies involved in rice production. The efforts have been successful in reducing crop damage by the snails but have failed to arrest their dispersal to new areas. Since 2002, the snails have infested almost 20,000 ha of rice growing areas (2008 data) and have threatened the livelihoods of farmers. In 2010, costs associated with apple snail damage were estimated as RM 82 million (US $28 million). Various management strategies involving the use of chemical, cultural, physical and biological control techniques have been developed and extended to the farmers. Success in controlling the snail infestation has been achieved but at higher crop production cost.

This contribution discusses the impacts of apple snail infestation in Malaysia, the various actions that have been developed and undertaken to manage the infestations, and the future outlook for the impact on rice production.

Additional keywords: Ampullariidae, integrated management strategies, Mollusca, *Pomacea*, rice

Introduction

Malaysia has an estimated wetland rice area of 350,357 ha, of which 262,809 ha is in Peninsular Malaysia, 63,064 ha in Sarawak and 24,524 ha in Sabah (Anonymous, 2012). Wetland rice constitutes about 85 % of the total rice area in the country, while the remaining 15 % is upland rice. In Peninsular Malaysia, 76 % of the wetland rice area has extensive irrigation and drainage facilities, while in Sabah and Sarawak only 15 % of the area is irrigated. About 72 % of rice production comes from the eight granary areas, all in Peninsular Malaysia. Two rice crops are cultivated per year in the granary areas, with the main season crop sown in August for a February harvest and the off-season crop sown in May for an October harvest. The national average yield for the main season crop of 2010 (combined granary and non-granary areas) was 3.9 tons/ha, while the average yield from the eight granary areas was 4.6 tons/ha (Anonymous, 2012). Currently, local rice production contributes about 72 % of total consumption, with the rest imported from other countries in the region. It is government policy to ensure that the country's rice output is maintained at 65 % of current need and increased to 90 % by 2020.

Rice is cultivated mainly by smallholders with an average farm size of 1.06 ha. Rice production in Malaysia is threatened by an acute labour shortage, leaving only the elderly to tend the crop. Furthermore, rice cultivation is not cost effective compared with other crops such as tobacco and melon. The rice growing area in Malaysia is not expected to increase; it is more likely that it will give way to human settlement and industrial development. Planted rice areas will also see a declining trend with the passing of the old generation of farmers. To maintain the current production capacity, productivity has to be increased.

Some farmers in the state of Selangor have even obtained yields exceeding 10 tons/ha. To reach higher productivity, yield-limiting factors such as diseases and pest infestations have to be reduced. The introduction and current infestation of apple snails is one of the main threats to rice production in Malaysia. The effort to bridge the gap between the current average yield of 3.9 tons/ha and the targeted sustainable yield of 7-8 tons/ha might be jeopardized if effective control measures are not implemented.

Current status of apple snail infestation

Apple snail infestations have spread to almost all of the rice granary and non-granary areas of Malaysia. The total area infested increased from 17,399 ha of rice in 2004 to almost 20,704 ha in late 2008. Estimated yield losses (in tons) caused by apple snails in rice granary and non-granary areas in the main season of 2009 are shown in Tables 1 and 2. Currently, the snails are present in all states except Johor in the south of Peninsular Malaysia. They have now become a key pest of rice in Malaysia (Yahaya *et al.*, 2010). Although the authorities have undertaken various control measures, the snails have been found spreading continuously to new areas. In 2010, costs associated with apple snail damage were estimated as RM 82 million (US $28 million). Since 2009, following intervention measures, the data suggest a fluctuating but stable area of around 2,500 ha from 2009 until 2012 (Fig. 1). The total infested area in off-season 2015 was about 1939 ha.

Table 1. Estimated yield losses caused by apple snails in rice granary areas in the main season of 2009.

Granary Area	Cultivated area (ha)	Yield losses (ton)	Value (RM)
MADA[1]	96,547	10,977	11,807,185
KADA[2]	28,137	18,178	19,995,910
IADA[3] Kerian Sungai Manik	26,846	918	1,009,470
IADA Barat Laut Selangor	18,729	376	526,540
IADA P. Pinang	10,305	1,029	1,142,190
IADA Seberang Perak	7,272	5,148	6,692,400
IADA Ketara	4,923	0	0
IADA Kemasin Semerak	4,520	56	56,000
Total	**197,279**	**36,682**	**41,229,695**

[1] Muda Agricultural Development Authority

[2] Kemubu Agricultural Development Authority

[3] Integrated Agricultural Development Authority

Table 2. Estimated yield losses caused by apple snails in rice in non-granary areas in the main season of 2009 in the various states of Malaysia.

Area	**Cultivated area (ha)**	**Yield losses (ton)**	**Value (RM)**
Kedah	30,488	2,747	1,442,043
Johor	1,228	0	0
Kelantan	10,164	19	19,200
Melaka	943	3	3,300
N.sembilan	298	8	6,160
Pahang	4,404	3	3,300
Perak	6,178	328	360,800
Perlis	7,403	262	288,200
Pulau pinang	2,477	236	259,600
*Selangor	-	-	-
Terengganu	3,842	0	0
TOTAL	**67,425**	**3,606**	**2,382,603**

*Data not available

The distribution of apple snails, however, does not necessarily correlate with the damage they cause. In some areas, such as in Perak, where more than 10,000 ha of rice were infested, damage to rice seedlings was minimal and mainly confined to poorly irrigated areas where water could not be drained off during the first 10 days of rice cultivation (Fig. 2). In other areas, such as in Kelantan, the total area infested was comparatively small, but damage could be quite extensive because of the recent introduction of apple snails and the indifferent attitude of the farmers towards the threat posed by them.

The impact of apple snail infestation is more serious in Malaysia than elsewhere because direct seeding is practiced by the majority of rice farmers to offset labour constraints. This practice exposes the vulnerable rice seedlings to attack. It is well known that the critical stage during which damage can be severe is basically confined to the first month after sowing, after which the rice plants are not adversely affected.

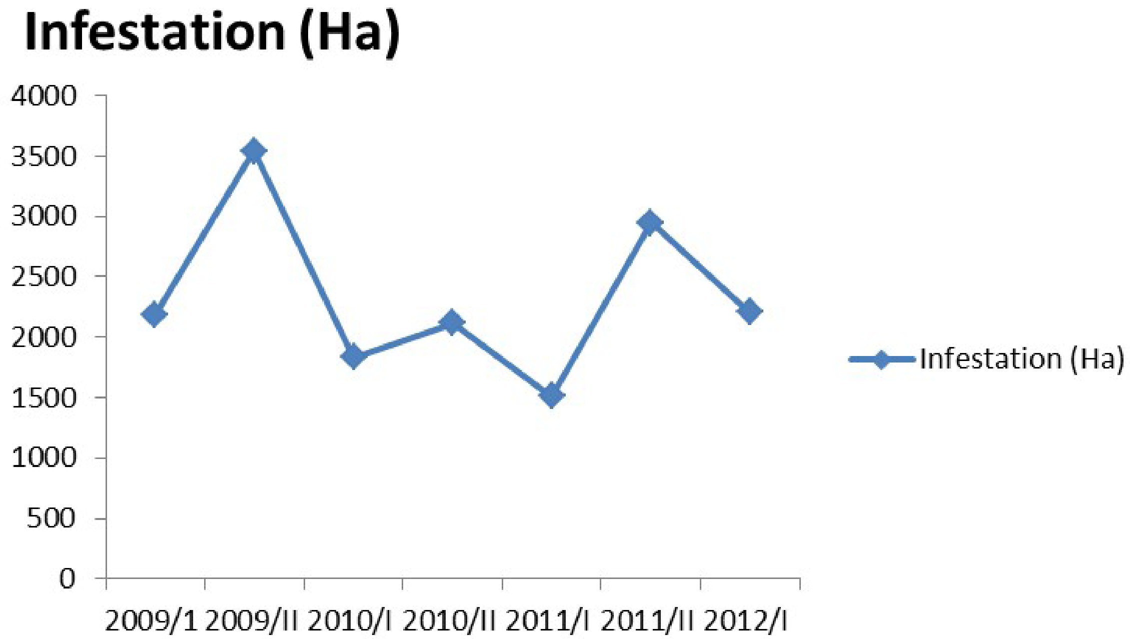

Fig. 1. Infestations by apple snails from 2009 to 2012. (Source: Palasupramaniam, DOA, Malaysia, 2013, pers. comm.).

Fig. 2. Damage is severe when the field cannot be drained before pre-germinated seed is broadcast. (Photo: H. Yahaya)

Review of research and training activities

Research

Although apple snails were first detected in rice fields in Malaysia as early as 1991, no substantial damage to rice crops was reported until the late 1990s. The species present in Malaysia were identified as *Pomacea canaliculata* and *P. insularum* (which has now been synonymized with *P. maculata*; Hayes *et al*., 2012). During the early phases of apple snail detection, containment and eradication programs were conducted using tea seed cake (TSC) and other molluscicides, based on experiences reported from other countries (Chang, 1992; Fouzi *et al.*, 1992). Molluscicide screening trials were conducted, and copper sulphate and salt water were found effective in controlling the snails. These trial results were used as the basis for eradicating an infestation in Kampung Pengkalan Semeling in Kuala Kedah by inundating the infested rice field with sea water followed by subsequent flushing with fresh water (Esa *et al.*, 1992). The attempt was successful in eradicating the snails in this locality primarily because of its close proximity to the sea and the availability of a fresh water supply nearby.

Research was also conducted on integrated management of apple snails, involving cultural, biological and chemical control (Teo, 1999a). To facilitate handpicking of snails by farmers, trials were conducted to evaluate various plant attractants as baits, with the leaves of papaya, sweet potato, tapioca (Fig. 3) and glyricidia found to be potent snail attractants (Teo, 1999b). Jackfruit and papaya also have potential as attractants to ease hand picking as well as spot spraying of molluscicide (Badrulhadza & Hafizi, 2014).

Water level management in rice fields after seeding or transplanting is critical in ensuring effective management of apple snails, as their damage potential depends on water depth, seedling age and pest density, in that order (Teo, 2005). Damage to direct seeded rice and to transplanted 21, 30 and 40 day old seedlings was 100 %, 89.2 %, 59.7 % and 46 %, respectively, at > 5 cm water depth, and insignificant damage was experienced at a water depth < 5 cm. When the water level was reduced to the saturated soil moisture level, insignificant damage occurred to direct-seeded and 21 day old transplanted seedlings even at a pest density of > 5 snails/m^2 (Teo, 2005).

Screening of plants with molluscicidal properties identified three local plants that are very effective against apple snails. Dry leaf powder of yellow furcraea or wild sisal (*Furcraea selloa var. marginata*) at a rate of 45 kg/ha can be used as an alternative to TSC, which is recommended at 51 kg/ha (Teo, 2002, 2005). Leaves of *Peltosporum*

Fig. 3. Tapioca leaves used as an attractant facilitates handpicking of snails and spot application of molluscicides. (Photo: H.Yahaya)

pterocarpum (locally known as *batai* or *jemerlang laut*) are effective but the dosage required to control 100 % of the snails was rather high at 150 kg/ha and might not be practical (Suryanto, 2000). Neem (*Azadirachta indica*) also has potential to be used as a biological molluscicide; seed and leaf extracts are being studied in terms of efficacy in controlling apple snails (Latip *et al.*, 2013).

Screening conducted to evaluate the efficacies of other chemical molluscicides showed that niclosamide, copper sulphate, metaldehyde (3 % and 5 %) were equally effective in controlling the snails (Yahaya, 2004).

Duck pasturing (Fig. 4) was introduced to farmers as a biological control for apple snails. Teo (2001) found that the effectiveness of duck varieties varied as follows: William Siam > Taiwan > Mallard > Peking > Khaki Campbell. Ducks generally reduced the snail population drastically. At the recommended rate of 5-10 ducks/ha, the snail population could be reduced from 5 snails/m^2 to less than 1 snail/m^2 after 1 month. Although other duck species were more effective, the Khaki Campbell was normally

Fig. 4. Duck pasturing can greatly reduce the snail population. (Photo: A. Badrulhadza)

provided to farmers because of its prolific egg-laying nature, thus providing additional income to the farmers.

A case study was carried out to evaluate the effectiveness of Integrated Pest Management (IPM) on 26 ha of rice fields in FELCRA Seberang Perak rice granary over four consecutive seasons (off season 2008 to main season 2009/2010). Apple snails were one of the targeted pests and the IPM approach reduced snail numbers (Saad *et al.*, 2010).

Training

Training activities were frequently organised by development authorities such as the Muda Agricultural Development Authority (MADA), the Kemubu Agricultural Development Authority (KADA) and the Integrated Agricultural Development Authority (IADA) to update extension officers on developments in apple snail management. Normally officers from the Department of Agriculture and the Malaysian Agricultural

Research and Development Institute (MARDI) were invited to conduct the briefings and trainings. A CD-based integrated pest management course organised by the International Rice Research Institute (IRRI), MARDI and MADA was conducted during 19-29 April 2004 at the MADA Training Centre, Alor Setar, Kedah. One of the subjects covered was management of apple snails. Twenty-nine officers from various government agencies involved in rice cultivation attended the course. In 2008, a similar course organised by MARDI, MADA, the Department of Agriculture (DOA), FELCRA Seberang Perak was held in Bahasa Malaysia on 4-8 August 2008 in MADA headquarters. On November 2008, another course was conducted at Tanjung Karang, Selangor, organised by MARDI and IADA Kerian-Sungai Manik. Both courses also included fieldwork training.

On 3-7 December 2012, a regional workshop on prevention of spread of apple snails and their control in rice was conducted in Kuala Lumpur, involving several participants from Southeast Asian countries. This workshop was co-organized by the DOA and the Asia and Pacific Plant Protection Commission (APPPC). The purpose of the workshop was to develop an action plan and to draft training materials for conducting a national awareness and management programme for *Pomacea* species in rice, with the following specific objectives.

- To provide opportunities for participating officers to gain expertise from experts in this field and share their experiences.
- To develop an action plan and management strategies to mitigate apple snail infestation.
- To develop a national awareness program and control measures.
- To collate all reference materials on *Pomacea* spp.

The workshop was attended by representatives of seven APPPC member countries (Malaysia, Philippines, Thailand, Myanmar, Cambodia, Lao PDR, and Vietnam) and the non-APPPC country Brunei Darussalam. From Malaysia, the participants were from various government agencies, research institutions and universities. The intent of the workshop was to produce a core group of competent trainers on apple snails in the region.

Realising the importance of the farmer's role in controlling apple snails, briefings are usually conducted by extension agents before the beginning of every planting season. Occasionally, farm machinery operators are also involved in the briefing to alert and remind them about their responsibility to clean farm machinery before transporting it to uninfected areas.

Patterns of spread of apple snails and invasion pathways

Apple snails were first detected in Malaysia in waterways in old tin mines and fishponds at Puchong and Subang, Selangor, in September 1991. The snail species found at Puchong was identified as *Pomacea maculata* (as "*insularum*"), while the species found at Subang was identified as *P. canaliculata* (Chang, 1992; Mat Hassan & Abdul Kadir, 2003). Mat Hassan & Abdul Kadir (2003) also reported *P. canaliculata* from Kedah and Perak and *P. maculata* (as the "black apple snail") from Sabah and Sarawak. None of these identifications was based on molecular or anatomical analysis (Hayes *et al.*, 2008, 2012), and they need to be confirmed. *Pomacea* spp. being quarantine pests, a nationwide search was conducted to detect the snails. The snails were present in abandoned tin mine ponds that were being used for rearing fish, prawns and ducks. Containment and eradication programmes were immediately undertaken by the DOA. However, introductions of the snails to other places went undetected and resulted in the spread of apple snails in Malaysia. In addition to commercial rearing or as food supplements for commercial duck rearing, the snails were also introduced through the aquarium trade. They were also intercepted at entry points in vegetables imported from a neighbouring country.

The first rice field found to be infested by apple snails in Malaysia was at Kampung Pengkalan Semeling in Kuala Muda, Kedah, in November 1991. The infestation was traced to a farmer who had brought back the snails from Thailand for aquarium rearing and had accidentally released them into the rice field. As described above, the snails were successfully eradicated by inundating the field with seawater followed by flushing with fresh water from a nearby pond. They may have been either *Pomacea canaliculata* or *P. maculata*.

In July 1992, *P. canaliculata* was found in a drain connected to rice irrigation canals next to a housing area. The source was an aquarium hobbyist who had brought back the snails from the Philippines and accidentally released them into the irrigation canals. This then became the source of infestation of the Wan Mat Saman irrigation canals and the surrounding fields. Identification as *P. canaliculata* is almost certainly correct, as only this species has been definitively recorded from the Philippines (Hayes *et al.*, 2008).

In Perak, where currently more than 10,000 ha of rice fields are infested, the source of infestation could be linked to an abandoned tin mine at Jelapang Kepayang, where *P. maculata* was detected in October 1991. The tin mine pond has a direct link to Sungai Kinta, a tributary of the Sungai Perak, the main river in the state of Perak. It is probably from this river and its tributaries that the snails found their way to the major rice granaries of FELCRA Seberang Perak and Sungai Manik-Labu Kubung.

Human activities, especially fishermen using throw nets and fish and prawn traps, also facilitated the spread of invasive apple snails. Numerous small, isolated infested areas were found next to the houses of these fishermen. Aquarium hobbyists who find apple snails to be alluring, beautiful creatures have also accidentally released them while changing the water in their aquaria. Apple snails have also been disseminated to new areas on farm machinery, especially combine harvesters, as in the case of their spread to the state of Kelantan and Penang. Combine harvesters from the infested areas in Kedah and Perak that were not properly cleaned transported either the egg masses or adults across the mountainous regions to Kelantan or across the straits to Penang Island. Snails also spread through flood waters during the monsoon season, as was the case of infestation in North Seberang Perai following a big flood in 2004, and via irrigation and drainage canals.

Government policies and regulations

Regulations on the status, spread and containment of *Pomacea* spp. in Malaysia fall under the Plant Quarantine Act of 1997, Section 6, Law of Malaysia, Act 167 (Anonymous, 1994), which provides power for direct destruction or treatment of plants and pests or treatment of land. The same law, under Section 12(1), provides the power to the authorities to take action against a dangerous pest. Normally, when *Pomacea* spp. are detected in small isolated areas, the local authorities, such as the District Department of Agriculture, MADA or KADA, will undertake a containment or eradication program. Such efforts have helped in preventing the spread of the snails to rice areas. However, when the snails have already spread and become established in rice areas, normal control in the rice fields and irrigation canals is organised, with participation of the farmers.

Since the first incursion of apple snails in in Kuala Muda, Kedah, the authorities have taken several measures to curb the spread of the pest. These measures include sending alerts to farmers during campaigns and highlighting the issues concerning the pests through electronic and printed media. Apple snails have been gazetted as dangerous pests and must be treated as quarantine pests such that a pest containment programme for the whole country must be put in place.

Potential impact of *Pomacea* spp.

Agriculture

In Malaysia apple snails are not expected to cause damage to cultivated crops other than rice. Only rice is cultivated under flooded conditions in the lowlands, with the only other crop that is planted under flooded conditions being watercress (*Nasturtium officinale*), which is cultivated in ponds in highland areas. So far no record of apple snail infestation on other economically important crops has been reported in Malaysia. In areas where *Pomacea* spp. are present in irrigation canals, weeds such as water hyacinth (*Eichhornia crassipes*), limnocharis (*Limnocharis flava*), water lettuce (*Pistia stratiotes*), *Salvinia* spp. and other aquatic weeds that normally clog the irrigation canals and waterways could possibly be controlled using the snails.

Native snails

The presence of *Pomacea* spp. in rice ecosystems may have a direct effect on indigenous snail species such as *Pila scutata*, *Pila ampullacea*, *Indoplanorbis exustus* and *Filopaludina* spp., as well as the bivalve *Corbicula perakensis*. Although no study has been conducted to evaluate the impact of *Pomacea* spp. on indigenous snails in Malaysia, the number of indigenous snails in the rice fields has been declining and *P. canaliculata* is known as a predator of native snails in Hong Kong (Kwong *et al*., 2013). Studies also need to be conducted to evaluate the effects of molluscicide applications used to control *Pomacea* spp. on the indigenous aquatic snails.

So far only two species of *Pomacea*, namely *P. canaliculata* and *P. maculata*, have been found in Malaysia. It is not known whether any hybridisation between the two species has occurred in Malaysia, as in Japan (Matsukura *et al*., 2013), but it is unlikely that hybridisation between the introduced *Pomacea* spp. and indigenous apple snails in the genus *Pila* has occurred.

Farm workers and health hazards

There has been no record of poisoning caused by molluscicide use in Malaysia. In Malaysia, only niclosamide and metaldehyde are registered for control of *Pomacea* spp.

The local farmers' organisations frequently advise farmers on the safety aspects of pesticide usage, especially during gatherings and briefings. In MADA areas, farmers who are members of the Pest Monitoring and Surveillance Scouts are mobilised to monitor the *Pomacea* spp. situation in their areas. They organise collection and destruction of the snails and their egg masses and are trained in pesticide application and safety aspects. They are also employed by MADA to conduct molluscicide applications in the main, secondary and tertiary irrigation canals. Scouts are supplied with rubber gloves, safety masks, safety boots and pails. The general public are notified of the control operations through public announcements by mobile units, letters or flyers distributed by group leaders and warning notices or signboards in public places. Normally one signboard is placed every 500 m in each operational area.

Poisoning, if it occurs, is primarily due to farmers' ignorance of safety procedures in the application of molluscicides, especially TSC. Although farmers are advised to soak TSC in water overnight before applying it to the field, some prefer to broadcast it directly onto their rice fields, resulting in exposure to the dust and causing them to suffer sore throats and coughs for a few days following application. Currently, TSC has been recommended to be removed from the approved list for use against *Pomacea* spp. Currently, farmers tend to use illegal molluscicides smuggled from neighbouring countries, such as fentin acetate and sodium salt of pentachlorophenol (NaPCP). The increased use of these illegal products to control *Pomacea* spp. brings more health hazards to farmers by way of acute and chronic poisoning. In fact there are many unreported cases of poisoning by these illegal molluscicides but only a few get reported officially.

Environment

The presence of *Pomacea* spp. and their egg masses on rice plants after the critical stage and on plants in recreational lakes and wetlands is an unwelcome sight and is considered as a visual irritant in the landscape. Application of molluscicides, especially those that are not recommended such as endosulfan, chlorpyrifos, copper sulphate, fentin acetate and NaPCP, pollute waterways and cause detrimental side effects on non-target organisms.

Economic losses

The severity of damage caused by apple snail infestation depends on the crop stage being attacked, the size and population density of the snails, the water level in the rice field and remedial actions taken to reseed or transplant to fill lost hills. The bigger the snail, the more damage it will cause; a 5 cm snail causes three times more damage than a 2 cm snail. Generally, a population density of 5 snails/m^2 can cause crop damage ranging from 50 % to 100 % depending upon the crop stage being attacked.

Without any control measures, it is estimated that a snail density of 1 snail/m^2 causes 20 % crop damage and monetary loss per hectare of US $168. A snail density of 8 snails/m^2 causes 90 % damage and monetary per hectare of US $758. When control measures are taken, additional costs will be incurred, while some crop damage will still be sustained. The cost of reseeding or transplanting to fill empty hills will normally be twice as much as initial costs; such costs are estimated to be US $79 per hectare. The cost of controlling snails, including molluscicide application, is estimated at US $53 per hectare and yield losses at 1-7 % cost US $8 per hectare. So when an attack by *Pomacea* spp. occurs, farmers will lose at least US $140 per hectare. These losses, however, do not take into consideration the uneven maturation (due to uneven growth) at harvest, which will affect rice quality and cause a subsequent reduction in selling price. Considering the total area of rice infested in Malaysia and two planting seasons a year, the total loss caused is estimated to be more than US $2.63 million per year.

Use of *Pomacea* spp.

The local apple snails, *Pila scutata* and *Pila ampullacea*, can sometimes be found for sale at farmers' markets. They are cooked and consumed as a special delicacy by local people. Traditionally, *P ampullacea* is consumed as a remedy for broken bones and backache. It is also commonly prescribed for the treatment of blood in the stool. From time to time, *Pomacea* spp. have been found being sold for culinary uses at farmers' markets, but action was immediately taken to contain and eradicate the snails at the farm rearing it. There are some indications that *Pomacea* spp. may have commercial value because of the number of applications to rear them commercially for culinary purposes submitted to the Department of Agriculture. Since 1997, five applications have been received, but only one was approved, in 1997, and permission was withdrawn in 2000 due to the species' pest status under the Quarantine Act.

The Department of Veterinary Services (DVS) in Yan, Kedah, has started a project on processing *Pomacea* spp. into livestock feed and fish food. This project is still at an early stage and further studies are still needed, especially in terms of their nutritional effects. MARDI considered using crushed *Pomacea* spp. shells as poultry feed because of their high calcium content. There is no information so far on any effort towards making *Pomacea* shells into handicraft products or to use the snails as aquarium pets.

Public education

Public education has always been given priority by the Department of Agriculture, MADA, KADA and other relevant authorities. Dialogues and briefings on *Pomacea* spp. are usually organised before the start of the rice planting season. Community efforts involving all farmers are organised for handpicking of snails and egg masses, especially in newly infested areas. During the earlier years of infestation, farmers were encouraged

Fig. 5. Public awareness campaign: competitions to collect adult snails and egg masses. (Photo: H.Yahaya)

to collect snails and egg masses for a reward. In one of the campaigns conducted by MADA along the Wan Mat Saman irrigation canal in 1998, 1.2 tons of snails and 15 kg of eggs were collected. In 1999, the same operation collected 532 kg snails and 26 kg of eggs. The payment for snails was US$0.13 per kg and that for egg masses was US$1.30 per kg. At other times competitions were held whereby farmers who collected the most snails and egg masses were given presents (Fig. 5). Video presentations on control techniques were normally shown and posters on apple snail problems and control measures were put up (Fig. 6). Sometimes colouring contests were held for preschool and primary school children. Normally, molluscicides such as metaldehyde and niclosamide were given to farmers to apply in their rice fields. In MADA and Sungai Manik areas ducklings and their starter feedstuffs were provided free during these occasions. In the MADA area alone, more than 5,000 Khaki Campbell ducklings were given to farmers in infested areas in 2002. The programme was continued in 2003, when 5,800 ducklings were provided free to farmers. Each farmer was normally given 50 ducklings. However, in 2004, the duckling programme was discontinued because of restriction on the import of ducklings from Thailand due to avian flu.

Fig. 6. Documentary video on control techniques. (Photo: H. Misrudin)

In most affected areas, local farmer associations and district officers of relevant authorities organised gatherings and briefings on invasive apple snail status and control strategies. Attendance was encouraging because fertiliser subsidies, free molluscicides and free ducklings were given out.

During the early stage of *Pomacea* spp. introduction, many farmers did not see the snails as a threat, as they look very similar to the indigenous apple snails, *Pila scutata* and *Pila ampullacea,* which do not cause problems in rice. *Pila* spp. differ from *Pomacea canaliculata* and *P. maculata* in the colour of the eggs, pink in the invasive species and white in the indigenous species. Shell colour is generally dark brown in *Pila* species of Malaysia and in *Pomacea maculata*, whereas in Malaysia *P. canaliculata* tends to be paler in colour. However, shell colour, especially of *P. canaliculata*, can vary widely. Since the indigenous *Pila* spp. have never caused any damage to rice seedlings, the farmers tend to view *Pomacea* spp. the same way. Also, it normally takes at least two seasons of rice cropping between initial introduction of *Pomacea* spp. into new areas and detection of their attack on rice seedlings. *Pomacea* spp. might initially be detected only in the irrigation canals, with very few entering the rice fields. Initial damage might be minimal and would be attributed to other factors such as deep standing water during the seedling stage or poor seed quality. The snails feed on the emerging plumules and radicle, which are not readily visible. The attacks may thus not be noticed, as the seeds may appear not to have germinated. Farmers realise the destructive nature of *Pomacea* spp. only after the damage becomes more obvious.

Currently, with aggressive campaigns and information dissemination organised by the Department of Agriculture and other local authorities through gatherings and briefings, leaflets, brochures, TV documentaries, and newspaper articles highlighting the threat of invasive *Pomacea* spp., the public has become increasingly aware of their presence and of the threat they pose. A manual on their management in rice was published by MARDI (2010). This manual is used as reference material for training and implementing the control programme and covered many aspects of managing threatened rice crops.

Future apple snail threats

Pomacea spp. are predicted to invade the entire lowland rice area of Malaysia in the near future. The concerted and continuous monitoring and control measures undertaken by the authorities succeed only in slowing the spread. Human activities and natural phenomena such as flooding will facilitate the spread. Since no local natural enemies are currently available to check the population build-up of the snails, it is foreseen that they will continue to spread without hindrance.

The threat is not limited to rice but extends to other aquatic plants growing in wetlands, commercial and recreational ponds, and lakes. The snails have already invaded the Putrajaya Wetland and are causing major management problems there, as it is much more difficult to control them in a permanently flooded situation than in rice fields with alternate flooded and dry conditions.

Limited availability of molluscicides will also pose a threat to management should the snails develop resistance to the current molluscicides. Farmers always prefer to use a single and convenient method for control, and molluscicides offer them the best option. Currently, only niclosamide ethanolamide 81.4 %, niclosamide-olamine 83.1 %, niclosamide 25 %, metaldehyde 5 %, metaldehyde 36.5 % and metaldehyde 50 % are registered for apple snail control. TSC was never registered for apple snail control and as such its use for that purpose is illegal under the Pesticides Act of 1974.

Management guidelines/options – traditional and new techniques

Dry or wet seeding followed by regulated flooding of rice fields beginning 7-10 days after seeding will normally provide effective control. However, in areas where weedy rice is a problem, this practice will result in abundant growth of weedy rice, which will cause severe yield losses. Weedy rice (*Oryza sativa* complex) encompasses variants of ordinary rice, but the grains are easily shattered, even before harvesting. Yield losses of more than

Fig. 7. Various kinds of strainers or traps can be used to prevent snail entry into rice fields. (Photos: H. Yahaya)

74 % have been reported in heavily snail-infested areas (Azmi & Abdullah, 1998). Water seeding, which is effective in controlling weedy rice, will expose the newly germinated rice seedlings to attack. A control technique that is compatible with weedy rice control is thus urgently needed in areas where both of these pests are prevalent. Duck pasturing is a technique that will be beneficial in controlling both the snails and weedy rice. Reverting to transplanting is also seen as an alternative for controlling snails and weedy rice. Since farm labour is one of the major constraints, use of mechanised transplanters is expected to alleviate the problem.

The management strategies employed by various agencies normally depend on the severity of infestation and the available options under specific conditions. The factors that influence the density of snail populations in rice fields and the effectiveness of control strategies need to be understood. Some of those factors are shown in Figure 8. Farmers as well as extension agents should know that only active apple snails in the rice fields can be handpicked, controlled by ducks or fish, or killed by molluscicides, while those

Fig. 8. Dead snails among rice seedlings, indicating effective control using molluscicides. (Photo: H. Yahaya)

that are aestivating in the soil cannot. Therefore, snails aestivating in the soil need to be activated by flooding the field for at least 2-3 days before such measures are applied. Using herbage attractants also can encourage the aestivating snails to come out during the flooding.

Farmers need to control apple snails only in their own rice fields. During land preparation, farmers should do proper ploughing and land levelling as these could reduce the snail population in the soil. Farmers also must avoid creating puddles in the rice fields. Aside from that, relevant authorities normally conduct control in the irrigation canals. However, not all snails in the irrigation canals can be controlled. Therefore, farmers have to put up strainers to prevent their entry into the rice fields (Fig. 7). Although some studies suggest the use of 2 mm mesh screen/strainers, bigger mesh strainers of less than 1.5 cm mesh screen can be used effectively, since only snails that are > 1.5 cm are capable of attacking rice seedlings. However, this does not prevent smaller snails from getting through the strainers and growing larger. Therefore, farmers are advised to be vigilant and manage any residual snails in the fields. Other cultural practices must be implemented accordingly, such as collection and destruction of the snails and egg masses before seeding or transplanting. Molluscicides are effective in controlling apple snails (Fig. 8), but damage to crops could occur through re-entry of snails from irrigation canals when the field is flooded or by snails emerging from aestivation in the soil once the field is flooded. Several options to manage apple snail infestation, as practiced in Malaysia, include the following.

- Dry seeding:
    a. First ploughing – dry
    b. Second ploughing – dry
    c. Seed broadcast – dry
    d. Water introduced 17-18 days after sowing and maintained at < 5 cm

This practice results in poor seed germination and poor initial crop growth and has given way to wet seeding.

- Wet seeding
    a. First ploughing – dry
    b. Second ploughing – dry
    c. Third ploughing – flooded
    d. Molluscicide treatment – water level 2-3 cm deep
    e. Perimeter drains constructed
    f. Fields drained to saturation

    g. Pre-germinated seed broadcast
    h. Strainers put up at water inlets into the fields
    i. Field flooded 12-15 days after seeding
    j. Water level maintained below 5 cm until the rice plants are about 1 month old

This planting technique is possible only where weedy rice is not a problem. Under both dry and wet seeding techniques, duck pasturing is done only before and after land preparation, prior to seed broadcasting.

- Pre-germinated seed broadcast in water
    a. Duck pasturing up to broadcasting pre-germinated seed
    b. First ploughing – flooded or dry
    c. Second ploughing – flooded
    d. Strainers put up at water inlets into the fields
    e. Molluscicide treatment – water level < 3 cm deep
    f. Third ploughing – flooded
    g. Pre-germinated seeds broadcast – water level > 10 cm

This option is the least effective in controlling apple snails but is very effective for controlling weedy rice.

- Transplanting
    a. Duck pasturing immediately after harvesting up to heading stage
    b. First ploughing – flooded or dry
    c. Second ploughing – flooded or dry
    d. Third ploughing – flooded
    e. Transplanting of 2-3 week old seedlings if done mechanically, or more than 1 month old seedlings if done manually
    f. Water level maintained at < 3 cm up to 1 month, after which water level can be increased to 10 cm

Transplanting offers the best management option for apple snails and weedy rice. However, due to labour shortage, manual transplanting using > 30 day old seedlings may not be possible unless a mechanical transplanter is used. In some infested areas, the local authorities such as MADA and enterprising farmers have already purchased a transplanter and are providing transplanting services to other farmers at a service rate of US $197 per hectare. However, transplanting will not be effective if water in the rice field is not drained off and snails are not controlled before transplanting. By reverting to transplanting, the cost of rice production will be increased substantially. Unless the cost of transplanting can be offset by higher yield, it is doubtful whether most farmers will be able to afford the additional costs and will be willing to accept the practice.

New paradigms

Although *Pomacea* spp. are introduced pests, they are well established in Malaysia. It has to be accepted that they are here to stay and we have to live with them. Management strategies have to be developed to minimise losses. We have to look for opportunities that could be created from the presence of apple snails in the rice ecosystems, such as using the snails for raising ducks and fish farming in the rice fields. Apple snails in the irrigation canals and in the rice fields after the critical stages could be used as biological control agents for weeds. At a time when the costs of pesticides are escalating at such a high rate, it would be wise to look for alternative measures that are more effective, environmentally friendly and cost effective and at the same time provide additional income to farmers. Ducklings could be provided free to individual farmers instead of molluscides and herbicides. Ducks could be released into the rice fields immediately after harvesting until new crops are planted, or in cases where transplanting is practiced, ducks could be released even after transplanting up to the heading stage. One of the problems with raising ducks to control apple snails is feeding the ducks during periods when they cannot be released into the rice fields. One of the ways to solve this problem is by herding the ducks from one field to another. In Kelantan, for example, commercial duck farmers transport and release their ducks into rice fields immediately after the crops are harvested, then move the ducks to other locations once the rice fields are seeded. Since the planting season varies among localities, the ducks can be moved and released accordingly.

Knowledge gaps

Research on apple snails in Malaysia has focused on management strategies to alleviate damage to the rice crop. More basic research in the areas of their taxonomy, biology and ecology has not been pursued in depth. The species diversity of apple snails present in Malaysia is not well established, although it is well accepted that two species, *Pomacea canaliculata* and *P. maculata*, are present. It is also not known whether hybridisation between these two species has occurred or whether hybridisation with the local apple snails has taken place (although that is highly unlikely). The impact of apple snail infestation on the local freshwater snails has not been assessed.

Lessons learned – what next?

The introduction of alien invasive species such as *Pomacea* spp. has shown that a small mistake committed with good intentions or an unintentional introduction can result in insurmountable problems. These apple snails may not be a problem in their native habitat because of the presence of natural enemies and other factors that regulate their populations. However, they have become serious pests when introduced to new habitats lacking natural enemies and other factors limiting population. This underlines the importance of regulations dealing with invasive alien species.

Apple snail infestations also highlight the importance of regional cooperation in combatting a common problem. Movement of people and invasive species across common borders should be closely monitored. The experiences of controlling apple snails

Fig. 9. Crop losses can be extensive if farmers fail to control apple snails effectively. (Photo: H. Yahaya)

in one country could be adapted to other countries. Information sharing and join research among neighbouring countries should also be encouraged.

The problems caused by apple snails also show that there is an imminent need for transformation of the rice production system in Malaysia. Small unproductive units of < 2 ha managed by elderly farmers should be consolidated and managed in mini-estates and estate systems, either by development authorities or farmers' associations. Severe apple snail damage (Fig. 9) normally occurs in rice fields managed by elderly farmers, who are less receptive to change and lack the resources to implement recommended control strategies. The problem is compounded by lack of hired farm labour. The majority of rice farmers in Malaysia are in the 50-70 years old group. They are involved with rice cultivation because of tradition and through inheritance of the land. In areas where rice cultivation is managed in estates and mini-estate systems, either by development authorities such as FELCRA and KADA or by farmers' associations such as in Penang, apple snail infestation has been effectively managed through concerted effort and availability of resources.

Rice farmers normally prefer to adopt only one simple method of controlling apple snails, such as the use of molluscicide. When other techniques such as handpicking and setting up strainers to prevent snail entry into the rice fields are recommended, farmers are reluctant to adopt them, as they have been accustomed to applying pesticides to manage rice pests and diseases. The farmers' attitude toward pest management needs to be changed from being dependent on pesticides to adopting non-chemical control.

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References

Anonymous. 1994. *Laws of Malaysia. Act 167. Plant Quarantine Act, 1976*. 22 p.

Anonymous. 2012. Paddy production survey report, main season 2010/2011. Department of Agriculture, Malaysia 208pp.

Azmi, M. & Abdullah, M.Z. 1998. A manual for the identification and control of padi angin (weedy rice) in Malaysia. Serdang: MARDI, 18pp.

Badrulhadza, A. & Hafizi, Y. 2014. Evaluation of several plant-based attractants for apple snail management. *Acta Biologica Malaysiana* 3(2): 49-57.

Chang, P.M. 1992. The golden apple snail, a new pest of rice in Malaysia. Unpublished mimeo.

Esa, Y.M., Wong, S.W. & Ibrahim, I.B.H. 1992. The occurrences and control of the golden apple snail, *Pomacea canaliculata* in Kedah Darulaman, Malaysia. Unpublished mimeo.

Fouzi, A., Esa, Y.M. & Ho, N.K. 1992. Golden apple snail control operations in MADA areas. Unpublished mimeo. [in Malay].

Hayes, K.A., Joshi, R.C., Thiengo, S.C. and Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hussain, Y., Abdullah, S., Man, A., Noor, M.H.M., Mamat, N, Setu, F. & Teo, S.S. 2010. *Manual Teknologi Pengurusan Siput Gondang Emas*. MARDI, Serdang, Selangor.

Kwong, K.L., Chan, R.K.Y. & Qiu, J.W. 2009. The potential of the invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia* 51: 343-356.

Latip, S. N. H. M., Mamat, M. and Keni, M. F. 2013. *Azadirachta indica* extract as biopesticide for controlling golden apple snail, *Pomacea canaliculata*. Advanced Science Letters. Vol. 19. American Scientific Publisher. p. 2992-2994.

Mat Hassan, O. & Abdul Kadir, A.H. 2003. Malaysia. In: *Invasive Alien Species in South-Southeast Asia National Reports & Directory of Resources* (Pallewatta, N., Reaser, J.K. & Gutierrez, A.T., ed.), p. 43-56. Global Invasive Species Programme, Cape Town, South Africa.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Saad, A., Yahaya, H., Muhammad, H., Azmi, M., Wan Zaki, W.M., Manim, A., Badrulhadza, A., Siti Norsuha, M., Maisarah, M.S., Noridiana, M.Y. & Sivapragasam, A. 2010. Integrated pest management approach towards sustainable rice production.

Unpublished (mimeo) paper presented at National Rice Conference, 28-30 June 2010. Lumut, Perak.

Suryanto, E. 2000. *Pomacea insularus* (Gastropoda: Pilidae): Its control under the integrated pest management (IPM) concept. PhD dissertation, Faculty of Science and Environmental Studies, University Putra Malaysia.

Teo, S.S. 1999a. Control of the golden apple snail in irrigated rice by integrated approach in Sabah. In: *Tropical Plant Protection in the Information Age. Proceedings of the 5th International Conference on Plant Protection in the Tropics. 15-18 March, 1999, Kuala Lumpur, Malaysia* (Sivapragasam, A., Ismail, A.A., Cheah, U.B., Chung, G.F., Chia, T.H., Omar, D., Ho, T.H., Lee, S.S., Lim, J.L., Lum, K.Y., Salleh, M., Ganapathi, N., Ong, C.A., Vijaysegeran, S. & Ahmad, Z.A.M., ed.), p. 84-88. Malaysian Plant Protection Society, Kuala Lumpur.

Teo, S.S. 1999b. Control of the golden apple snail (*Pomacea* spp.) by handpicking using herbage as attractants. In: *Sustainable Crop Protection Practices in the Next Millennium. Proceedings of the MCB-MAPPS Plant Protection Conference '99. 2-3 November, 1999, Kota Kinabalu, Sabah, Malaysia* (Sidek, Z., Bong, C.L., Kanapathipillai, V.S., Ong, C.A. & Kadir, H.A., ed.), p. 78-81. Malaysian Plant Protection Society, Kota Kinabalu.

Teo, S.S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* 20: 599-604.

Teo, S.S. 2002. Selecting plants with molluscicidal properties for the control of the golden apple snail *Pomacea canaliculata* (Lamarck). In: *Biopesticides: Positioning Biopesticides in Pest Management Systems. Proceedings of the 3rd International Conference on Biopesticides, 22-26 April 2002, Kuala Lumpur, Malaysia* (Mulla, M.S., ed.), p. 237-243. Malaysian Plant Protection Society, Kuala Lumpur.

Teo, S.S. 2005. Integrated control of the golden apple snail, *Pomacea canaliculata* (Lamarck, 1822), in rice. In: *APEC Symposium on the Management of the Golden Apple Snail. September 6-11, 2004, Chinese Taipei* (Lai, P.Y, Chang, Y.F. & Cowie, R.H., ed.), p. 61-68. National Pingtung University of Science and Technology, Pingtung, Taiwan.

Yahaya, H. 2004. Management of golden apple snail, *Pomacea* spp. in Malaysia. Unpublished paper (poster) presented at International Rice Conference, 13-16 October 2003, Alor Setar, MARDI: IRRI.

Yahaya, H. and Badrulhadza, A. 2012. Pengurusan siput gondang. In: Saad, A., Badrulhadza, A., Sariam, O., Azmi, M., Yahaya, H., Siti Norsuha, M., and Maisarah, M. S. (eds.). Pengurusan Perosak Bersepadu Tanaman Padi ke Arah Pengeluaran Berlestari. MARDI. Serdang. Ms. 141-154. (in Malay).

Yahaya, H., Saad, A., Azmi, M., Muhamad Hisham, M. N., Nordin, M., Fakrulabadi, S., and Teo, S. S. 2010. Manual teknologi pengurusan siput gondang emas. MARDI. 54 pp. (in Malay).

The apple snail *Pomacea canaliculata* in East Malaysia – past, present and future

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Abstract

This paper discusses the invasion of the apple snail *Pomacea canaliculata* in East Malaysia in the 1990s. As soon as the snail was noticed in rice fields the Department of Agriculture immediately initiated a research program to study the new pest and launched a control operation comprising cultural, biological and chemical components to contain outbreaks in rice. The control operation successfully contained the pest within two years with pest population density decreased from 5 snails/m^2 to less than 1 snail/m^2 in most places. Since then many natural enemies of *P. canaliculata* have emerged and have helped to maintain the snail population density at a tolerable level. At this juncture, it is essential to conserve and to preserve beneficial species (possible natural enemies) through the judicious use of pesticides to enhance the development of ecosystem services.

Additional keywords: Ampullariidae, biological control, chemical control, cultural control, ecosystem services, integrated pest management, Mollusca, molluscicides, Sabah, Sarawak, tea seed cake

Introduction

The apple snail *Pomacea canaliculata*, native to South America, is a freshwater snail in the family Ampullaridae (Cowie, 2002). In the 1980s it was introduced from Argentina to Taiwan for commercial production (Mochida, 1991). From there, it was distributed to other countries to improve the living conditions of the rural poor (Matienzo, 1984; Anderson, 1993). However, the introductions were done in haste with no prior studies on the economics and possible impacts on the new environments (Acosta & Pullin, 1989).

When demand for the snails was poor, farmers abandoned their snail-farming projects and in many instances the snails escaped and ravaged rice crops, with losses running into millions of dollars (Naylor, 1996). Apple snails soon became major rice pests in Asia (Halwart, 1994). The estimated snail-infested areas were 171,425 ha in Taiwan in 1986, 16,195 ha in Japan in 1989 and 400,000 ha in the Philippines in 1989 (Mochida, 1991).

In its native environment *Pomacea canaliculata* inhabits swamps, marshes, ponds, lakes and riversides with shallow slow moving or stagnant water. It is extremely polyphagous, feeding on vegetal, detrital and animal matter with a voracious appetite. Temperature is the main growth-limiting factor, and significantly influences many aspects of *Pomacea* biology (Cowie, 2002; Hayes *et al*., 2015). In Malaysia, with no significant seasonal temperature fluctuation and an ecosystem resembling its natural habitat, the snails can continue to breed for as long as the water level is as deep as its shell height. When water becomes limiting the snails will bury into the mud to aestivate and can remain alive for at least 10 months (Teo, 2003a). Therefore, once infested, the situation is seemingly irreversible. The most active period occurs during the rainy seasons. This is manifested by the numerous pinkish egg masses on emergent vegetation. Hatching success is high, ranging from 87 to 100 % (Teo, 2004).

Apple snails are most damaging to seedlings planted by direct seeding. They readily consume young rice seedlings that still have accessible soft tissue. The seedlings become tolerant to snails at 40 days old if the water level is 5-7 cm deep, that is, below the height of the hardened culms that the snails cannot eat. The snails must be in the water to be able to attack the plants. If the water is deeper than 5-7 cm, the 40 day old seedlings become susceptible to the pest if the upper, tender tissues are submerged. Thus, water level is the most important factor determining the extent of damage. The damage potential of the snails in rice depends on water depth > seedling age > pest density in this decreasing order (Teo, 2003b).

Invasion of apple snails in East Malaysia (Sabah and Sarawak)

In Sabah, the snails were first sighted in Keningau in the early 1990s, and in Sarawak they were reported in Ba Kelalan in the Limbang Division in 1997. A few years later, they spread to most of the rice growing districts in Sabah with a total infested area of 5000 ha. They were not so widespread in Sarawak; by 2011 the total infested area was only 278 ha. The scenes during the early invasion were alarming, with snails of various sizes and densities as high as 50 snails/m^2, and with the strikingly pinkish egg masses covering the stalks and stems of the vegetation in every newly infested area. Farmers panicked when their crops failed to grow and the unusual sight of bright pinkish colour appeared all over their fields. District agriculture offices became packed with farmers with samples of snails and egg masses to report the strange incidents. Many of these farmers applied chemicals arbitrarily against the snails, with variable effects. Some came and complained that the seeds supplied by the Department of Agriculture (DOA) were not good because they failed to germinate. The offices became busier than ever with crowds of farmers seeking help daily. Visits were then made to the farmers' fields and the same causal agent was identified in all places. In the beginning not much could be recommended to the farmers apart from handpicking because there was nothing in the literature and not much information could be gathered from neighbouring countries as they also faced the same problems. Often, a meeting was called by the district agriculture office to organize a handpicking operation in which every community member was encouraged to participate. Huge quantities of snails amounting to several hundreds of kilos were collected from each location. Farmers insisted that somehow the DOA should come up with a chemical for snail control. But at that time there was none and eventually someone suggested tea seed cake, which had been used by the Fisheries Department to remove unwanted fish in fishponds. This product was tested and proven effective by DOA, so it was used and is used to this day. While searching for information and control measures, the DOA also initiated a research program to develop control techniques against the pest. The research program adopted a user sensitive approach to enhance transfer of technology to the end users. Research findings were pooled to form an integrated pest management (IPM) package comprising cultural, biological and chemical components. The package was applied in the snail control operation launched by the DOA, which was implemented throughout the state of Sabah (Teo, 1999a).

Management strategy for apple snails in the past

Following the apple snail invasion, awareness campaigns consisting of briefing and dialogue sessions were organized repeatedly by DOA extension staff and research personnel to disseminate information to the farming community on the new rice pest. The main objectives were to motivate farmers to participate in the control operation and to enhance transfer of technologies. Participation of farmers was paramount to the success of the control operation as they were the primary source of manpower for implementing recommended research findings. Without farmers' participation, the control operation would not have been able to progress. In meet-the-farmer sessions, farmers were taught how to control the pest. A control program was formulated for the area where the meeting was held, usually covering water depth management, transplanting of older seedlings, handpicking by the community with or without herbage attractants, tea seed powder applications, installation of snail filters and duck herding. To start with, resource-poor farmers were provided with the materials they lacked, with the aim of encouraging them to participate in the control operation. For example, tea seed powder was given free to those farmers whose rice fields were heavily infested. The meeting sessions also provided an opportunity for the exchange of ideas and for receiving feedback from the farmers. The meetings were also venues for rectifying mistakes and clarifying confusion. For example, in the beginning, many farmers mistook the invasive apple snails for the local snails (*Pila* spp.), which are harmless to rice. They could not differentiate between them. After a series of briefings and field visits the differences were explained to them and they were willing to participate in the control operation.

A Pest Surveillance Committee was set up at the DOA headquarters in all districts and villages, with the objectives of supervising and monitoring the control operation. The committee consisted of DOA research and extension personnel and the farmers. The committees called for meeting monthly, initially, to assess the progress of the control operation. A leader would be picked from among the farmers and one extension agent was assigned to supervise the control program for each location. The extension agent would report the progress of the control operation to the district Agriculture Officer who would in turn report to the chairperson of the Pest Surveillance Committee at the DOA headquarters.

A census was conducted and snail population density estimated during the following pre-planting season to assess the effectiveness of the control operation. Snail density was estimated in 15 randomly located 1 m^2 quadrats. As backup support, mass media were used to disseminate information, together with flyers, posters and signboards, which were set up at strategic locations to enhance the control operation and to caution the public not to spread the snails.

Control measures developed for apple snails

Cultural control

The most common cultural controls practised by farmers were handpicking and transplanting of 40-day-old seedlings, with water depth maintained at 5 cm for the first few weeks. At booting, the plants become tolerant to the pest so the water level is raised to more than 10 cm at this stage. For direct seeding, the water in the field is drained to a saturated soil moisture condition followed by broadcasting pre-germinated seeds. The seeds are prepared by soaking in water inside a gunnysack for two nights and on the third day the sack is lifted from the water to drain off excess water. On the fourth day, the seeds are broadcast onto the field. If a machine is used to broadcast the pre-germinated seeds, the seeds should not be soaked for more than one night, as this results in a longer root that would be prone to damage when broadcast by the machine. Pre-germinated seeds are used so that the seedlings provide a complete ground cover and have an advantage over weeds. At day 28, water is introduced to a depth of 5 cm only and to a deeper depth when the crop reaches the booting stage. These methods are widely used by farmers because they are cheap and simple to apply. Handpicking is carried out occasionally through a community effort when a big crowd turns up to handpick the snails and egg masses. Handpicking is now easier with the use of herbage attractants. Leaves of papaya (*Carica papaya*), sweet potato (*Ipomea batatas*), tapioca (*Manihot esculenta*) and gliricidia (*Glyricidia sepium*) are potent snail attractants (Teo, 1999b). A small bundle of the leaves is submerged in stagnant water at the edge of the field. Significant numbers of snails are attracted to the leaves after 24 hours and are collected and destroyed the following day. This method significantly reduces the time and labour required in the normal handpicking operation.

While controlling snails in the field, filters such as wire mesh screens were set up at the water inlets to prevent re-infestation through the flow of snail-infested water. However, the smallest (newly hatched) snails are only 2 mm in size and only screens with mesh size smaller than 2 mm are 100 % snail proof. So this measure was not widely used because the filters quickly became clogged with dirt and rubbish. Once the filters are clogged, water will not flow and flooding will soon occur as the water overflows the banks of the irrigation canals. Unless manpower is available for cleaning the filters at frequent intervals, it is not practical to set up filters at the irrigation inlets.

During off-planting seasons farmers are encouraged to keep the fields dry to prevent snails from breeding. Dry ploughing and rotavating are carried out just before the pre-planting season to kill snails aestivating underground by exposing them to heat and dryness. After wet ploughing, farm machines are required to go through a sanitation procedure. After operating in an infested area, tractors and combine harvesters are required to be washed thoroughly at a washing pavement prepared by the district's agriculture extension personnel before moving on to their next destination. This regulation is enforced to curb the spread of apple snails by farm machines.

Biological control

Ducks are recommended to farmers for biological control of snails in rice fields at a density of 5 to 10 ducks/ha[1]. At these densities the ducks can reduce the pest population from 5 snails/m^2 to less than 1 snail/m^2 after 1 month. The recommended varieties of duck include William Siam > Taiwan > Mallard > Peking > Khaki Campbell in this decreasing order (Teo, 2001). However, ducks are damaging to young rice seedlings so should not be released when the seedlings are still young. For transplanted rice, the ducks can be released 4 weeks after transplanting and for direct seeding, 6 weeks after sowing. Ducks are also damaging to the crop at the ripening stage, so they must be removed from the fields when the crop matures. Biological control of snails with ducks is highly effective and consistent. Snails and their egg masses can hardly be noticed in rice fields in which duck herding has been practiced. Duck droppings also helped to fertilize the field.

Chemical control

Although chemical control is included in the IPM package, the Department of Agriculture discourages farmers from using chemicals because they are hazardous to the environment, and may also endanger public health. Chemical control is allowed only when the pest population density exceeds 5 snails/m^2 and when there is manpower shortage or when time is a constraint for other means of control. The chemicals recommended for snail control include tea seed powder, pellets of metaldehyde 5 % and niclosamide. Tea seed powder is recommended at 51 kg/ha in a stagnant water of depth 5-7 cm. Tea seed powder is a by-product of oil extraction from tea seeds. It contains 5.2-7.2 % saponin, which causes hemolysis in animals (Minsalan & Chiu, 1988). It is toxic to most aquatic organisms such as fish and frogs. However, its residual period is only 2-3 days. Pellets of metaldehyde 5 % are used when tea seed powder is not available. The recommended rate is 15 kg/ha but usually a smaller quantity is used because it is spot-applied in waterlogged areas or small ponds following field draining in the rice plots where there are snails. Niclosamide is rarely used unless the other two molluscicides are in short supply.

A trial on the screening of plants with molluscicidal properties identified a plant species known as yellow furcraea or wild sisal (*Furcraea selloa var. marginata*) that is highly effective against apple snails. Dry leaf powder of yellow furcraea is recommended for apple snail control at 45 kg/ha (Teo, 2002), much lower than the recommended rate of tea seed powder. The seedlings of this plant have been distributed to the farmers for planting in their backyards or in patches of uncultivated land.

Management of apple snails present and future

The DOA control operation managed to contain the pest within two years. The pest population density in most of the districts was less than 1 snail/m^2. At around the same time, natural enemies of the snails began to appear, even in our experimental plots. Thus a survey was carried out to identify and record as many as possible of the natural enemies of the snails. These organisms were not harmed in the control operation as the use of molluscicides was not prioritized because of their hazardous effects on the environment, particularly to beneficial species. Tea seed powder, which is harmful to frogs and fish, was widely used in the beginning, but because the residue of this product was short-lived (2-3 days) fish and other aquatic organisms could be seen again in the rice fields a few days later.

No natural enemies of the apple snails were noted in the rice fields at the start of the infestations. Attempts were made to identify natural enemies in the rice ecosystem but to no avail. It appeared that time was required for many species to discover the snails and shift their feeding preferences to them. The absence of natural enemies in the early phase of the invasion may explain why there was such a phenomenal increase in the pest population density in the newly infested areas. The natural enemies documented in East Malaysia (Teo, 2005) include the species listed in Table 1. Many of these species, such as the Fulvous Whistling-Duck, (*Dendrocygna bicolor*) and Asian Swamp Eel (*Monopterus albus*), have great potential for biological control of apple snails. Thus, a

Table 1. Natural enemies of apple snails in East Malaysia, from Teo (2005).

Mammal	Rice field rat (*Rattus argentiventer*)
Birds	Collared kingfisher (*Todirhampphus chloris chloroptera*) Domestic duck (*Anas platyrhnchos*) Fulvous whistling-duck (*Dendrocygna bicolor*) Purple swamphen (*Porphyrio porphyrio*)
Amphibian	Frog (*Rana rugulosa*)
Fish	Common carp (*Cyprinus carpio*) African catfish (*Clarias gariepinus*) Tilapia (*Oreochromis niloticus*) Kerok (*Abas testudineus*) Snakehead (*Ocephalus striatus*) Asian Swamp Eel (*Monopterus albus*)
Insects	Red ant (*Tridomyrmex myrmecodiae*) Fire ant (*Solenopsis geminata*) Stink bug (*Scotinophora cinera*)
Spider	Wolf spider (*Pardosa pseudoannulata*)

judicious use of pesticides is essential to preserve their natural habitat and to sustain the predatory activities of the beneficial species, which has been considered as an ecosystem service (K.L. Heong, unpublished). Many of these natural enemies are found in the areas infested with apple snails, such as ponds, lakes, swamplands, marshes, drains and streams, which are also the sources of water for irrigation of rice fields. Thus, if it is necessary to control the snail pest in these locations, duck herding is a good option if there are facilities to house the ducks. Fish are another potential biocontrol agent against snails in such water bodies. African Catfish (*Clarias gariepinus*) and Common Carp (*Cyprinus carpio*) are effective predators of apple snails but the former is not readily adaptable to rice field conditions (Teo, 2006). Ponds and lakes with larger area and deeper water may be more suitable for African Catfish. As it is also a carnivorous fish, it can probably defend itself better than Common Carp from other carnivorous fish species such as the Snakehead (*Ocephalus striatus*). The list of natural enemies of the invasive apple snails may not stop here. In years to come more beneficial species are expected to emerge if pesticides are used judiciously in the rice ecosystem. Under no choice conditions, pellets of metaldehyde 5 % are highly recommended, as no fish or frogs were killed when this product was used in the field. A new formulation is now available, which is more effective and environmentally friendly. In this survey, minute organisms were not included in the study. In fact some microorganisms are also emerging as beneficial species. A nematophagous fungus has been reported attacking the snails' egg masses (Maketon *et al*., 2009). Thus, ecosystem services should be exploited even more in the future in order to maintain the snail population density at a tolerable level. Molluscicides with long lasting residues should not be used so as not to harm and to preserve natural enemies.

Conclusions

Invasive apple snails are now under control in East Malaysia. The control operation comprising cultural, biological and chemical components was meant to cut down chemical applications but in some cases this could not be avoided, particularly in the beginning when no other control measures were available. Fortunately, only tea seed powder was used, which was more environmentally friendly than many of the synthetic chemicals. Fish, frogs and other aquatic organisms came back after a short while in fields to which tea seed powder was applied. We are glad that many natural enemies of the snails emerged shortly after the snails were brought under control. One of the reasons why so many natural predators appeared within a short period of time was probably associated with the slow movement of the pest, which made it vulnerable to them. The snail population density in most districts is now less than 1 snail/m^2 as a result of these ecosystem services. Chemical control is no longer necessary. Cultural and biological controls are highly recommended, as they are environmentally friendly and harmless to beneficial species. However, chemical application has become the norm for many farmers. If chemicals are the only choice, it is advisable to use environmentally friendly molluscicides such as pellets of metaldehyde 5 %. This product is partially resistant to water and if it is applied at an appropriate rate, most of the pellets are taken up by the snails with little left in the water to dissolve.

During the control operation, we felt that the people dimension, particularly the farmers, was the most important frontier. Many briefing and dialogue sessions were carried out with the aim to instil a spirit of cooperation among extension agents, researchers and farmers. These were once thought not necessary but as confusion, misconception and miscommunication became rampant, these were immediately reinstated. Cooperation among the different groups of people is paramount to the success of the control operation. When we meet more often, it helps us to know the farming community better and when friendship is established, communication becomes easier. Messages disseminate and spread faster when there is bonding among people, which facilitates cooperation and progress in the control operation.

The control measures introduced must match the capability and affordability of the farmers to implement them. Something that is difficult to use or expensive to apply will not be adopted by them. But once a problem crops up, the key is to discuss the problem with the farmers and listen to them. However, a control operation involving thousands of farmers may not always run smoothly. Problems such as poor farmer attendance in briefing sessions, preference for using chemical control over other control measures and refusal to implement the recommended control measures in their own fields can be expected. However, these problems were only seen among a handful of farmers. The more committed farmers usually implemented all the control measures recommended by the Department of Agriculture.

References

Acosta, B.O. & Pullin, R.S.V. (eds). 1989. Summary report of a workshop on environment impact of the golden apple snail (*Pomacea* sp.) on rice farming systems in the Philippines. International Centre for Living Aquatic Resources Management, Manila.

Anderson, B. 1993. The Philippines snail disaster. *The Ecologist* 23: 70-72.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CABI Publishing, Wallingford.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Maketon, M., Suttichart, K. & Domhom, J. 2009. Effective control of invasive apple snail (*Pomacea canaliculata* Lamarck) using *Paecilomyces lilacinus* (Thom) Samson. *Malacologia* 51: 181-190.

Matienzo, L.H. 1984. Wilson Ang's big foot snails. *Greenfields* 14: 24-29.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-622 pls.

Minsalan, C.L.O & Chiu, Y.N. 1988. *Effects of Teaseed Cake on Selective Elimination of Finfish in Shrimps Ponds. CRSP Research Report 88-11*. Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis.

Naylor, R. 1996. Invasion in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Teo, S.S. 1999a. Control of the golden apple snail in irrigated rice by integrated approach in Sabah. In: *Tropical Plant Protection in the Information Age. Proceedings of the 5th International Conference on Plant Protection in the Tropics. 15-18 March, 1999, Kuala Lumpur, Malaysia* (Sivapragasam, A., Ismail, A.A., Cheah, U.B., Chung, G.F., Chia, T.H., Omar, D., Ho, T.H., Lee, S.S., Lim, J.L., Lum, K.Y., Salleh, M., Ganapathi, N., Ong, C.A., Vijaysegeran, S. & Ahmad, Z.A.M., ed.), p. 84-88. Malaysian Plant Protection Society, Kuala Lumpur.

Teo, S.S. 1999b. Control of the golden apple snail (*Pomacea* spp.) by handpicking using herbage as attractants. In: *Sustainable Crop Protection Practices in the Next Millennium. Proceedings of the MCB-MAPPS Plant Protection Conference '99. 2-3 November, 1999, Kota Kinabalu, Sabah, Malaysia* (Sidek, Z., Bong, C.L., Kanapathipillai, V.S., Ong, C.A. & Kadir, H.A., ed.), p. 78-81. Malaysian Plant Protection Society, Kota Kinabalu.

Teo, S.S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* 20: 599-604.

Teo, S.S. 2002. Selecting plants with molluscicidal properties for the control of the golden apple snail *Pomacea canaliculata* (Lamarck). In: *Biopesticides: Positioning Biopesticides in Pest Management Systems. Proceedings of the 3rd International Conference on Biopesticides, 22-26 April 2002, Kuala Lumpur, Malaysia* (Mulla, M.S., ed.), p. 237-243. Malaysian Plant Protection Society, Kuala Lumpur.

Teo, S. S. 2003a. Aestivation behaviour of the golden apple snail (*Pomacea canaliculata*) under glasshouse conditions. *Sabah Agriculture Research Journal* 1: 75-80.

Teo, S.S. 2003b. Damage potential of the golden apple snail *Pomacea canaliculata* (Lamarck) in irrigated rice and its control by cultural approaches. *International Journal of Pest Management* 49: 49-55.

Teo, S.S. 2004. Biology of the golden apple snail, *Pomacea canaliculata* (Lamarck, 1822), with emphasis on responses to certain environmental conditions in Sabah, Malaysia. *Molluscan Research* 24: 139-148.

Teo, S.S. 2005. A survey on the natural enemies of the golden apple snail (*Pomacea canaliculata* Lamarck) in Sabah. *Sabah Agriculture Research Journal* 2: 81-88.

Teo, S.S. 2006. Evaluation of different fish species for biological control of golden apple snail *Pomacea canaliculata* (Lamarck) in rice. *Crop Protection* 25: 1004-1012.

Apple snails (*Pomacea* spp.) in Myanmar: current status and integrated management activities

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Abstract

Non-native apple snails were introduced to Myanmar in the early 1990s. They quickly spread to many of the states and regions of the eastern part of the country, becoming major pests of irrigated rice. A range of cultural, biological and chemical control measures have been used to control the snails, with hand picking being the most effective.

Additional keywords: Ampullariidae, biological control, Burma, chemical control, cultural control, IPM, Mollusca, pest, rice

Introduction

Apple snails, probably *Pomacea maculata* (Fig. 1) and perhaps also *P. canaliculata*, were first introduced to North Shan State, Myanmar, from the People's Republic of China in the early 1990s as food for humans. A few years after of the introduction, they had spread to many parts of the country via irrigated fields, canals, waterlogged areas and rivers, and had become major rice pests, causing damage in nurseries and to newly transplanted and direct-seeded rice.

Based on the monthly Plant Protection Division reports of pest incidence on various seasonal crops from all States and Divisions of Myanmar, by 2011 apple snails had spread to rice fields in both North and South Shan State, Kachin State, the eastern part of Bago Region, Mon State, Kayin State and Tanintharyin Region, and in 2013 they were found in rice in Ayeyarwaddy Division (Table 1). There was an especially serious outbreak in Kayin State in 2011 monsoon paddy. Many egg masses can also be seen in Inle Lake in South Shan State.

Fig. 1. *Pomacea* sp., probably *P. maculata* in Myanmar. Left, female laying eggs; right, mating pair; insert, a single egg.

Table 1. Total area (ha) of rice-growing areas in Myanmar infested by apple snails, by state or region.

State/Region	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016
Mon	230	12	15	38	6
Shan (North)	84	14	123	1	-
Kachin	27	69	16	22	50
Kayin	52	66	17	13	12
Bago	13	-	-	-	13
Tanintharyi	6	-	2	4	0.2
Ayeyarwaddy	-	-	49	272	189

Current management activities

Both the public and private sectors are aware of the apple snail infestations in paddy fields and non-rice habitats in the above regions of Myanmar. Generally, apple snail management involves farmer training and development of leaflets by Plant Protection technicians, with funds coming from Government. Leaders of local government have also organized annual community snail control campaigns involving soldiers, students and large numbers of farmers to reduce snail populations in community waterways by hand picking. The snails are collected in the morning and evening when they are most active and easy to find and then destroyed.

As in other countries, various cultural practices are implemented to reduce apple snail numbers. Snails are trapped using baits such as the leaves of papaya, banana and eucalyptus, and water melon peel. These are put on bamboo or plastic netting that is placed alongside the bunds of the rice fields. Large numbers of snails can then easily be destroyed. Bamboo stakes are also placed in water-logged areas or near the rice field irrigation canals and any egg masses laid on these stakes are readily seen and destroyed.

Young transplanted seedlings are most vulnerable during the first ten days after transplanting. Planting older seedlings therefore helps to minimize damage. Snails prefer to be in standing water, so that alternately draining and irrigating the rice fields after transplanting helps to reduce their activity. Ditches are constructed within the field and the snails congregate in them and can be easily collected and destroyed. To prevent snails spreading from field to field, screens are set up at the water inlets and outlets.

Biological control may involve ducks and/or fish. Ducks are pastured in the rice field at a density of about 200 ducks/ha (80/acre) 30 days after transplanting. Rice-fish farming can also be practiced using fish that prey on the snails. Stakes that snail-eating birds can use as perches are placed near the bunds of rice fields in waterlogged areas.

Niclosamide 70 WP applied at a rate of about 440-590 g/ha (180-240 g/acre) and Metaldehyde 5G at a rate of about 4.4-5.9 kg/ha (1.8-2.4 kg/acre) are used for chemical control. Neem cake, Jatropha cake and tobacco leaves as botanical pesticides are broadcast at the rate of about 450 kg/ha.

Conclusion

In 2011-2012, the heaviest apple snail infestation occurred in Kayin State and the lightest in Taninthayi Region. However, in 2012-2013, infestations only occurred in Kachin, Kayin and Shan (North) States. Infestations were first noted in Ayeyarwaddy Division in 2013. The infested areas were reduced in Kayin State using IPM strategies. Overall in Myanmar, among the control measures mentioned above, hand picking is most widely implemented and can control 80 % of the snail populations.

Country report (Pakistan) on non-native apple snails

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Abstract

Apple snails in the genus *Pomacea* are not native to Pakistan. They were first discovered in Pakistan in 2009, in Haleji Lake, Sindh. Initially, the species was identified as *P. canaliculata* but subsequently this was corrected to *P. maculata*. The introduction of these snails to Haleji Lake was a result of the aquarium trade. The snails were imported to Pakistan by an aquarium fish trader and were raised in the vicinity of Haleji Lake, from where they were introduced to the lake. In a few years they completely invaded the lake, establishing populations throughout the lake as a result of their rapid growth and high rate of egg production. Since then they have spread beyond the lake and reached rice fields. However, dense populations of the snails are not evident so far in the paddy fields and there has been no record of agricultural losses due to apple snails as yet. There is need for stricter controls and enhanced education as a priority to prevent their future invasion of highly economically valued agricultural fields.

Additional keywords: Ampullariidae, invasive species, Mollusca, *Pomacea*

Introduction

The genus *Pomacea* is native to South and Central America, parts of the Caribbean, and the south-eastern USA. Species of *Pomacea* have become widely established in many areas in Southeast Asia, Sri Lanka, Guam, Hawaii, Papua New Guinea, the Dominican Republic, parts of the mainland USA and Australia, mostly as a result of escape or release from aquaculture operations or through the pet trade (Rawlings *et al*., 2007; Hayes *et al*., 2008, 2012).

The two most important invasive species of *Pomacea* in Asia are *P. canaliculata* and *P. maculata* (*P. insularum* is a junior synonym of *P. maculata*) (Hayes *et al*., 2008, 2012). *Pomacea canaliculata* is native to Argentina and Uruguay, while *P. maculata* is more widely distributed from the La Plata region of Argentina to the Amazon basin of Brasil, including Uruguay and Paraguay, and possibly Bolivia, Ecuador and Peru (Hayes *et al*., 2012). Often these two species have not been distinguished in Asia, with much of the literature referring to them as just *P. canaliculata*. They became major agricultural pests, especially of wetland rice, resulting in great economic losses (Naylor, 1996). They have a high rate of reproduction (Hayes *et al*., 2015) and can survive harsh environmental conditions such as pollution (Lach & Cowie, 1999) or low oxygen levels (because they can breathe air).

Among the countries of southern and eastern Asia, only the subcontinent (Pakistan, India, Bangladesh and Sri Lanka) had until recently not been invaded by *P. canaliculata* or *P. maculata*, although they constantly faced the threat of invasion. Another species, *Pomacea diffusa*, is present in Sri Lanka (Hayes *et al*., 2008).

Discovery of *Pomacea* in Pakistan

However, a species of *Pomacea* was discovered in Pakistan in 2009 and identified as *P. canaliculata* (Baloch *et al*., 2012). It is now known that this identification was incorrect due to unavailability of detailed taxonomic information at that time, and the species is in fact *P. maculata*. The present identification is based on the distinguishing characters described by Hayes *et al.* (2012). These characters include the large size and reddish colour inside the aperture (Fig. 1), and the size of the eggs and number of eggs per clutch.

Fig. 1. *Pomacea maculata* from Haleji Lake, Sindh, Pakistan.

The population of *P. maculata* was found in Haleji Lake, Sindh (24°47.230 N, 67°45.435 E; Figs. 2, 3), at an altitude of 20 m above sea level during a limnological survey. Originally the lake was a salt-water lake. During World War II, additional water was required for troops stationed in Karachi. The salt water was drained out and an embankment was constructed around the lake, which was fed by fresh water through a canal. As a result, Haleji Lake became one of the major sources of water for the increasing population of Karachi as well as an exquisite refuge for water-fowl. The complete circumference of the lake is about 19 km. The main reservoir covers an area of about 10.5 km^2 and the maximum depth is about 5 m. The lake was declared a wildlife sanctuary in 1977. It is a Ramsar site and hunting, fishing and recreational activities are prohibited.

The introduction of *P. maculata* to Haleji Lake was a result of the aquarium trade. The snails were imported to Pakistan by an aquarium fish trader in the area and were raised along with

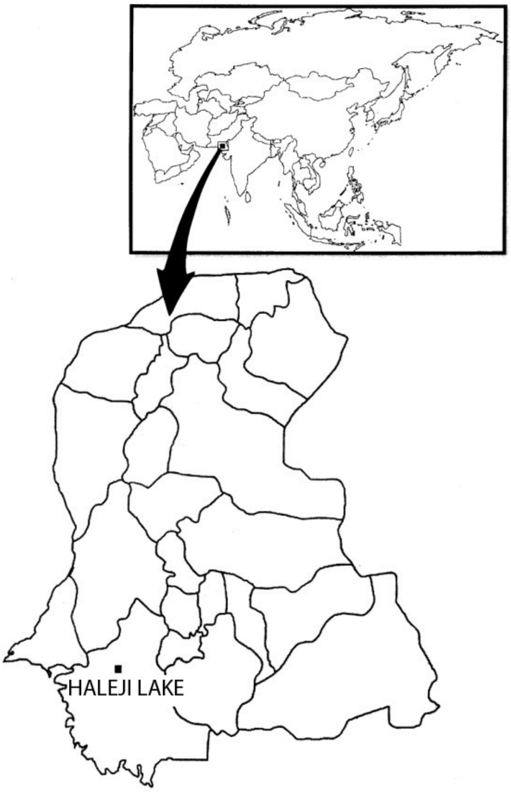

Fig. 2. Sindh Province, Pakistan, showing the location of Haleji Lake.

Fig. 3. Haleji Lake, Sindh, Pakistan.

aquarium fish for sale. However, some snails were taken by an unknown person and introduced to the lake, with the fallacious idea that they would produce pearls. There is now a well-established snail population throughout the lake. The impact of the snails on the lake ecosystem and on local fishers has not yet been studied.

Observations on *Pomacea maculata* in Haleji Lake

Baloch *et al*. (2012) reported observations on 17 live specimens collected from shallow water in the lake. Maximum shell height (length) of the collected specimens was 80 mm. The shells were dull yellow to brown, with longitudinal darker bands. In younger animals the bands were wider than in older ones relative to shell size. The colour of the largest snails was more yellowish golden with a brown tinge.

In laboratory aquaria, the snails were generally fed in the morning; as soon as food was provided they started to move towards it and began to feed. Generally, they preferred broad leaved plants. The snails inhaled air with the siphon when grazing on the surface (surface film feeding; Saveanu & Martín, 2013). When at rest the snails were either settled on the aquarium bottom or sometimes they floated at the surface. Copulation generally began in the morning and lasted more than 2 h, sometimes much longer. Eggs were laid mostly at night but sometimes during the day or evening, with egg-laying taking about 3-4 h to complete (Fig. 4). The egg masses were laid about 7-10 cm above the water on the aquarium sides. Clutches averaged 300-400 eggs. The female uses her foot to make a canal or groove in which the eggs are transferred by muscular movement. The snails would feed on their own eggs when there was a shortage of food. When the snails were maintained in bulk in one large aquarium, they never bred. Most eggs

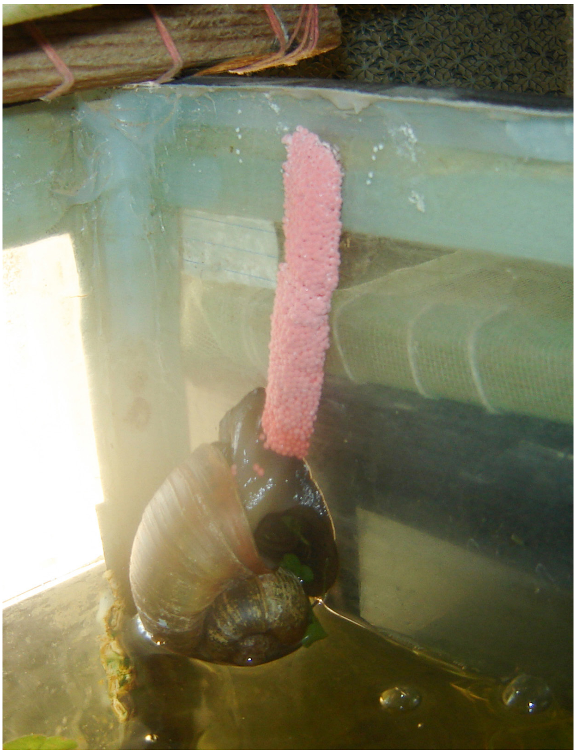

Fig. 4. *Pomacea maculata* laying eggs in an aquarium.

hatched in 1-2 weeks, although it could take up to about 20 days for all eggs in a clutch to hatch.

Ecological role, use and significance of invasion

In general, apple snails are herbivores with a wide range of food plants (e.g. rice seedlings, taro, duck weed, water hyacinth, algae, azolla and other succulent leafy plants), often preferring young, soft plants (Cowie, 2002; Hayes *et al*., 2015). However, they will also eat eggs and juveniles of other snails, other invertebrates, and decomposing organic matter, including carrion (Hayes *et al*., 2015). They are preyed upon by many other animals, including ants, dragonfly larvae, other insects, spiders, various birds, numerous fish and rats (Yusa, 2006). Some of these animals, such as carp or ducks, are intentionally introduced to control the snails (Joshi & Sebastian, 2006) as few natural predators may be present in the non-native range of the snails.

Apple snails are eaten by people and animals, and used in aquariums, but not to the beneficial extent intended. They have little commercial value as a human food source. In Pakistan, these snails could be used as raw food material for poultry and fish feed. They could perhaps also be developed as bio-indicators of water pollution from pesticides and trace metals (Hayes *et al*., 2015).

There are also human health threats associated with *Pomacea maculata*. It and other apple snail species are vectors of disease-causing parasites such as the rat lungworm, *Angiostrongylus cantonensis*, which causes the sometimes fatal eosinophilic meningitis (or meningoencephalitis) in humans (Hayes *et al*., 2015). It may also contribute to skin irritations by acting as an intermediate host of trematodes and it may also act as a host of the trematodes causing human echinostomiasis (Hayes *et al*., 2015). In Pakistan no study on human health threats from *Pomacea maculata* has been undertaken.

Pomacea canaliculata is listed among 100 of the world's worst invasive species (Lowe *et al*., 2000). This list was created prior to the full clarification of the distinction in Asia of *P. maculata* from *P. canaliculata*, and the widespread occurrence of *P. maculata* (Hayes *et al*., 2008). Both species should be considered highest priority based on their wide distribution in many countries and their potential to destroy crops and harm people. Stopping the spread of *P. maculata* in Pakistan with stricter controls and enhanced education should be a priority.

In many countries, diverse control measures have been used (Cowie, 2002). Various biological control methods have been tried, including the use of ducks and fish such as carp and Nile tilapia. Some plants with toxic substances, if grown in the rice paddies, or left floating near the snails, are being used. The most effective control method seems to be hand-picking the snails, but this is labour-intensive. In Pakistan these snails have expanded beyond Haleji Lake to nearby rice fields but dense populations of the snails have not yet become established and no estimates of agricultural losses are so far available. Therefore, hand picking in the paddy fields is being used and no use of chemicals or pesticides is so far recorded.

References

Baloch, W.A., Memon, U.N., Burdi, G.H., Soomro, A.N., Tunio, G.R. & Khatian, A.A. 2012. Invasion of channeled apple snail *Pomacea canaliculata*, Lamarck Gastropoda: Ampullariidae) in Haleji Lake, Pakistan. *Sindh University Research Journal (Science Series)* 44: 263-266.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed), p. 145-192. CABI Publishing, Wallingford.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.-W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Pilar Cadierno, M., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2013. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Joshi, R.C. 2007. Problems with the management of the golden apple snail *Pomacea canaliculata*: an important exotic pest of rice in Asia. In: *Area-wide Control of Insect*

Pests (Vreysen, M.J.B., Robinson, A.S. & Hendrichs, J., ed.), p. 257-264. Springer, Dordrecht.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija.

Lach, L. & Cowie, R.H. 1999. The spread of the introduced freshwater apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) on O‘ahu, Hawai‘i. *Bishop Museum Occasional Papers* 58: 66-71.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species*. Invasive Species Specialist Group, Auckland.

Naylor, R. 1996. Invasion in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology* 7: 97. doi:10.1186/1471-2148-7-97

Saveanu, L. & Martín, P.R. 2013. Pedal surface collecting as an alternative feeding mechanism of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Journal of Molluscan Studies* 79: 11-18.

Yusa, Y. 2006. Predators of the introduced apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae): their effectiveness and utilization in biological control. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 345-361. Philippine Rice Research Institute, Nueva Ecija.

South American apple snails, *Pomacea* spp. (Ampullariidae), in Singapore

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Abstract

South American apple snails, *Pomacea* spp. (Ampullariidae) have been established in Singapore since the late 1980s. Based on molecular analyses and shell morphology, two species of *Pomacea* (*P. canaliculata* and *P. maculata*) have been found. The introduction of *Pomacea* to Singapore was probably accidental via the aquarium trade. Their current status and distribution in Singapore are summarised herein. In highly urbanised Singapore, the species have not caused obvious environmental damage, in contrast to the agricultural damage they have caused in rice plantations throughout neighbouring Southeast Asian countries. Nevertheless, the introduced *Pomacea* are now widespread throughout the island city-state, and may be competing with the native Southeast Asian ampullariid, *Pila scutata*, which has declined since the arrival of *Pomacea*. Other potential impacts of *Pomacea* spp. in Singapore include modification of wetland habitat and acting as vectors of human disease. Some measures to remove *Pomacea* in localised areas have been initiated. However, eradication of these introduced apple snails appears difficult, if not impossible.

Additional keywords: introduced, invasive, gastropod, freshwater, Mollusca

Introduction

There are some 25 species of freshwater gastropods in the island city-state of Singapore, the largest of which are species of the family Ampullariidae (Tan & Woo, 2010; Tan *et al*., 2012). There are currently at least three species of ampullariids extant in Singapore: the native Southeast Asian species *Pila scutata*, and two introduced South American species *Pomacea canaliculata* and *Pomacea maculata* (Ng *et al*., 2014; Fig. 1). *Pomacea canaliculata* has been listed among 100 of the world's worst invasive species (Lowe *et al*., 2000) and is typically regarded as a pest in countries where it has been introduced, damaging crops and threatening native ecosystems (Naylor, 1996; Cowie, 2002; Carlsson *et al*., 2004). Although introduced apple snails are most commonly called *Pomacea canaliculata*, more than one species is in fact present in Asia (Hayes *et al*., 2008, 2012).

Hayes *et al*. (2008) had identified *Pomacea* species from Singapore to be *Pomacea maculata* (as *Pomacea insularum* in Hayes *et al.* 2008), but the specimens used in that study were obtained from a local aquarium shop rather than from locally established populations (R.C. Joshi, pers. comm.). The aquarium shops in Singapore mostly sell imported non-native species, rather than locally collected ones (Ng, pers. obs.). While molecular methods have previously been the most effective means of distinguishing between *Pomacea canaliculata* and *Pomacea maculata* (see Hayes *et al*., 2012), in Southeast Asia, the situation has recently become more complicated, as introduced populations of the two species appear to be hybridising (Matsukura *et al*., 2013). Snails collected in Singapore were

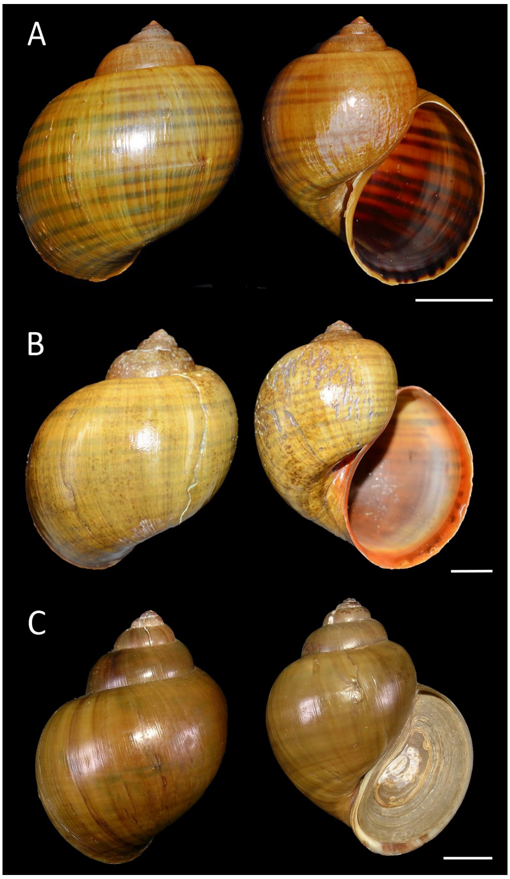

Fig. 1. Ampullariidae in Singapore. A: *Pomacea canaliculata*; B: *Pomacea maculata*;C: *Pila scutata*. Scale bars 10mm.(Photos: S.K. Tan [A and C] and T.H. Ng [B])

recently re-examined, and individuals of *Pomacea maculata* and *Pomacea canaliculata* were distinguished based on both conchological characteristics and molecular methods (Ng *et al*., 2014; unpublished data).

Unlike neighbouring Southeast Asian countries, e.g. Malaysia (see Yahaya *et al*., 2006), where the species have been documented to cause damage to rice fields, *Pomacea* species have not caused similar problems in highly-urbanised Singapore, which does not have large-scale agriculture. Nonetheless, the introduced snails may be impacting the confamilial species *Pila scutata* (Fig. 1). This article reviews the introduction of *Pomacea* to Singapore, its current status and distribution, its possible environmental impacts, especially on native biodiversity, and existing management approaches to control the species.

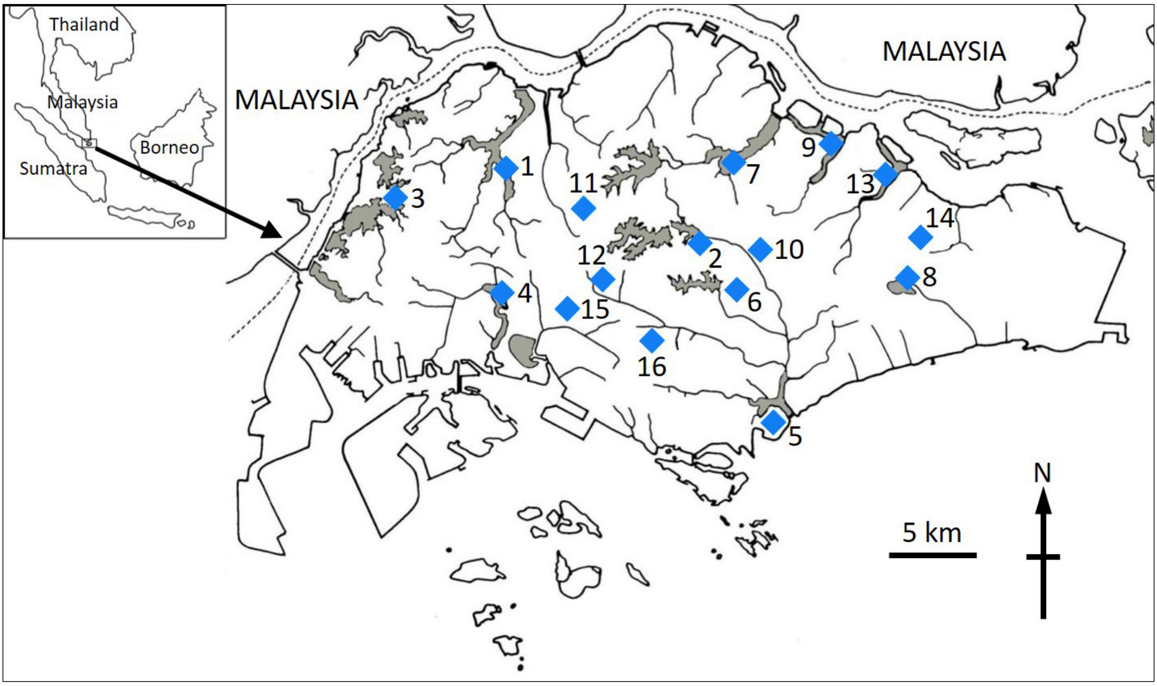

Fig. 2. Approximate localities where *Pomacea* specieshave been recorded in Singapore. 1, Kranji Reservoir and tributaries (Kranji marshes, Sungei Tengah); 2, Lower Peirce Reservoir; 3, Western Catchment Reservoirs; 4, Jurong Lake and tributaries (Sungei Jurong; Sungei Lanchar); 5, formerly Marina City Park, now Gardens by the Bay; 6, Upper Thomson Road; 7, Lower Seletar Reservoir and tributaries (Sungei Seletar, Sungei Seletar Simpang Kanan); 8, Bedok Reservoir; 9, Punggol Reservoir and tributaries (Seletar Wet Gap); 10, Bishan-Ang Mo Kio park; 11, Pang Sua pond; 12, Singapore Quarry; 13, Serangoon Reservoir; 14, Tampines Eco Green; 15, Bukit Batok Nature Park; 16, Botanic Gardens.

Status and distribution

Pomacea has successfully spread throughout Singapore over the past two decades (Fig. 2). Until recently, it was assumed that all *Pomacea* in Singapore were *Pomacea canaliculata*. The presence of both *Pomacea canaliculata* and *Pomacea maculata* among established populations in Singapore has since been confirmed by sequencing the mitochondrial CO1 gene of a few individuals (Ng, unpublished). The results also indicated that both species were present in at least one locality, and it is likely that many other *Pomacea* populations consist of mixed species. Without extensive molecular sequencing of all populations in Singapore, it is currently impossible to determine the species or species composition at localities where the snails are established (see also Ng *et al*., 2014).

Pomacea are commonly found grazing at the littoral zone of reservoirs and in shallow water along the edges of canals. Females leave the water to lay eggs, and clusters of these bright pink eggs can often be found on the surfaces of rocks or concrete banks, and on

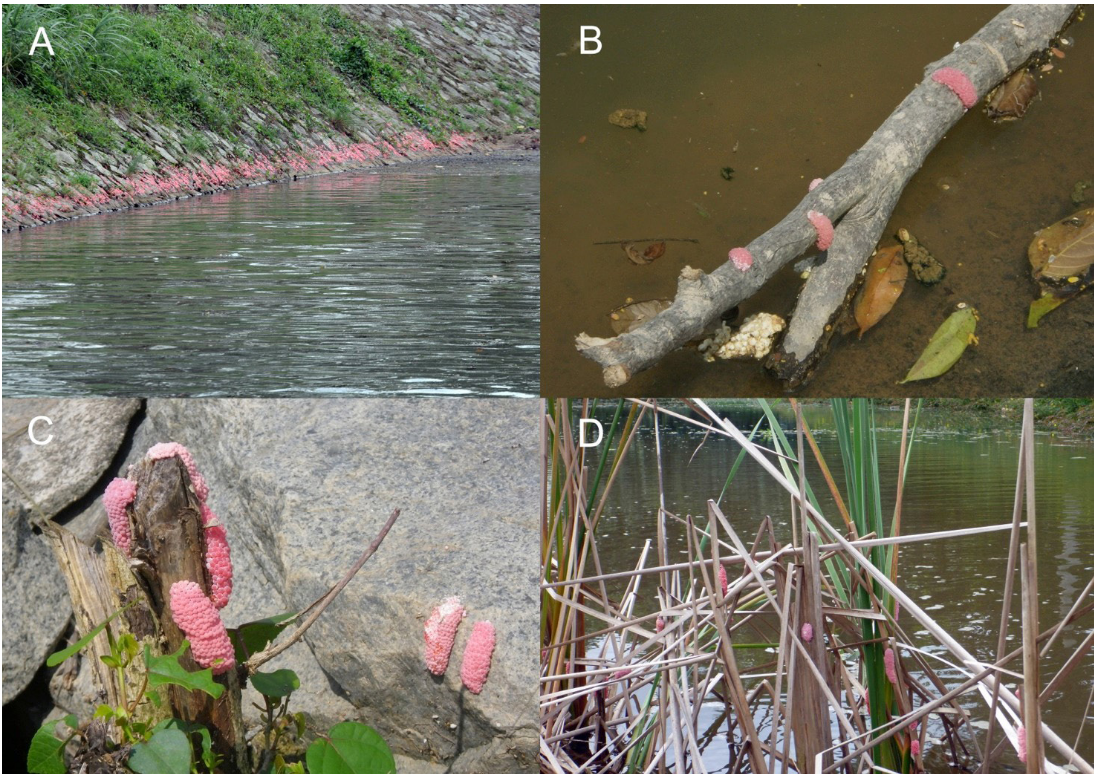

Fig. 3. Habitats where *Pomacea* are found in Singapore. A,B, canals; C, banks of reservoirs; D, freshwater marshes.(Photos: H.H. Tan [A], S.K. Tan [B andC],T.H. Ng [D]).

emergent vegetation (Fig. 3). Various animals have been reported to prey on *Pomacea* species, including numerous birds, e.g. crows and ducks (Yusa, 2006). In Singapore, the snails were recently reported to be preyed on by the Asian openbill, *Anastomus oscitans*, a species of stork that specialises on molluscan prey and that has apparently spread into the Malay Peninsula from Thailand, thanks in part to the success of introduced *Pomacea* in the region (Low *et al*., 2013). Although *Pomacea* eggs contain anti-predatory chemicals (Dreon *et al*., 2010), they are also eaten by insects (Yusa, 2001, 2006). A terrestrial snail, *Bradybaena similaris*, was observed apparently feeding on a cluster of *Pomacea* eggs on the banks of a reservoir in Singapore, and another snail, *Quantula striata*, was subsequently recorded in the laboratory to be able to feed on the eggs (Ng & Tan, 2011).

In spite of predation pressure, populations are widespread and mainly established in modified, urban freshwater habitats. *Pomacea* are now found in at least 13 of the 17 reservoirs in Singapore, their associated tributaries and canals, and in various artificial ponds in parks and golf courses around the island (Ng *et al.*, 2014; Ng, unpublished; Fig. 3). These apple snails do not appear to have any particular preference for type of habitat; they thrive at sites regardless of bank type-granite rock walls or more natural vegetated shorelines (e.g. Lower Seletar and Kranji Reservoirs) as well as steep concrete banks (e.g. canals and storm-water drains [Pang Sua pond]), or bottom substrate type-soft muddy (e.g. Jurong Lake), sandy (e.g. Sungei Seletar Simpang Kanan) or concrete (e.g. canals). Records from the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum at the National University of Singapore, published literature and unpublished data (including personal communications) were reviewed, as summarised below.

Museum specimens examined

Sungei Kranji, upper stream: ZRC1989.2295–1989.2296, 2 Sep 1988; Kranji Reservoir, upstream: ZRC1990.843–1990.851, 2 Sep 1988; Sungei Tengah Road: ZRC1990.17328–1990.17331, 24 Feb 1989; Kranji Reservoir: ZRC1990.17012–1990.17039, ZRC1990.17049–1990.17054, ZRC1992.3111, 5 Oct 1989; Jurong Lake: ZRC1991.19276–1991.19280, Apr 1991; Marina City Park, pond: ZRC1995.676, 28 May 1995; Chinese Gardens: ZRC.MOL.8, 2 May 2002; Lower Seletar Reservoir: ZRC.MOL.9, 24 May 2002; Upper Thomson Road: ZRC.MOL.12, 29 Jun 2002; Kranji

Reservoir: ZRC.MOL.7, 7 Jul 2002; Jurong Lake: ZRC.MOL.11, 11 Jul 2002; Lower Peirce Reservoir: ZRC.MOL.10, 8 Aug 2002.

Literature records

Ng, 1991: 130 (remarks on the species or local distribution; sold in aquarium trade); Ng *et al*., 1993 (prefers open areas); Chan, 1996 (shares same habitat as *Pila scutata*); Maassen, 2001 (as *Pomacea lineata*); Goh *et al*., 2002 (able to survive harsher environments than *Pila scutata*); Clements *et al*., 2006 (in reservoirs and rural streams); Hayes *et al*., 2008 (as *Pomacea insularum*, Singapore specimens obtained from aquarium trade [R.C. Joshi, pers. comm.]); Tan *et al*., 2012 (found in most reservoirs and tributaries); Ng *et al*., 2014 (a review of the family Ampullariidae in Singapore, including *Pomacea canaliculata* and *Pomacea maculata*).

Other records

Lower Seletar Reservoir, reservoir with granite banks, 8 Mar 2007, 4 Mar 2009 (Ng & Tan, pers. obs.); Poyan Reservoir, reservoir with granite and natural banks, 13 May 2009 (Ng & Tan, pers. obs.); Kranji Reservoir, reservoir with granite and natural banks, 3 Jun 2009 (Ng & Tan, pers. obs.); Murai Reservoir, reservoir with granite banks, 2 Dec 2009 (Ng, pers. obs.); Sarimbun Reservoir, reservoir with granite and natural banks, 8 Dec 2009 (Ng, pers. obs.); Tengeh Reservoir, reservoir with granite and natural banks, 31 Mar 2010 (Ng, pers. obs.); Seletar Wet Gap, tributary of Punggol Reservoir, 20 Nov 2011 (Ng, pers. obs.); Kranji marshes, freshwater marshes, adjacent to Kranji Reservoir, 23 Dec 2011 (Ng & Tan, pers. obs.); Sungei Buloh Wetland Reserve, freshwater ponds, 19 Jan 2012 (National Parks staff, pers. comm.); Sungei Pang Sua, storm-water pond, 12 Mar 2012 (J.T.B. Kwik, pers. comm.); Sungei Tengah Road, concrete canal, 26 Apr 2012 (Ng, pers. obs.); concrete canal off Bishan Street 14, 27 Jul 2012 (J.T.B. Kwik, pers. comm.); Chinese Gardens, artificial ponds in park, 15 Aug 2012 (T.D.L. Lee, pers. comm.); Sungei Seletar Simpang Kanan, canal with natural banks, leading to reservoir, 24 Oct 2012 (J.T.B. Kwik, pers. comm.); Sungei Jurong, canal with natural banks, 26 Nov 2012 (J.K.I. Ho, pers. comm.); Raffles Country Club, golf course ponds, 10 Jan 2013 (Yeo, pers. obs.); Serangoon Reservoir, 12 May 2013 (Tan, pers. obs.); Gardens by the Bay, artificial pond in park, 31 May 2013 (D.J.J. Ng, pers. comm.); Sungei Lanchar, concrete canal, off Jurong Central Park, 3 Jun 2013 (Ng, pers. obs.); Sungei Seletar, canal with natural

Fig. 4. Other species of Ampullariidae found in the aquarium trade in Singapore. Left, *Pomacea diffusa*; right, *Marisa cornuarietis*. Scale bars 10mm. (Photos: T.H. Ng)

banks, 11 Jun 2013 (Yeo, pers. obs.); Singapore Quarry, artificial lake, remnant granite quarry, within nature reserve, 12 Jun 2013 (Ng, pers. obs.).

Pathways of introduction and spread

Numerous sources have erroneously at times considered *Pomacea canaliculata* to range throughout much of South America east of the Andes from temperate Argentina to the Amazon basin (e.g. Cazzaniga, 2002; Cowie & Thiengo, 2003), with some suggesting it was introduced to parts of Southeast Asia from the Amazon (e.g. FAO, 2015). These misconceptions stem from the inability to distinguish it from other *Pomacea* species until the late 2000s. In fact, the native range of *Pomacea canaliculata* is much more restricted, to the Lower Paraná, Uruguay and La Plata basins (Hayes *et al*., 2012). It was initially introduced to Asia from Argentina (Hayes *et al*., 2008). *Pomacea maculata* has a wider native range, overlapping with *Pomacea canaliculata* in the northern part of the range of the latter and extending to the Amazon basin (Hayes *et al*., 2012). *Pomacea maculata* was introduced to Asia from Argentina or southern Brasil (Hayes *et al*., 2008).

Pomacea originally appeared in the wild in Singapore in the late 1980s, with the first specimens collected in 1988 in the northwest, at Sungei Kranji (presumably part of Kranji

Reservoir, see Fig. 2) (ZRC1989.2295–1989.2296). By 1989, the snails had already established in a canal leading to Kranji Reservoir, though they were misidentified as *Pila* species in an unpublished report (Xu *et al*., 1989). The first published record of *Pomacea* in Singapore was by Ng (1991). Museum records are the only other source of information regarding the timing of the snails' initial introduction to Singapore. The specific identity of the initial introduction(s), whether *Pomacea canaliculata* or *Pomacea maculata*, is not known.

The aquarium trade is believed to be the main pathway for the introduction of the established *Pomacea* species to Singapore (Ng, 1991; Yeo & Chia, 2010). The golden-coloured varieties identified probably correctly as *Pomacea canaliculata*, but also possibly *Pomacea maculata*, were first recorded in the trade (Ng, 1991), possibly during the early 1980s (H.E. Ng, pers. comm.) and are still sold. Other ampullariids were later imported for sale in the aquarium trade, *Pomacea diffusa* (since late 1995) and more recently *Marisa cornuarietis* (Fig. 4), but these have thus far not been found in the wild. A *Pomacea* species, identified as *Pomacea canaliculata*, has also been successfully cultivated in Singapore by the Agri-Food and Veterinary Authority (AVA, the government agency responsible for food safety and the health of food and ornamental animals and plants) from at least the mid-1990s, apparently for the live food trade (Tan & Ho, 2001). The snails are not commonly eaten in Singapore, although the cultured golden-coloured varieties are occasionally available at local seafood restaurants (Ng *et al*., 2014).

Pomacea species are currently very common and widespread in Singapore (Fig. 2). Many aquatic introductions in Singapore have been attributed to intentional releases by religious devotees seeking spiritual merit (Yeo & Chia, 2010), though there are no documented cases of people releasing apple snails for this purpose. Although local introduction pathways have yet to be studied, it is likely that the snails have been introduced into Singapore's fresh waters accidentally, either escaped from cultivation ponds or introduced via the disposal of aquatic plants or aquarium water into waterways (Ng *et al*. 2015). Eggs and snails may also be introduced together with ornamental aquatic plants that are planted in various water bodies for landscaping purposes (Tan *et al.* 2012).

Once introduced, *Pomacea* could have spread via active and passive (human-or animal-mediated) means (cf. Cowie & Robinson, 2003). Modified waterways are major anthropogenic dispersal pathways for alien aquatic species (Lintermans, 2004; Leuven *et al*., 2009). *Pomacea canaliculata* is able to actively disperse by moving upstream or downstream, over considerable distances, even in flowing waters (Seuffert & Martín, 2012). The current Singapore landscape of highly connected waterways and managed inter-reservoir water transfers (Fielding *et al*., 2010) may be important in aiding the dispersal of species. Apple snails could also have been spread by the introduction of aquatic plants for ornamental purposes (Cowie & Robinson, 2003). Juvenile snails may be accidentally translocated by recreational activities, for instance associated with fishing gear being brought from one water body to another (Alonso & Castro-Díez, 2008), or by hitchhiking on other animals or birds (Van Leeuwen *et al*., 2013). The physico-chemical characteristics of waterways may also influence the spread of snails (Schreiber *et al*., 2003; Kwong *et al*., 2008). Further work is required to better understand the dispersal mechanisms of these species in Singapore.

Potential impacts on native species

Pomacea canaliculata can prey on smaller species of snails (Kwong *et al*., 2009). Populations of the Southeast Asian native confamilial *Pila* species have been reported to be declining because of the introduced *Pomacea*, although relying on anecdotal accounts (Halwart, 1994), and some declines may be due to the use of molluscides to eradicate *Pomacea* (Anderson, 1993). Marwoto *et al*. (2011), however, reported complete replacement of *Pila virescens* (as *Pila polita* in Marwoto *et al.*, 2011; for explanation of the correct name see Cowie & Héros [2012]), last collected in the 1970s, by the invasive *Pomacea canaliculata* in an Indonesian swamp. There may have been competition even where the species of *Pila* and *Pomacea* are both introduced. For instance, in Hawaii, *Pomacea canaliculata* is a widespread invasive species on many of its islands, but *Pila scutata* (also introduced) is only found on one island where *Pomacea canaliculata* is absent (Cowie *et al*., 2007; as the junior synonym *Pila conica*). It is possible that *Pila* were unsuccessful in establishing on other islands because of competition from *Pomacea* (Cowie *et al*., 2007). In Singapore, *Pila scutata* populations and numbers have been declining, seemingly coincident with the arrival of *Pomacea* species, circumstances that suggest the latter may be outcompeting *Pila scutata* (see also Chan, 1996; Tan & Yeo, 2010; Tan *et al*., 2012; but see below).

Nonetheless, there is thus far no direct evidence or clear consensus as to whether the introduced *Pomacea* species have outcompeted species of *Pila*, or whether the decline of *Pila* species has been caused by habitat loss instead or as well (see Ng *et al*., 1993; Chan, 1996; Tan *et al*., 2012). *Pila scutata* was formerly widespread and commonly found in ponds and ditches in Singapore (e.g. Ponniah, 1962; Johnson, 1973; Xu *et al*., 1989). Surveys in the 2000s revealed that the species remained extant in only a small number of localities (Tan *et al*., 2013). Recent records at some sites were of single empty shells, while populations have not been recorded in recent years from localities that were sampled prior to 2009. Wide-scale modification of freshwater habitats, especially into concrete-lined canals, have been proposed as one reason for the disappearing populations of *Pila scutata* (Ng *et al*., 1993; Chan, 1996). Reservoirs have been suggested as important for the conservation of freshwater molluscs (Clements *et al*., 2006), but at least one type of habitat modification, granite rock wall banks (see Fig. 3C), which is prevalent in Singapore and has occurred in many reservoirs, may not be favoured by *Pila scutata*, which appears to prefer shaded, natural vegetated banks (Tan *et al*., 2013; Ng *et al*., 2014).

Land use change alone, however, does not satisfactorily explain the declining numbers of *Pila scutata*. The species is pollution-tolerant and used to be found in degraded habitats like septic ponds well before the introduction of *Pomacea* in the late 1980s (Ponniah, 1962; Johnson, 1973). Suitable habitats for *Pila scutata* still exist around Singapore, with many artificial or modified waterways being restored or rehabilitated into a more natural state for aesthetic and recreational purposes (Lee *et al*., 2010). These efforts have resulted in canals with natural, vegetated banks and that seem suitable for the native species. However, these new habitats are now being rapidly colonised by *Pomacea*. *Pomacea* has been very quick to establish in newly-created freshwater habitats like the Punggol Reservoir, a former estuarine river that was dammed in December 2009 (Ng & Tan, 2013), with an established population of *Pomacea* detected by November 2011 in a tributary of the reservoir (Seletar Wet Gap) (Ng, pers. obs.).

The invasion success of *Pomacea* species, in both Asia and elsewhere, has been attributed to traits like their faster growth, higher fecundity, and larger appetite for native aquatic macrophytes, compared to native species (Cowie, 2002; Estoy *et al*., 2002; Carlsson *et al*., 2004; Morrison & Hay, 2011). Introduced *Pomacea maculata* in the USA, which are larger in size than their native congener, *Pomacea paludosa*, impact the growth of the native juveniles (Conner *et al*., 2008). If *Pila scutata* is indeed prone to being outcompeted, the few localities in Singapore where it is still extant must be regarded as under severe threat, as these areas are also occupied by *Pomacea*, putting the long-term survival of the native species in Singapore at risk. The gravity of the situation is exacerbated by the fact that the status of this Southeast Asian native remains largely unknown in Peninsular Malaysia. There have not been wide scale surveys of the species since the 1970s and 1980s, when snails, being parasite hosts, were studied mainly for medical reasons (e.g. Lim *et al*., 1977; Palmieri *et al*., 1979).

Other impacts

Diseases like angiostrongyliasis have been linked to consumption of *Pomacea canaliculata*, which is a host of the parasite, *Angiostrongylus cantonensis*, that causes the disease (Lv *et al*., 2010). In Singapore, the parasite has been detected in the giant African snail, *Achatina fulica* (Bisseru, 1971), but not in *Pomacea* species (Tan & Ho, 2001). Despite *Pomacea* being cultured locally and occasionally available at local restaurants, there is no established culture of consuming the aquatic snails in Singapore. Nonetheless, foreign labourers may be collecting the snails for food (Fig. 5). Thus, a potential outbreak of angiostrongyliasis and other diseases associated with *Pomacea* species (Komalamisra *et al*., 2009) in Singapore may be unlikely but not impossible.

Fig. 5. A, remnants of a fire pit in a forest clearing; B, empty apple snail shells found next to the pit (apparently discarded after consumption). (Photos: J.T.B. Kwik)

Pomacea species have the potential to modify wetland ecosystems by eliminating macrophytes, and facilitating phytoplankton blooms (Carlsson *et al*., 2004; Fang *et al*., 2009). Aquarium hobbyists are in fact wary of putting apple snails in aquariums with valuable aquatic vegetation, as *Pomacea* are voracious herbivores (Perera & Walls, 1996). This is potentially less of a problem in Singapore since *Pomacea* are mainly found in artificial or modified habitats with little to no natural or native aquatic vegetation, although there are some populations in more natural marsh-like habitats, along the fringes of more rural reservoirs like Kranji Reservoir. However, restoration or rehabilitation of waterways to a more natural, vegetated state (as mentioned above), for example through installation of floating wetlands for water purification and habitat creation will necessarily involve introduction of macrophytes (Lee *et al*., 2010). The irony is that these efforts may in fact be aiding the spread of *Pomacea*, while the ultimate aim to rehabilitate waterways with vegetation may be hindered by the presence of *Pomacea*, which have the potential to become ornamental plant pests, possibly causing large-scale damage to the aquatic plants. Another nuisance associated with the species are the bright pink egg masses along manicured water features, which are generally considered to be unsightly.

Management measures

While the European Union has recently banned the import of *Pomacea* species because of their known invasiveness (European Commission, 2012), such regulations have not been enacted in Singapore. Among other Southeast Asian nations, various directives have been issued to prevent the spread of the species but not all are legally binding; only Vietnam has issued a directive that mandates the destruction of *Pomacea* (see Joshi & Sebastian, 2006). In Singapore, while there are regulations that prohibit the introduction of animals, they only apply to nature reserves, reservoirs and selected parks (Tan & Tan, 2013). Despite a considerable number of aquatic introductions being attributed to the aquarium trade (e.g. Ng & Tan, 2010; Ng & Lim, 2010; Yeo, 2010), the existing legislation relating to the import of ornamental species is relatively less strict than that of countries like Australia and the USA (Yeo & Chia, 2010; Tan & Tan, 2013). The AVA regulates the import of flora and fauna for ornamental purposes or human consumption, but species of *Pomacea* are not among the list of the few species that are banned (Yeo & Chia, 2010; Tan & Tan, 2013).

In recent years, the National Parks Board of Singapore, the government agency charged with administering local nature conservation, has pioneered efforts to increase public awareness regarding the dangers of releasing alien species through ‘Operation No Release’, organised in conjunction with the Public Utilities Board (which manages the reservoirs) (see Yeo & Chia, 2010). Also, volunteers have been engaged to remove apple snails and their eggs from some parks (G. Pereira, pers. comm.; T.L. Koa, pers. comm.). Local-level decisions may be made to clear particular areas of the snails and their eggs for various reasons, including conservation in some cases and aesthetics in others. However, there are currently no concerted, large-scale efforts, nor national-level policies that target the removal of these introduced species.

Conclusions

For successful eradication of a non-native species, the invasion should ideally be identified early and action taken swiftly (Simberloff, 2008). As *Pomacea* snails have been widely established in Singapore for over two decades (including in the island nation's water catchment reservoirs), it is likely to be difficult for the species to be easily or successfully eradicated. The interconnectedness of water bodies and high frequency of introduction of ornamental aquatic plants for aesthetic purposes in parks and canals increases the likelihood of the snails constantly being transferred from one locality to another. It is uncertain how effective the local active removal of *Pomacea* has been (or will be, in the longer term). In isolated habitats with minimal connectivity, it may still be possible to keep the populations small or to remove the species effectively (although monitoring to prevent re-introductions/re-invasions will be necessary considering the propagule pressure, which is likely to be high). The lack of baseline population data and ecological studies on native ampullariids in this region may also be obscuring comprehension of the true extent of the invasiveness of *Pomacea* species. Further work to fill these knowledge gaps is urgently needed to assess the biological impacts of these invasive species in Southeast Asia and to avoid any potential ecological disaster going unnoticed.

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References

Anderson, B. 1993. The Philippine snail disaster. *The Ecologist* 23: 70-72.

Alonso, A. & Castro-Díez, P. 2008. What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? *Hydrobiologia* 614: 107-116.

Biseru, B. 1971. The prevalence of *Angiostrongylus cantonensis* larvae collected from the giant African snail, *Achatina fulica* in West Malaysia and Singapore. *The Southeast Asian Journal of Tropical Medicine and Public Health* 2: 523-526.

Carlsson, N.O.L., Brönmark, C. & Hansson, L.-A. 2004. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575-1580.

Cazzaniga, N.J. 2002. Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda: Ampullariidae). *Biocell* 26: 71-81.

Chan, S.Y. 1996. Some freshwater gastropods of Singapore. *Of Sea and Shore* 18: 184-187.

Clements, R., Koh, L.P., Lee, T.M., Meier, R. & Li, D. 2006. Importance of reservoirs for the conservation of freshwater molluscs in a tropical urban landscape. *Biological Conservation* 128: 136-146.

Conner, S.L., Pomory, C.M. & Darby, P.C. 2008. Density effects of native and exotic snails on growth in juvenile apple snails *Pomacea paludosa* (Gastropoda: Ampullariidae): a laboratory experiment. *Journal of Molluscan Studies* 74: 355-362.

Cowie, R.H. 2002. Apple snails as agricultural pests: their biology impact and management. In: *Mollusca as Crop Pests* (Barker, G.M., ed.), p. 145-192. CAB International, Wallingford, UK.

Cowie, R.H. & Héros, V. 2012. Annotated catalogue of the types of Ampullariidae (Mollusca: Gastropoda) in the Muséum national d'Histoire naturelle, Paris, with lectotype designations. *Zoosystema* 34: 793-824.

Cowie, R.H. & Robinson, D.G. 2003. Pathways of introduction of nonindigenous land and freshwater snails and slugs. In *Invasive Species: Vectors and Management Strategies* (Ruiz, G. & Carlton, J., ed.), p. 93-122. Island Press, Washington DC.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastropoda: Ampullariidae: *Asolene*, *Felipponea*, *Marisa*, *Pomacea*, *Pomella*): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A., Tran, C.T. & Levin, P. 2007. Distribution of the invasive apple snail *Pomacea canaliculata* (Lamarck) in the Hawaiian Islands (Gastropoda:

Ampullariidae). *Bishop Museum Occasional Papers* 96: 48-51.

Dreon, M.S., Ituarte, S. & Heras, H. 2010. The role of the proteinase inhibitor ovorubin in apple snail eggs resembles plant embryo defense against predation. *PLoS ONE* 5: e15059.

Estoy, G.F., Jr, Yusa, Y., Wada, T., Sakurai, H. & Tsuchida, K. 2002. Effects of food availability and age on the reproductive effort of the apple snail, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae). *Applied Entomology and Zoology* 37: 543-550.

European Commission 2012. 2012/697/EU: Commission implementing decision of 8 November 2012 as regards measures to prevent the introduction into and the spread within the Union of the genus *Pomacea* (Perry) (notified under document C(2012) 7803). *Official Journal of the European Union L311* 55: 14-17.

Fang, L., Wong, P.K., Lin, L., Lan, C. & Qiu, J.-W. 2009. Impact of invasive apple snails in Hong Kong on wetland macrophytes, nutrients, phytoplankton and filamentous algae. *Freshwater Biology* 55: 1191-1204.

FAO (Food and Agriculture Organization of the United Nations) 2015. *Pomacea canaliculata* introduced to Singapore from Amazon basin. Invasive Species Factsheets, Fisheries and Aquaculture Department. http://www.fao.org/fishery/introsp/5954/en. Accessed 6 November 2015.

Fielding, M., Schwanenberg, D., Twigt, D.J., Eikaas, H., Pinho, J.L.S. & Viera, J.M.P. 2010. DSS for water quality management of Marina Reservoir system in Singapore. In: *Proceedings of the 9th International Conference on Hydroinformatics, 7–10 September 2010. Tianjin, China* (Tao, J., Chen, Q. & Liong, S.-Y., ed.), p. 2417-2424. Chemical Industry Press, Beijing.

Goh, B.P.L., Sodhi, N.S., Li, D., Ho, S.H. & Ng, P.K.L. 2002. *A Guide to Urban Creatures*. Singapore Science Centre, Singapore.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hayes, K.A., Joshi, R.C., Thiengo S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distribution* 14: 701-712.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Johnson, D.S. 1973. Freshwater life. In: *Animal Life and Nature in Singapore* (Chuang, S.H., ed.), p. 103-127. Singapore University Press, Singapore.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija.

Komalamisra, C., Nuamtanong, S. & Dekumyoy, P. 2009. *Pila ampullacea* and *Pomacea canaliculata*, as new paratenic hosts of *Gnathostoma spinigerum*. *The Southeast Asian Journal of Tropical Medicine and Public Health* 40: 243-246.

Kwong, K.L., Wong, P.K., Lau, S.S.S. & Qiu, J.-W. 2008. Determinants of the distribution of apple snails in Hong Kong two decades after their initial invasion. *Malacologia* 50: 293-302.

Kwong, K.L., Chan, R.K.Y. & Qiu, J.-W. 2009. The potential of the invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia* 51: 343-356.

Lee, E., Soh, T. & Kalyanaraman, G. 2010. Where do we come in? In: *Private Lives. An Exposé of Singapore's Freshwaters* (Yeo, D.C.J., Wang, L.K. & Lim, K.K.P., ed.), p. 234-239. Raffles Museum of Biodiversity Research, Singapore.

Leuven, R., Van Der Velde, G., Baijens, I., Snijders, J., van der Zwart, C., Lenders, H. & bij de Vaate, A. 2009. The river Rhine: a global highway for dispersal of aquatic invasive species. *Biological Invasions* 11: 1989-2008.

Lim, B.L., Yap L.F. & Krishnansamy, M. 1977. Studies of *Angiostrongylus malaysiensis* (Nematoda, Metastrongylidae) in Peninsular Malaysia: natural infection in freshwater snails and rodents in ricefields and infectivity experiments. *The Southeast Asian Journal of Tropical Medicine and Public Health* 8: 1-9.

Lintermans, M. 2004. Human assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research* 38: 481-501.

Low, B.W., Lim, K.S., Yap, F., Lee, T.K., Lim, K.C. & Yong, D.L. 2013. First record of the Asian openbill, *Anastomus oscitans* (Aves: Ciconiidae) in Singapore, with notes on foraging and dispersive movements. *Nature in Singapore* 6: 25-29.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species.* Invasive Species Specialist Group (ISSG), Auckland, New Zealand.

Lv, S., Zhang, Y., Steinmann, P., Zhou, X.-N. & Utzinger, J. 2010. Helminth infections of the central nervous system occurring in Southeast Asia and the Far East. *Advances in Parasitology* 72: 351-408.

Maassen, W.J.M. 2001. A preliminary checklist of the non-marine molluscs of West-Malaysia. “A handlist”. *De Kreukel* Extra Edition: 1-155.

Marwoto, R.M., Isaningsih, N.R., Mujiono, N., Heryanto, Alfiah & Riena 2011. *Keong Air Tawar Pulau Jawa (Moluska, Gastropoda)*. Pusat Penelitian Biologi—LIPI, Cibinong. [in Indonesian]

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Morrison, W.E. & Hay, M.E. 2011. Feeding and growth of native, invasive and non-invasive alien apple snails (Ampullariidae) in the United States: invasives eat more and grow more. *Biological Invasions* 13: 945-955.

Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Ng, H.H. & Tan, H.H. 2010. An annotated checklist of the non-native freshwater fish species in the reservoirs of Singapore. *COSMOS* 6: 95-116.

Ng, P.K.L. 1991. *A Guide to Freshwater Life in Singapore*. Singapore Science Centre, Singapore.

Ng, P.K.L., Chou, L.M. & Lam, T.J. 1993. The status and impact of introduced freshwater animals in Singapore. *Biological Conservation* 64: 19-24.

Ng, P.X. & Tan, H.H. 2013. Fish diversity before and after construction of the Punggol and Serangoon Reservoirs, Singapore. *Nature in Singapore* 6: 19-24.

Ng, T.H. & Lim, K.K.P. 2010. Introduced aquatic herpetofauna of Singapore’s reservoirs. *COSMOS* 6: 117-127.

Ng, T.H. & Tan, S.K. 2011. Observations of land snails feeding on eggs of *Pomacea canaliculata*. *Nature in Singapore* 4: 79-83.

Ng T.H., Tan, S.K. & Low, M.E.Y. 2014. Singapore Mollusca: 7. The family Ampullariidae (Gastropoda: Caenogastropoda: Ampullarioidea). *Nature in Singapore* 7: 31-47.

Ng T.H., Tan, S.K. & Yeo, D.C.J. 2015. Clarifying the identity of the long-established, globally-invasive *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae) in Singapore. *Bio Invasions Records* 4: 189-194.

Palmieri, M., Palmeiri, J. & Sullivan, J. 1979. The natural diet of *Pila scutata* (Mousson 1848) a common Malaysian prosobranch snail. *The Malayan Nature Journal* 32: 343-345.

Perera, G. & Walls, J.G. 1996. *Apple Snails in the Aquarium*. T.F.H. Publications, Neptune City, USA.

Ponniah, A.J. 1962. *Studies on some Non-Marine Aquatic Gastropods of Singapore Island, with a Note on Five Species Collected from Malacca*. B.Sc. (Hons.) Thesis. University of Singapore, Singapore.

Schreiber, E.S.G., Quinn, G.P. & Lake, P.S. 2003. Distribution of an alien aquatic snail in relation to flow variability, human activities and water quality. *Freshwater Biology* 48: 951-961.

Simberloff, D. 2008. We can eliminate invasions or live with them. Successful management projects. *Biological Invasions* 11: 149-157.

Seuffert, M. & Martín, P. 2012. A lentic dweller in lotic habitats: the behavior of the invasive South American apple snail *Pomacea canaliculata* in flowing water. *Aquatic Ecology* 46: 129-142.

Tan, C.C.A. & Ho, H.F. 2001. A parasitological survey of apple snails (*Pomacea* species) in Singapore. *Singapore Journal of Primary Industries* 29: 27-29.

Tan, M.B.N. & Tan, H.T.W. 2013. *The Laws Relating to Biodiversity in Singapore*. Raffles Museum of Biodiversity Research, Singapore.

Tan, S.K. & Woo, H.P.M. 2010. *A Preliminary Checklist of the Molluscs of Singapore*. Raffles Museum of Biodiversity Research, Singapore.

Tan, S.K. & Yeo, D.C.J. 2010. The softies. In: *Private Lives. An Exposé of Singapore's Freshwaters* (Yeo, D.C.J. Wang, L.K. & Lim, K.K.P., ed.), p. 94-101. Raffles Museum of Biodiversity Research, Singapore.

Tan, S.K., Chan, S.Y. & Clements, G.R. 2012. *A Guide to Snails and Other Non-marine Molluscs of Singapore*. Science Centre Singapore, Singapore.

Tan, S.K., Lee, Y.-L. & Ng, T.H. 2013. The status of *Pila scutata* (Gastropoda: Ampullariidae) in Singapore. *Nature in Singapore* 6: 135-141.

Van Leeuwen, C.H.A., Huig, N., Van Der Velde, G., van Alen, T.A., Wagemaker, C.A.M., Sherman, C.D.H., Klaassen, M. & Figuerola, J. 2013. How did this snail get here? Several dispersal vectors inferred for an aquatic invasive species. *Freshwater Biology* 58: 89-99.

Xu, Z., Yang, X., Huang, S. & Chen, J. 1989. *Pila* as a Biological Resource. Unpublished student report. River Valley High School, Singapore.

Yahaya, H., Nordin, M., Muhamad Hisham, M.N. & Sivapragasam, A. 2006. Golden apple snails in Malaysia. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 215-230. Philippine Rice Research

Institute, Nueva Ecija.

Yeo, D.C.J. 2010. Introduced decapod crustaceans in Singapore's reservoirs. *COSMOS* 6: 85-90.

Yeo, D.C.J. & Chia, C.S.W. 2010. Introduced species in Singapore: an overview. *COSMOS* 6: 23-37.

Yusa, Y. 2001. Predation on eggs of the apple snail *Pomacea canaliculata* by the fire ant *Solenopsis geminata*. *Journal of Molluscan Studies* 67: 1-6.

Yusa, Y. 2006. Predators of the introduced apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae): their effectiveness and utilization in biological control. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 345-362. Philippine Rice Research Institute, Nueva Ecija.

Behaviour and ecology of *Pomacea canaliculata* from Southern Pampas (Argentina)

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Abstract

Pomacea canaliculata is in many respects the best known species of apple snails (family Ampullariidae), although the available information is both fragmentary and geographically biased. Most studies in its non-native range have focused on applied aspects in managed or artificial wetlands in various countries in Southeast Asia. In its natural range the emphasis has been on basic studies of its reproductive biology, ecology and behaviour in populations from small streams at the southernmost extreme of its distribution (Southern Pampas, Argentina). The extreme geographic position and the lotic nature of these populations may have biased some conclusions about the behavioural and ecological traits of *P. canaliculata*; contemporary evolution and genetic exchange may also have diversified these traits in the non-native range. Even though the ecological information from native populations may not be directly applicable elsewhere, it nevertheless remains as a necessary reference to understand the full potential of adaptation and spread of *P. canaliculata* to new environments around the world. Surprisingly enough, comparative studies of native and non-native populations of *Pomacea* spp. are almost lacking. This short review focuses on the distribution, thermal biology, aerial respiration, feeding, reproduction, phenotypic plasticity and shell shape of *Pomacea canaliculata* in its native range in Argentina.

Additional keywords: Ampullariidae, distribution, feeding, life-history, Mollusca, phenotypic plasticity, respiration, thermal biology, shell shape

Introduction

In many respects *Pomacea canaliculata* is the best known species in the species-rich family Ampullariidae. Most of the interest in this species in recent decades has been spurred by its fast spread and major ecological and agricultural impacts outside its native range (Cowie, 2002; Joshi & Sebastian, 2006; Horgan *et al*., 2014). The IUCN Invasive Species Specialist Group listed *P. canaliculata* among *100 of the World's Worst Invasive Alien Species* (Lowe *et al*., 2000) because of its serious impacts, but it is also as an illuminating example of patterns and processes in biological invasions. The publication of ecological studies on this species in its non-native range have flourished in the 21st century; however, several of these studies erroneously or dubiously identified *P. canaliculata* as their object of study, probably because of the unnoticed presence of other alien *Pomacea* spp. and a mostly shell-based taxonomy (Cowie *et al*., 2006; Hayes *et al*., 2012, 2015).

Fig. 1. A section of the Pigué stream, one of the long streams inhabited by *Pomacea canaliculata* in the plains of the Encadenadas del Oeste basin (Buenos Aires province, Argentina). (Photo: S. Burela)

Most studies in the non-native range have focused on applied aspects of the ecology of *P. canaliculata* and other *Pomacea* spp. in managed or artificial wetlands. In the natural range of *P. canaliculata* (Río de la Plata basin, South America; Hayes *et al*., 2012), in contrast, the emphasis has been on basic studies of its reproductive biology, ecology and behaviour in natural habitats. Ecological studies of apple snails in the Pampas region, Argentina, from which apparently most *Pomacea* invaders have originated (Hayes *et al*., 2008, 2012; Lv *et al*., 2013), have concentrated on *P. canaliculata* (reviewed by Estebenet & Martín, 2002).

The aim of this contribution is to review the research done since around 2002 on the behaviour and ecology of *P. canaliculata* in Southern Pampas and to highlight those aspects that may be useful to understand, predict and control the invasions as well as to understand the ecological and agricultural impacts in the non-native range.

Determinants of distribution

The natural range of *P. canaliculata* extends from the Río de la Plata basin southwards to the Encadenadas del Oeste basin in Southern Pampas (37°S, Buenos Aires Province, Argentina), making it the southernmost apple snail in the world (Martín *et al*., 2001). Factors determining the presence and abundance of *P. canaliculata* in lotic and lentic waterbodies at its southernmost limit have been studied at different spatial scales. It is generally found in shallow, quiet and turbid sites with low $Na^+/(K^{++} + Mg^{++})$ ratios (Martín *et al*., 2001). The arid conditions in western Buenos Aires Province and the high salinity and temporary nature of most waterbodies probably limit its expansion westwards. On the other hand, the main factor that limits its southward spread has been the hydrological barrier imposed by the Tandilia and Ventania Mountains of southern Buenos Aires Province and not temperature (Martín *et al*., 2001; Martín & De Francesco, 2006).

The upper reaches of streams (altitudes and slopes higher than 230 m above sea level and 3.2 %, respectively) arising from the Tandilia and Ventania Mountains are mostly uninhabited by *P. canaliculata* (Martín *et al*., 2001, Seuffert & Martín, 2013a). Heavy rains in the steeper headwater sections result in floods that sometimes eradicate *P. canaliculata* populations, making headwaters unfavourable habitats for their mid and long term persistence.

In the plains of the Encadenadas del Oeste basin, *P. canaliculata* is generally found in the long streams arising from the Ventania Mountains (Fig. 1), while the short streams that arise from springs in the plains are rarely inhabited (Seuffert & Martín, 2013a). In long streams, *P. canaliculata* is most frequently recorded in places close to the shore, with low current velocities, fine sediments rich in organic matter and abundance of submersed macrophytes. The association with microhabitats in proximity to the shore or with available emergent substrata is due in part to the need to ventilate the lung periodically (Seuffert & Martín, 2009, 2010). Short streams are characterized by higher conductivities, more alkaline pH, very low or zero current velocities and the absence of trees on the margins (Seuffert & Martín 2013a); during hot summer days water temperatures are high enough to induce snail inactivation (Seuffert *et al*., 2010). Evidence from two sink populations showed that *P. canaliculata* can survive and reproduce in the short streams, although these streams are not suitable for long term persistence of the populations.

Knowledge of the determinants of the distribution of *P. canaliculata* in its natural realm is necessary to predict its potential habitat range in invaded areas and for an efficient design of barriers to its spread. However, unpredictable variation in climatic and hydrological conditions generates stochastic patterns of extinction-colonization that blur the relationships between snail abundance and environmental factors (Seuffert & Martín, 2013a). A metapopulational approach seems necessary to fully understand and predict the distribution of this snail in its natural and invaded ranges.

Thermal biology

The activity of *P. canaliculata* decreases sharply below water temperatures of 15 °C, and the snails are totally inactive at 10 °C. Activity levels also decrease above 30 °C, especially in poorly aerated or fouled waters (Seuffert & Martín, 2010). Crawling speed increases linearly with temperature, but the time spent crawling is nearly constant. The time spent feeding increases with temperature between 10 and 25 °C but drops at higher temperatures (Seuffert *et al*., 2010). Juvenile growth rates increase with temperature from 15 to 25 °C but do not show a further increase up to 35 °C, probably because of the decline in feeding time and the increase in metabolic rates (Seuffert & Martín, 2013b).

The duration of incubation of the aerial egg masses of *P. canaliculata* is highly dependent on air temperature (Pizani *et al*., 2005). The lower threshold for embryonic development is around 16 °C, while the cumulative degree-days until hatching range between 120 and 132 (Seuffert *et al*., 2012). The egg masses of *P. canaliculata* have been the target of various chemical and mechanical control methods (Wang *et al*., 2012). Degree-day models of egg development coupled with field temperature data can be used to predict time required to hatch and thereby to optimize the frequency and timing of the application of control methods to improve their effectiveness (Seuffert *et al*., 2012).

At the southernmost limit of its distribution (37ºS), *P. canaliculata* remains inactive during the five or six colder months but does not fall into deep dormancy, as the snails soon become active if water temperature rises (Seuffert *et al*., 2010). Even though *P. canaliculata* can survive in somewhat colder regions (Colorado river, 39.5°S; Martín *et al*., 2001), low temperatures are probably one of the main impediments to its expansion to higher latitudes in invaded areas.

Behaviour in flowing water

Pomacea canaliculata has traditionally been considered a lentic dweller but it also thrives in lotic systems (Martín *et al*., 2001; Seuffert & Martín, 2013a). Current plays an important role in the use of microhabitats in the streams of Encadenadas del Oeste basin, because almost all snails occur in places with current velocities below 0.3 m.s^{-1} (Seuffert & Martín, 2013a). However, in an experimental flume, *P. canaliculata* showed a high resistance to being dislodged during short periods with current velocities up to 1.64 m.s^{-1} (Seuffert & Martín, 2012). Hence, in spite of the water velocities that the snails are able to resist if they become exposed, they tend to avoid fast-flowing places probably because the current interferes with their activities and movements.

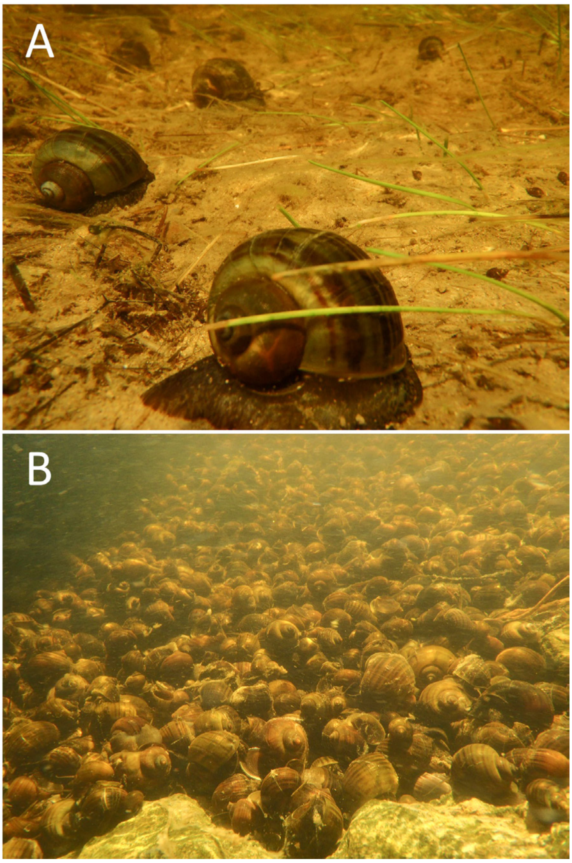

Fig. 2. A: *Pomacea canaliculata* crawling upstream in El Huáscar stream. B: massive concentration of *P. canaliculata* snails downstream of a fast current zone below a bridge in El Huáscar stream. (Subaquatic photos: P.R. Martín)

The dispersal of *P. canaliculata* within Southern Pampas streams depends on both crawling and drifting. During field observations, the snails did not show a positive rheotactic response as the mean net displacement at different velocities did not differ from zero; snails crawl more often upstream (Fig. 2A), but at a slower pace than downstream (Seuffert & Martín, 2012). At the population level, a slow upstream spread seems possible in streams in the plain, being probably enough to compensate for downstream drift but not sufficient to permit colonizing of headwaters.

In invaded regions irrigation channels probably constitute favourable environments for the active and passive dispersal of snails, acting as pathways for the spread of *P. canaliculata*. They also seem to be suitable places to practice control measures, by promotion of downstream drift and subsequent capture of the snails, or the placing of hydrodynamic barriers to prevent upstream spread (Fig. 2B).

Aerial respiration and the dependence on air access

As other apple snails, *P. canaliculata* is commonly considered "amphibious" as it has both a gill and a lung, which is ventilated through a long extensible siphon. The lung allows the snails to dwell in habitats with poorly oxygenated waters or that dry out frequently. Aerial respiration begins within three hours of hatching and is performed routinely as long as the snails are active, even in oxygen saturated water. Lung ventilation frequency increases with water temperature and snail size, probably because of an increase in metabolic rate and a decrease in the relative surface of gas exchange (Seuffert & Martín, 2009).

Restriction of aerial respiration negatively affects activity, feeding and survivorship, its effects being worst at high temperatures and in foul water, which probably hinders oxygen uptake through the gill. Under certain conditions, such as in stagnant waters with heavy organic loads and high temperatures, *P. canaliculata* is an obligate aerial breather. Nevertheless, its distribution is also restricted under less extreme conditions since in the highly oxygenated waters of the temperate streams of Southern Pampas most snails are usually found near emergent substrata (Seuffert & Martín, 2010). Accessibility to air would be an important feature of waterbodies prone to invasion by this species, especially in lentic tropical environments. The effectiveness of some chemical or physical control measures could be increased by focusing them on areas where snails concentrate because of their dependence on air access.

Feeding mechanisms and food spectrum

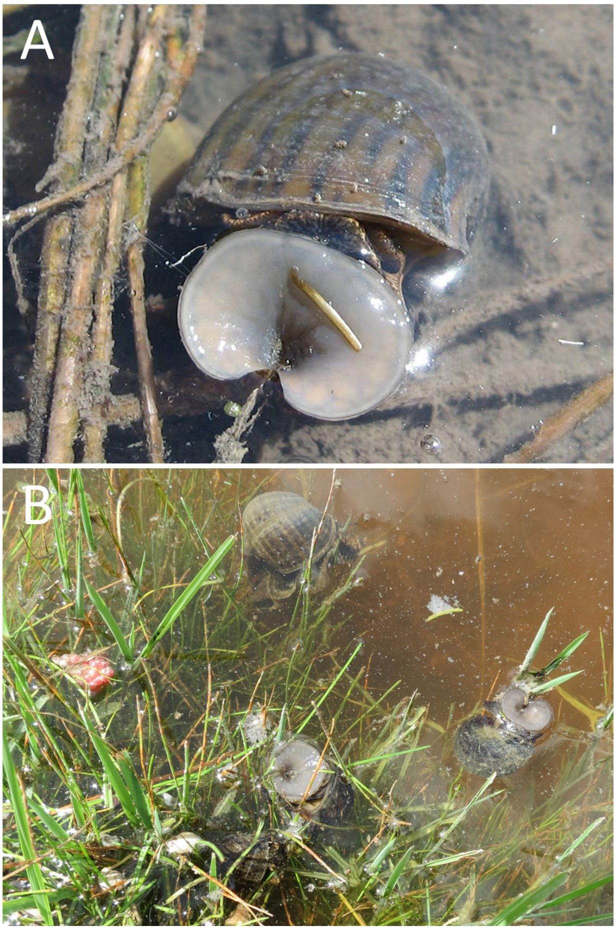

Fig. 3. A: *Pomacea canaliculata* performing pedal surface collecting from a muddy bottom. B: two *P. canaliculata* performing pedal surface collecting from leaves of submerged grasses. (Photos: L. Saveanu)

Pomacea canaliculata is a polyphagous snail with diverse and flexible feeding mechanisms, which, according to the functional feeding groups of Cummins & Klug (1979), can be classified as scraping, shredding and collecting. By scraping, *P. canaliculata* gets material adhering to submersed surfaces mainly by use of its radula; by shredding, employing the jaws, it obtains pieces of submersed materials; and by collecting, it gathers materials from the water surface by action of a temporary ciliated funnel formed by the foot (Fig. 3A). The latter mechanism has been renamed pedal surface collecting (Saveanu & Martín, 2013) as the more widely used name (ciliary feeding) is also applied to the capture of suspended particles. In the field, pedal surface collecting is performed even when submersed macrophytes are abundant (Fig. 3B) and shows a peak during the night but negligible levels during daylight hours. In the laboratory, snails bigger than 10 mm in shell length frequently capture materials of diverse nature and size from the surface by pedal surface collecting (e.g. animal and vegetal parts or individuals, liquids and biofilms). These snails also feed on their own eggs when the egg masses become submerged both in the field and in the laboratory (Saveanu & Martín, 2014).

Apple snails are regarded mainly as phytophagous shredders and scrapers but pedal surface collecting is an adaptable and efficient way to capture diverse trophic resources. This alternative feeding mechanism should not be neglected when evaluating the suitability of water bodies for *P. canaliculata*, as it may permit establishment even in the absence of submersed plants and benthic algae (Saveanu & Martín, 2015).

Feeding rates and growth efficiency

Smaller snails have higher specific ingestion rates than bigger ones regardless of the feeding mechanism used (shredding, scraping or pedal surface collecting) and the trophic resource consumed (Tamburi & Martín 2009a; Saveanu & Martín 2013, 2014). By pedal surface collecting, snails of 12 mm shell length ingested 14 times more per unit biomass than 50 mm snails (Saveanu & Martín, 2013), while by shredding and scraping they ingested only 5 times more than the bigger ones (Tamburi & Martín, 2009a). The ingestion rates of their own eggs were around eight times higher for juveniles (16 mm shell length) than for adults (38 mm). The same ontogenetic pattern was observed in food conversion efficiencies (Tamburi & Martín, 2009a). The higher specific ingestion rates of small snails indicate that they are probably responsible for an important part of the damage to aquatic crops during the reproductive season (Tamburi & Martín, 2009a).

Energy costs of reproduction are carried by females and, consistently, they have higher feeding rates by shredding and scraping and also a higher conversion efficiency of the food obtained (Tamburi & Martín, 2009a). However, intersexual differences in specific capture rates were not detected in snails performing pedal surface collecting (Saveanu & Martín, 2013).

Reproductive biology

Mating is one of the aspects of *P. canaliculata* behaviour that has been studied most thoroughly. Males and females mate frequently with different partners, and copulations, which are subaquatic, are among the longest in the Caenogastropoda (12.15 ± 3.97 h; Burela & Martín, 2011). During copulation the male's foot is firmly adhered to the right side of the female's last shell whorl (Fig. 4A) and the penis sheath is inserted in the pallial cavity, gripping the rim of the shell (Burela & Martín, 2009), a pattern shared by most ampullariids (Burela & Martín, 2011, Tiecher *et al*., 2014). The most remarkable behavioural component of the mating of *P. canaliculata* is the nuptial gift-giving, a phenomenon as yet only known among gastropods in this species (Burela & Martín, 2007, 2014).

Oviposition takes place out of water on any firm emergent substrate, mostly during night or early morning, but sometimes during late morning. The eggs are laid usually one by one to build an egg mass but sometimes a row of two to four is seen on the right

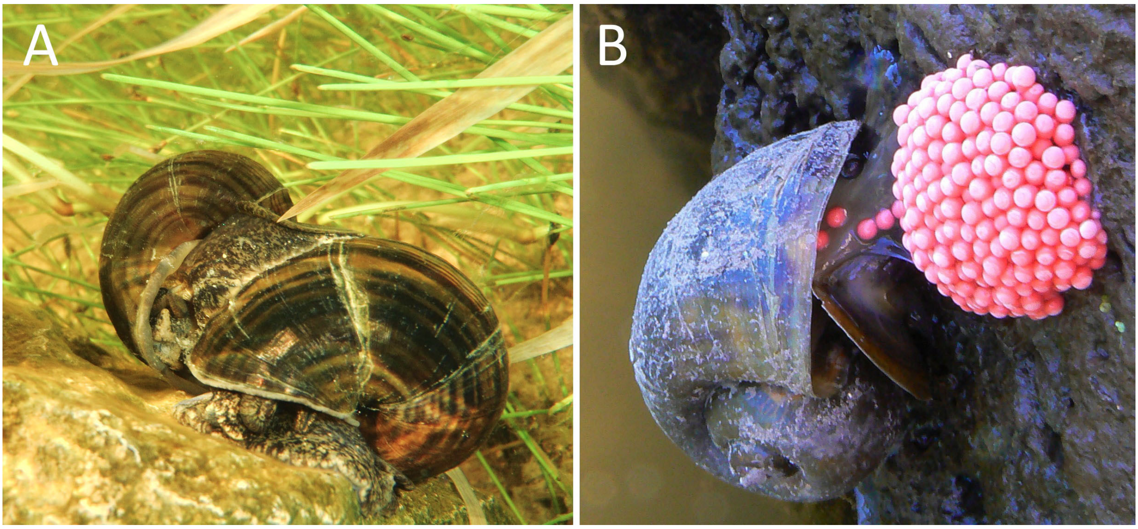

Fig. 4. A: *Pomacea canaliculata*in copulation, male on the left (subaquatic photograph by P.R. Martín). B: female *P. canaliculata* laying eggs on a vertical rocky stream bank. (Photo: S. Burela).

side of the foot (Fig. 4B). The total number of eggs and egg masses laid by females that copulated once are the same as those of females that copulated repeatedly with the same male (Burela & Martín, 2011), indicating that after a single copulation a female can store and maintain enough viable sperm to fertilize ova for the rest of her fertile life (i.e. 2244 eggs during eight weeks on average between the first and the last egg mass). This capacity has been considered a key feature in the invasiveness of *P. canaliculata* since it greatly increases the potential of a single female to establish a new population (Jerde *et al*., 2009). Even though *P. canaliculata* females can fully express their reproductive potential after a single copulation and are also able to reject males (Burela & Martín, 2009), they frequently engage in long-lasting copulations. A possible benefit from multiple copulations for females is a higher genetic diversity of progeny, a feature that probably enhances the capacity to adapt to novel environments in invaded areas.

Food availability and life-history plasticity

The life history traits of *P. canaliculata* show important inter-population variation mostly attributable to phenotypically plastic responses to multiple environmental factors (Martín & Estebenet, 2002) but so far only the effect of food availability has been experimentally tested. When full sibling snails were reared in a gradient of food availability (between 20 and 100 % of the *ad libitum* ingestion rate) the reaction norms of size and age at maturity were very different in the two sexes. Males matured at an age of around 15 weeks irrespective of size and food availability (15 mm at 20 %, 29 mm at 100 %); for females it was necessary to reach sizes larger than 32 mm to reproduce (Tamburi & Martín, 2009b), which was attained at very different ages according to food availability (15 weeks at 100 %, 50 weeks at 20 %).

The male strategy seems to be to copulate as soon as possible and when food availability is chronically low they sacrifice size, although apparently this does not affect their reproductive success (Burela & Martín, 2011). In contrast, under extreme food deprivation females delay their maturity for up to three times as long to attain a minimum size compatible with the high energetic costs of producing big and complex eggs without jeopardizing their own survival (Tamburi & Martín, 2011). Females accumulate resources during the pre-reproductive period to spend them intensively when they mature.

The food availability at which males grew (20 to 100 %) did not affect the eggs, egg masses and progeny of their consort females (reared with food *ad libitum*; Tamburi & Martín, 2011). In contrast, when food availability is chronically low, females lay fewer eggs although without reducing their size or the number of egg masses; however, the survival time of hatchlings under starvation increases with the trophic deprivation suffered by their mothers. Hence, the female strategy seems to be to assure hatchling size and survivorship and to spread the risk of total failure of progeny (e.g. due to the submersion of the egg masses or to hatching beyond the shoreline; Pizani *et al*., 2005) among several egg masses.

Reduction in food availability would reduce population growth in paddy fields through a delay in female maturity (Estoy *et al*., 2002; Tamburi & Martín, 2009a). However, a decrease in fecundity would appear only at deprivation levels higher than 50 % and fecundity would fall just 27 % and would be partially compensated for by the increase in hatchling survival (Tamburi & Martín, 2011).

Food availability and shell plasticity

Shell shape is used for primary taxonomic identification of apple snails in the field but its high variability (Estebenet *et al*., 2006) may lead to misidentifications because of a general lack of knowledge about the limits of specific variation and the factors affecting it. Shell shape can also be used to sex *P. canaliculata* with a high degree of confidence, which is useful when they cannot be dissected during field or laboratory studies. Many ecological and life history traits of *P. canaliculata* are sexually dimorphic and hence it is important to analyse the information for each sex separately (Seuffert & Martín, 2010; Tamburi & Martín, 2009a, b).

Shells of *P. canaliculata* exhibit inter-population variation that is both genetic and environmental in origin (Estebenet & Martín, 2003). Food availability increases shell variability at maturity, increasing the aperture width relative to total shell length in both sexes (Tamburi & Martín, 2012). Female shells are relatively more globose, with less expanded apertures and a wider last whorl, and these differences are not blurred by food availability (Tamburi & Martín, 2012). Snails growing fast with food *ad libitum* have thinner shells than those with some degree of food deprivation and females growing fast have thinner shells than males, probable because of the development of the albumen gland complex, which is rich in calcium carbonate.

Interpretation of trophic effects on shell shape is complicated by the allometric growth of the shell of *P. canaliculata* (Estebenet *et al*., 2006) and the effect of food availability on size at maturity (Tamburi & Martín, 2009b). Using geometric morphometrics techniques based on landmarks a significant static allometry (shape differences among adults of different size) has been detected in *P. canaliculata* (Tamburi & Martín, 2013). After statistically removing the allometric effects, only females showed a direct trophic effect on shell shape, being more globose and with a larger aperture when grown at high food availabilities.

Concluding remarks

Ecological studies on *P. canaliculata* in its native range have focused on small streams of Southern Pampas, where the southernmost populations are located (Martín *et al*., 2001, Martín & De Francesco, 2006). Since *P. canaliculata* is the only apple snail present in the area, the problems resulting from misidentifications (Hayes *et al*., 2012) or genetic exchange (Matsukura *et al*., 2013) have been avoided. On the other hand, the extreme location of Southern Pampas within the native range of *P. canaliculata*, its semiarid and highly seasonal climate and the lotic nature of the habitats of the most studied populations may have biased some conclusions regarding its behavioural and ecological traits. However, the life history and population dynamics of an invader at the boundaries of its native range may provide especially interesting information related to its potential spread in its non-native range (Guo, 2006).

The reliability of comparisons among many published studies on *Pomacea* spp., especially those undertaken outside the natural range, is hampered by misidentifications, nomenclatural problems and the diverse and generally unknown origin of the alien apple snails (Cowie *et al*., 2006; Lv *et al*., 2013). In addition, contemporary evolution and genetic exchange in the invaded range (Guo, 2006) may have diversified the behavioural and ecological traits of *P. canaliculata* through the generation of novel genotypes with their own plastic reactions to the environment. The information from native populations nevertheless stands as a necessary reference to comprehend the full potential of adaptation and spread to new environments around the world. Surprisingly enough, comparative studies of native and non-native populations of *Pomacea* spp. are almost lacking.

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References

Burela, S. & Martín, P.R. 2007. Nuptial feeding in the freshwater snail *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Malacologia* 49: 465-470.

Burela, S. & Martín, P.R. 2009. Sequential pathways in the mating behavior of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Malacologia* 51: 157-164.

Burela, S. & Martín, P.R. 2011. Evolutionary and functional significance of lengthy copulations in a promiscuous apple snail, *Pomacea canaliculata* (Ampullariidae, Caenogastropoda). *Journal of Molluscan Studies* 77: 54-64.

Burela, S. & Martín, P.R. 2014. Nuptial gifts in *Pomacea canaliculata* (Ampullariidae, Caenogastropoda): experimental and field evidence about their function. *Malacologia*, 57: 319-327.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CAB International, Wallingford, UK.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-23. Philippine Rice Research Institute, Nueva Ecija, Philippines.

Cummins, K.W. & Klug, M.J. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10: 147-172.

Estebenet, A.L. & Martín, P.R.. 2002. *Pomacea canaliculata* (Gastropoda: Ampullariidae): life-history traits and their plasticity. *Biocell* 26: 83-89.

Estebenet, A.L. & Martín, P.R. 2003. Shell interpopulation variation and its origin in *Pomacea canaliculata* (Gastropoda: Ampullariidae) from southwestern Buenos Aires Province, Argentina. *Journal of Molluscan Studies* 69: 301-310.

Estebenet, A.L., Martín, P.R & Burela, S. 2006. Conchological variation in *Pomacea canaliculata* and other South American Ampullariidae (Caenogastropoda). *Biocell* 30: 329-335.

Estoy, G.F., Yusa, Y., Wada, T., Sakurai, H. & Tsuchida, K. 2002. Effect of food availability and age on the reproductive effort of the apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Applied Entomology and Zoology* 37: 543-550.

Guo, Q. 2006. Intercontinental biotic invasions: what can we learn from native populations and habitats? *Biological Invasions* 8: 1451-1459.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Horgan, F.G., Stuart, A.M. & Kudavidanage, E.P. 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica* 54: 90-100.

Jerde, C.L., Bampfylde, C.J. & Lewis, M.A. 2009. Chance establishment for sexual, semelparous species: overcoming the Allee effect. *American Naturalist* 173: 734-746.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija, Philippines.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species Database*. IUCN Invasive Species Specialist Group, Auckland, New Zealand.

Lv, S., Zhang, Y., Liu, H.X., Hu, L., Liu, Q., Wei, F.R., Guo, Y.H., Steinmann, P., Hu, W., Zhou, X.N. & Utzinger, J. 2013. Phylogenetic evidence for multiple and secondary introductions of invasive snails: *Pomacea* species in the People's Republic of China. *Diversity and Distributions* 19: 147-156.

Martín, P.R. & Estebenet, A.L. 2002. Inter-population variation of life-history traits in *Pomacea canaliculata* (Gastropoda: Ampullariidae) in southwestern Buenos Aires Province, Argentina. *Malacologia* 44: 153-163.

Martín, P.R. & De Francesco, C.G. 2006. Fossil record of *Pomacea* (Caenogastropoda: Ampullariidae) in Argentina and its palaeoenvironmental implications. *Biocell* 30: 337-343.

Martín, P.R., Estebenet, A.L. & Cazzaniga, N.J. 2001. Factors affecting the distribution of *Pomacea canaliculata* (Gastropoda: Ampullariidae) along its southernmost natural limit. *Malacologia* 43: 13-23.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Pizani, N., Estebenet, A.L. & Martín, P.R. 2005. Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae). *American Malacological Bulletin* 20: 55-63.

Saveanu, L. & Martín, P.R. 2013. Pedal surface collecting as an alternative feeding mechanism of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Journal of Molluscan Studies* 79: 11-18.

Saveanu, L. & Martín, P.R. 2014. Egg cannibalism in *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) from the Southern Pampas: an alternative trophic strategy? *Malacologia* 57: 341-351.

Saveanu, L. & Martín, P.R. 2015. Neuston: a relevant trophic resource for apple snails? *Limnologica* 52: 75-82.

Seuffert, M.E. & Martín, P.R. 2009. Influence of temperature, size and sex on aerial respiration of *Pomacea canaliculata* (Gastropoda: Ampullariidae) from Southern Pampas, Argentina. *Malacologia* 51: 191-200.

Seuffert, M.E & Martín, P.R. 2010. Dependence on aerial respiration and its influence on microdistribution in the invasive freshwater snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Biological Invasions* 12: 1695-1708.

Seuffert, M.E. & Martín, P.R. 2012. A lentic dweller in lotic habitats: the behavior of the invasive South American apple snail *Pomacea canaliculata* in flowing water. *Aquatic Ecology* 46: 129-142.

Seuffert, M.E. & Martín, P.R. 2013a. Distribution of the apple snail *Pomacea canaliculata* in Pampean streams (Argentina) at different spatial scales. *Limnologica* 43: 91-99.

Seuffert, M.E. & Martín, P.R. 2013b. Juvenile growth and survival of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) reared at different constant temperatures. *SpringerPlus* 2: 312.

Seuffert, M.E., Burela, S. & Martín, P.R. 2010. Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *Journal of Thermal Biology* 35: 77-84.

Seuffert, M.E., Saveanu, L. & Martín, P.R. 2012. Threshold temperatures and degree-day estimates for embryonic development of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Malacologia* 55: 209-217.

Tamburi, N.E. & Martín, P.R. 2009a. Feeding rates and food conversion efficiencies of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Malacologia* 51: 221-232.

Tamburi, N.E. & Martín, P.R. 2009b. Reaction norms of size and age at maturity of *Pomacea canaliculata* (Gastropoda: Ampullariidae) under a gradient of food deprivation. *Journal of Molluscan Studies* 74: 19-26.

Tamburi, N.E. & Martín, P.R. 2011. Effects of food availability on reproductive output, offspring quality and reproductive efficiency in the apple snail *Pomacea canaliculata*. *Biological Invasions* 13: 2351-2360.

Tamburi, N.E. & Martín, P.R. 2012. Effect of food availability on morphometric and somatic indices of the apple snail *Pomacea canaliculata* (Caenogastopoda, Ampullariidae). *Malacologia* 55: 33-41.

Tamburi, N.E. & Martín, P.R. 2013. Allometric and trophic effects on shell morphology of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) from a geometric morphometrics viewpoint. *Molluscan Research* 33: 223-229.

Tiecher, M.J., Burela, S. & Martín, P.R. 2014. Mating behavior, egg laying and embryonic development in the South American apple snail *Asolene pulchella* (Ampullariidae, Caenogastropoda). *Invertebrate Reproduction and Development* 58: 13-22.

Wang, Z., Tan, J., Tan, L., Liu, J. & Zhong, L. 2012. Control the egg hatchling process of *Pomacea canaliculata* (Lamarck) by water spraying and submersion. *Acta Ecologica Sinica* 32: 184-188.

Pomacea canaliculata in Ecuador: a recent pest with multiple implications

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Abstract

This article characterises and analyses the presence of the alien invasive species *Pomacea canaliculata* in Ecuador, a pest present in many countries that has severe impacts on agriculture, human health and the natural environment. For the first time, a list of the native species of the genus *Pomacea* in Ecuador is provided, as well as an occurrence map, based on review of existing (but few) bibliographic data, museum collections and recent field work. There is a lack of information on other mollusc species in Ecuador, but there is the potential for ecological impact of *P. canaliculata* on the native mollusc fauna, especially other *Pomacea* species, which may already be in decline. Other biological threats and consequences are considered, highlighting the impacts that the invasion has had in this Andean country. Also, events that have occurred since the detection of the pest are reviewed, in particular, the decisions adopted by the rice agricultural sector in comparison with those reported by other countries facing a similar situation.

The epidemiological role of *P. canaliculata* in Ecuador is analysed following confirmation that *P. canaliculata* can carry the nematode *Angiostrongylus cantonensis*, which causes eosinophilic meningitis. Cases of human infection and the possible routes of transmission are discussed, confirming that Ecuador was the first South American country to have cases of the disease. These results are compared with those for the giant African snail (*Lissachatina fulica*), a mollusc that can also transmit the disease.

It isprobable that native *Pomacea* species can also be infected with the nematode. Finally, a wide range of measures and management actions that should be considered, and possibly adopted, by Ecuador are proposed with the goal of controlling this dangerous pest.

Additional keywords: Ampullariidae, *Angiostrongylus*, apple snail, giant African snail, invasive species, South America

Introduction

In 2005, *Pomacea canaliculata*, a member of the family Ampullariidae, commonly known as apple snails, was discovered in Ecuador, where it causes serious damage to rice (Horgan *et al*., 2014a). It is an invasive alien species in Ecuador. *Pomacea canaliculata* has generalist feeding habits, often lacks natural enemies in places that it invades, can tolerate a wide range of temperatures, is very resistant to water pollution and has high reproductive potential (Lach *et al*., 2001; Cowie, 2002). It also has diverse impacts, not only agricultural but also ecological and socio-medical, and therefore is considered among 100 of the world’s worst alien invasive species (Lowe *et al*., 2000). Its harmful effects include destruction of native aquatic vegetation resulting in serious habitat modification, competition with native mollusc species and acting as a host of several parasites, including the rat lungworm, *Angiostrongylus cantonensis* (Cowie 2002; Carlsson *et al*., 2004, 2009; Hollingsworth & Cowie, 2006; Levin, 2006; Lv *et al*., 2011; Vazquez-Silva *et al*., 2011).

Pomacea canaliculata is a South American species. Its native range has been thought to extend throughout much of South America east of the Andes (Hylton Scott, 1958) but it is now known to be restricted to the Lower Paraná, Uruguay and La Plata basins, and perhaps lower parts of the upper Paraná and southern parts of Brasil (Hayes *et al*., 2008, 2009a, 2012). It was introduced from Argentina, probably more than once (Hayes *et al*., 2008), to many Asian countries, where it became a serious agricultural pest, especially of rice (Naylor, 1996; Cowie, 2002; Wada, 2004; Joshi & Sebastian, 2006). Later, it was introduced and became established in other areas of the Pacific (Hawaii, Guam, Papua New Guinea) (Cowie, 2002), the USA (Rawlings *et al*., 2007), the Dominican Republic (Rosario & Moquete,2006), Chile (Letelier & Soto-Acuña, 2008; Jackson & Jackson, 2009) and Spain (López et al., 2010; MMAMA, 2015). The first introductions to Asia were based on the potential of the snails as a commercial product for human consumption, through cultivation (aquaculture). However, major commercial production was not successful and the snails escaped or were released from the facilities and colonised various aquatic ecosystems, becoming a major pest, especially in wetland rice. The snails were also imported for use in aquaria (Cowie, 2002). Climate models applied to the potential expansion of the species, inferred high colonisation ability of many non-infested areas of the world (Baker, 1998), facilitated by the increased activity of the snails at higher temperatures (Heiler *et al*., 2008).

The confused taxonomy and difficulty of identifying species of *Pomacea* (Ramirez *et al*., 2003; Cowie *et al*., 2006; EFSA, 2012) has caused much uncertainty regarding the identities of the pest species. The main consequence of this is that even though it is possible to identify some species quickly on the basis of a combination of morphological characters of the shell and eggs, for others it is only possible by investigating detailed internal anatomy and by applying molecular techniques. This was especially the case in distinguishing *Pomacea maculata* (formerly often referred to as its junior synonym *Pomacea insularum*) from *P. canaliculata* (Rawlings *et al*., 2007; Hayes *et al*., 2008, 2009a, 2012; Matsukura *et al*., 2008; EFSA, 2012). The use of common names for *P. canaliculata*, in both English and other languages has also caused confusion (Cowie *et al*., 2006).

In 2009 the presence of an unknown snail was confirmed in rice fields in Guayas province of Ecuador. Subsequently, this species was confirmed as *Pomacea canaliculata* by Dr. David Robinson of the USDA-APHIS, in communication to the Training and Design Workshop of the Andean Regional Program for the Control of Mollusc Pests event conducted by Agrocalidad in Guayaquil in 2011.

One of the most important actions when a pest, such as this one, is detected is informing and training citizens to help them contribute to reducing the impact. Therefore, in 2010, we presented various proposals for action to Agrocalidad based on educating people about snails as rice pests in the Ecuadorian coastal region. We developed specialised reports on the pest (unpublished) for the Ministry of Environment and made information presentations. Also, the first author participated in preparation of the preliminary list of introduced and invasive species in continental Ecuador, including *P. canaliculata* (MAE, 2011). The expanding distribution of the pest, the damage caused and the interest generated among the relevant authorities in Ecuador, led in 2011 toAgrocalidad organising the first workshop on the pest, in Guayaquil, attended by experts, including Dr. Robinson (above), national guests and others from the Andean region.

The information in this contribution is derived from a bibliographic review (scientific and non-scientific), reviews of collections, surveys and field investigations and activities. The aim is to contribute to the knowledge of the genus *Pomacea* in terms of diversity and distribution of the species in Ecuador, and in this way to document, evaluate and participate in lessening its environmental, agricultural and human health impacts.

The genus *Pomacea* in Ecuador and the appearance of *P. canaliculata*

In Ecuador, native apple snails (Ampullariidae) are important in terms of their diversity and representation in the freshwater mollusc fauna (Correoso, 2002, 2008; Lasso *et al*., 2016). Unfortunately, molluscan faunal studies have been limited and the lack of information does not help in the protection and conservation of this natural heritage. This lack of knowledge may lead indirectly to significant impacts of *P. canaliculata* on the native fauna, as indiscriminate and hasty pest management actions are implemented.

The diversity and abundance of *Pomacea* species is particularly high in the Andean and Amazonian regions, including in Peru (Ramírez *et al*., 2003), Colombia (Ardila, 2008), Venezuela and Brasil (Simone, 2006), although the genus extends through Central America and the Caribbean to the southeastern USA. Ecuador, in the Neotropical Andean region and with distinct mountain, coastal and Amazonian areas, has fewer species than Brasil, Venezuela and Colombia (Table 1), although with a high number of endemics (Table 2). There is a pronounced drop in diversity in the coastal region.

There have been few surveys of snails in Ecuador and there are few records of *Pomacea* in Ecuador in the literature. A number of species are mentioned as occurring in Ecuador by modern authors (Cowie & Thiengo, 2003; Ramirez *et al*., 2003; Simone, 2006), referencing the classical authors, who are noted in Table 2. There are no large national reference collections of Ampullariidae or other non-marine molluscs in Ecuador, because much of the collected material (especially type series) was taken to foreign museums, mostly in Europe, including the Museum national d'Histoire naturelle, Paris (Cowie & Héros, 2012), the Natural History Museum, London, the Museum für Naturkunde, Berlin (Köhler & Glaubrecht, 2006) and the Museo Nacional de Ciencias Naturales, Madrid, among others (see also Cowie & Thiengo, 2003; Correoso, 2008).

Table 1. Number of native species of *Pomacea* in various countries.

Country	Number of *Pomacea* species	Reference
USA	1	Vazquez-Silva *et al*., 2011
Mexico	1	Vázquez-Silva *et al*., 2011
Cuba	2	Vázquez Perera & Perera Valderrama, 2010
Ecuador	21	Present text
Peru	22 or fewer	Ramírez *et al*., 2003
Venezuela	26 (and 6 subspecies)	Wikipedia contributors, 2017
Brasil	32	Simone, 2006
Colombia	32	Linares & Vera, 2012
Argentina	3	Rumi *et al*., 2008

Table 2. Species of *Pomacea* known from Ecuador according to literature sources, review of collections and our own data (collected by the first author). Species are considered endemic to Ecuador if no records are known from other countries. Synonyms listed are only those by which the species has been reported in Ecuador. Synonymy according to Cowie & Thiengo (2003) unless otherwise stated.

Species	Synonyms	Native/Endemic to Ecuador	References
Pomacea aldersoni (Pain, 1946)	—	Endemic, described from Santa Barbara	Pain, 1946; Cowie & Thiengo, 2003; Simone, 2006
Pomacea aulanieri (Deville & Huppé, 1850)	—	Native, reported from Santa Rosa	Germain, 1910
Pomacea canaliculata (Lamarck, 1822)	—	Introduced	MAE, 2011
Pomacea chemnitzii (Philippi, 1852)	*Ampullaria porphyrostoma* Reeve, 1856; as "*porplustoma*" in Museu de Historia Natural	Native, Guayas Province and the coast	Cowie & Thiengo, 2003; Museo de Historia Natural "Gustavo Orcés V"
Pomacea columbiensis (Philippi, 1851)	—	Native, reported from Rio Pastaza (Miller, 1879)	Miller, 1879; Cousin, 1887; Germain, 1910; Cowie & Thiengo, 2003
Pomacea columellaris (Gould, 1848)	*Ampullaria sprucei* Reeve, 1876	Native, Pastaza province	Pain, 1960 (tentative record); Museo de Historia Natural "Gustavo Orcés V"
Pomacea cousini (Jousseaume, 1887)	—	Endemic, without precise locality	Jousseaume, 1887; Cowie & Thiengo, 2003; Simone, 2006
Pomacea cumingii (King & Broderip, 1831)	*Ampullaria quitensis* Busch, 1859	Native, without precise locality (Quito is higher than the altitudinal limit of the genus)	Miller, 1879; Cousin, 1887; Cowie & Thiengo, 2003 (tentative record)

Pomacea cf. cumingii (Philippi, 1851 *non* King & Broderip, 1831)	—	Native, reported from Rio Pastasa (Miller, 1879)	Miller, 1879; Cousin, 1887
Pomacea expansa (Miller, 1879)	—	Endemic, described from Rio Santiago, Esmeraldas Province.	Miller, 1879; Cousin, 1887; Cowie & Thiengo, 2003; Simone, 2006
Pomacea cf. lineata (Spix, 1827)	—	Native, coastal region	Simone, 2006 (incorrectly synonymized *Ampullaria chemnitzii* Philippi, 1851 with *P. lineata*); Martini *et al*., 2008
Pomacea maculata Perry 1810	*Ampullaria haustrum* Reeve, 1856	Native, Pastaza province	Jácome, 2001b; Correoso, 2008; Hayes *et al*., 2012 (synonymy)
Pomacea martinezi (Hidalgo, 1866)	—	Endemic, described from Santa Rosa	Hidalgo, 1866; Cousin, 1887; Germain, 1910
Pomacea modesta (Busch, 1859)		Native, without precise locality	Miller 1879; Cousin, 1887; Simone 2006
Pomacea pealiana (Lea, 1838)	—	Native	Cowie & Thiengo, 2003
Pomacea cf. producta (Reeve, 1856)	—	Native, without precise locality	Museo de Historia Natural "Gustavo Orcés V", collected before 1960
Pomacea puntaplaya (Cousin, 1887)	—	Endemic, described from Punta-Playa, without precise locality	Cousin, 1887; Simone 2006
Pomacea quinindensis (Miller, 1879)	—	Endemic, possibly specifically to the coast, described from Rio Quinindé	Miller, 1879; Cousin, 1887; Germain, 1910; Simone, 2006
Pomacea reyrei (Cousin, 1887)	? *Ampullaria aulanieri* Deville & Huppé, 1850	Possibly endemic, described from Napo, reported from Santa Rosa	Cousin, 1887; Germain, 1910; Simone 2006
? *Pomacea sordida* (Swainson, 1823)	—	Native, reported from Babahoyo (as "Bodegas"), identification uncertain	Cousin, 1887 (tentative identification)
Pomacea tenuissima (Jousseaume, 1894)	—	Endemic, described from Coca	Jousseaume, 1894; Simone, 2006
Pomacea sp.	—	Native, Rio Napo	Correoso, 2008 (unidentified species)

Nonetheless, some specimens are present in Ecuador in the Museo de Historia Natural "Gustavo Orcés V" at the Institute for Biological Sciences, National Polytechnic School, also in the Museum of Zoology of invertebrates (QCAZ) at the Faculty of Natural Sciences at the Catholic University Ecuador, and in the Ecuadorian Museum of Natural Sciences (MECN) (Correoso, 2002, 2008). Table 2 also lists the results of review of material in these Ecuadorian museum collections (in many cases without any georeference) and surveys conducted by the first author, including synonyms and tentatively whether each species is endemic, native but not endemic, or introduced in Ecuador.

Undoubtedly, the most important historical reports of *Pomacea* (as "*Ampullaria*", which is now not considered a synonym of *Pomacea*; Cowie, 1997) in Ecuador are due to Miller (1879), Cousin (1887), Jousseaume (1887) and Germain (1910). Unfortunately, for most species these publications only mention Ecuador without a more precise locality.

The genus *Pomacea* in Ecuador includes preliminarily 22 species, of which 13 are native, eight endemic or possibly endemic and one introduced (*P. canaliculata*). Future research may change the number of species and their status. Thus, species described many years ago may be synonymised, as has happened with *P. flagellata* in Central America, for which Pain (1964) identified 30 synonyms, while new species may be described, as many areas are still unexplored.

Despite the above difficulties, the knowledge we do have of the distributions of the species is valuable for pest management in Ecuador. We can infer a wide geographical distribution of both native *Pomacea* species (Fig. 1, Appendix 1) and *P. canaliculata* (Fig. 2, Appendix 2) in Ecuador. In addition, *Pomacea* spp. have been recorded at many other locations that are not shown in the figures nor listed in the appendices, as they are not well georeferenced. Together, these data indicate an overall distribution of 120 localities in 13 provinces of Ecuador with at least one species at each locality.

Appendix 1. Localities in Ecuador where native species of *Pomacea* have been recorded (for map see Fig. 1).

No.	Locality	Province	Latitude	Longitude
1	Santa Rosa	Esmeraldas	0°45'37.6" N	79°37'27" W
2	Quininde	Esmeraldas	0°19'14.6" N	79°28'4.9" W
3	SantaRosa	Guayas	1°54'24.5" S	79°57'44" W
4	Cerro Colorado	Guayas	2°40'0" S	80°45'0" W
	CerroColorado	Guayas	2°30' 0" S	80°30'0" W
5	La Puntilla	Guayas	2°9'40.3" S	79°48'8.4" W
6	Mindo	Pichincha	0°3'30.2" S	78°46'30.3" W
7	Rio Blanco	Pichincha	0°23'53" S	78°48'43" W
8	Gualea	Pichincha	0°6'41" N	78°45'1.3" W
9	Nanegal	Pichincha	0°8'25.3" N	78°40'29.5" W
10	Tandayapa vertiente	Pichincha	0º0'2.6" N	78º40'35.3" W
11	Gualea	Pichincha	0º6'41" N	78º45'1.3" W
12	Parroquia Tena	Napo	0º59'27.7" S	77º48'53.2" W
13	Instituto Lingüístico de Verano Limoncocha	Napo	0º22'27" N	76º33'37" W
14	Pozo Shushuqui	Sucumbíos	0º2'28" N	76°37'58.8" W
15	Lago Agrio Ciudad	Sucumbíos	0º5'17.2" N	76º53'12.8" W
16	Laguna Cuyabeno	Pastaza	0°1'0" S	76°49'58.8" W
17	Macas	Morona Santiago	2º18'15.5" S	78º7'0.4" W
18	Chiguaza	Morona Santiago	2º2'20.2" S	77º59'0.5" W
19	Puerto Francisco Orellana	Orellana	0º28'7.1" S	76º59'9.9" W

Fig. 1. Locations corresponding to *Pomacea* spp. native to Ecuador based on records in the literature, museum collections and field surveys (see Appendix 1 for location details).

Pomacea species occur in Ecuador on both sides of the Andes (and are separated by them) occupying the two lower altitude regions of the country, that is, Amazonia and some locations in the eastern foothills of the Andes, and in the provinces of Pichincha (mainly), Esmeraldas and Guayas, west of the Andes. According to the literature records, the altitudinal limit of the genus in Ecuador is around 1500-1600 m above sea level, although in our survey the maximum altitude reached was about 1250-1450 m in Pichincha province in waterways of Nanegal, Mindo and Gualea, and in the Rio Blanco. Altitude acts as a limiting factor in the distribution of the species, because of the temperatures and other factors that influence the lives of these ectothermal organisms (Correoso, 2008).

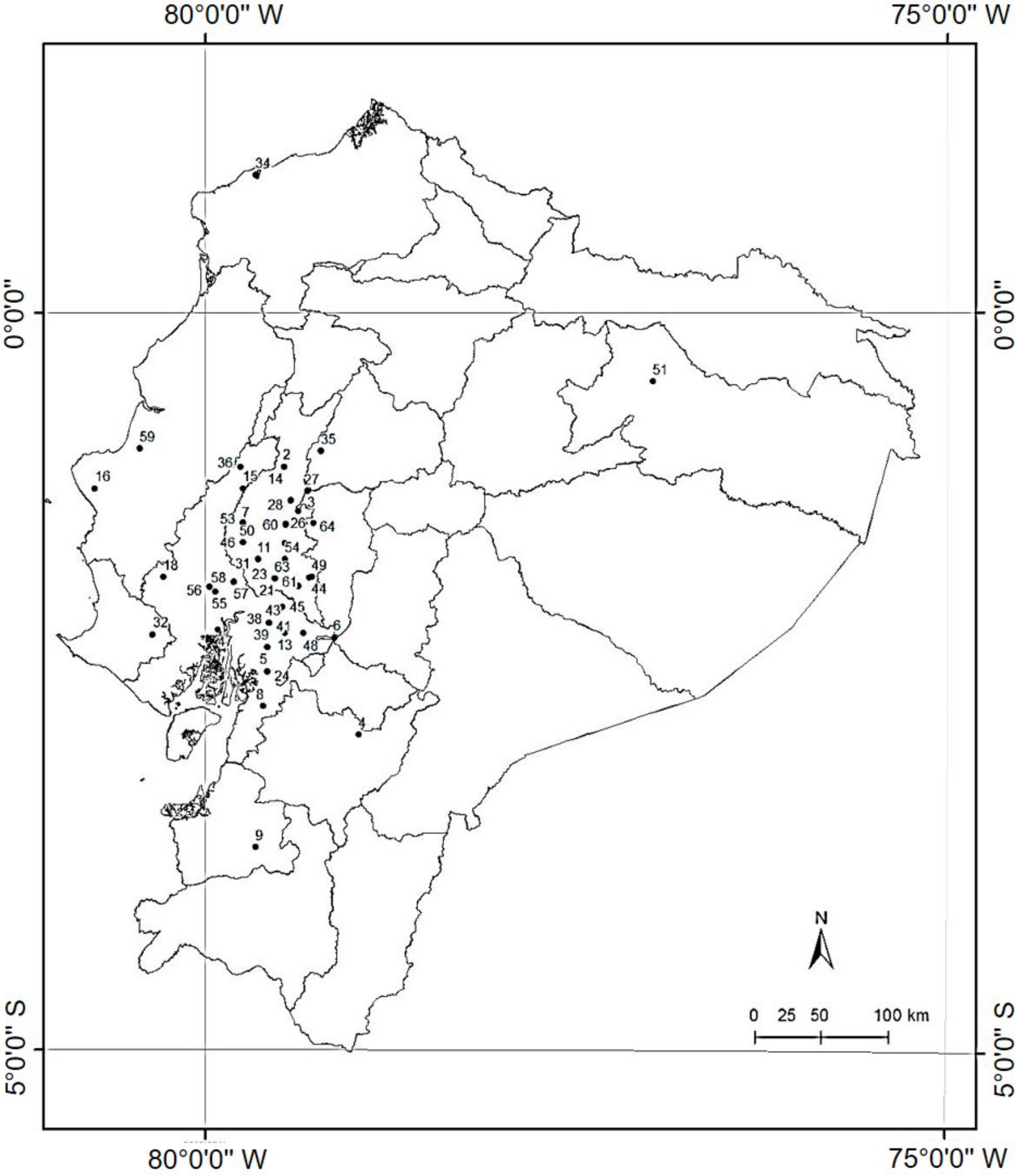

Fig. 2. Locations where *P. canaliculata* has been recorded in Ecuador from 2008 to 2012 (see Appendix 2 for location details).

Appendix 2. Cities in Ecuador where *P. canaliculata* has been recorded (2008-2012) (for map see Fig. 2).

No.	Locality	Province	Latitude	Longitude
1	Ventanas	Los Ríos	1°26'04.4" S	79°27'27.8" W
2	Quevedo	Los Ríos	1°02'35.4" S	79°28'23.5" W
3	Quinsaloma	Los Ríos	1°12'20" S	79°18'49.28" W
4	Ricaurte	Los Ríos	2°51'47.5" S	78°57'50.4" W
5	Tarifa	Guayas	2°16'12.8" S	79°34'58.1" W
6	Cumandá	Chimborazo	2°12'21.1" S	79°08'02.25" W
7	Palenque	Los Ríos	1°26'09.2" S	79°45'04.4" W
8	Naranjal	Guayas	2°40'22.7" S	79°36'51.96" W
9	Milagro	Guayas	3°37'42" S	79°39'44.6" W
10	Jauneche	Guayas	1°26' 04.3" S	79°27'36" W
11	Baba	Los Ríos	1°40' 26.7" S	79°38'54.3" W
12	Babahoyo	Los Ríos	1°48'05.5" S	79°32'01.4" W
13	Naranjito	Guayas	2°10'26.9" S	79°28'03.3" W
14	Quevedo	Los Ríos	1°02'35.3" S	79°28'23.5" W
15	Pichincha	Manabí	1°11'31.5" S	79°44'57.1" W
16	Pichincha	Manabí	1°11'31.5" S	80°44'57.1" W
17	Babahoyo - La Huaquilla	Los Ríos	1°47'38.2" S	79°17'09.6" W
18	Babahoyo - La Huaquilla	Los Ríos	1°47'38.2" S	80°17'09.6" W
19	Babahoyo - La Huaquilla	Los Ríos	1°47'38.2" S	81°17'09.6" W
20	Babahoyo - La Huaquilla	Los Ríos	1°47'38.2" S	82°17'09.6" W
21	Babahoyo	Los Ríos	1°48'05.5" S	79°32'01.4" W
22	Babahoyo - La huaquilla	Los Ríos	1°47'38.2" S	79°17'09.6" W
23	Babahoyo	Los Ríos	1°48'05.5" S	79°32'01.4" W
24	Samborondon (H. El Encanto)	Guayas	2°16'12.8" S	79°34'58.1" W
25	Ventanas (La Poza)	Los Ríos	1°26’04.3” S	79°27’36.0” W
26	El Cristal - Ventanas	Los Ríos	1º20’43.6” S	79°22’33.1” W
27	Quinsaloma - Ventanas	Los Ríos	1°12’33.9” S	79°18’41.5” W
28	La Ercilia - Ventanas	Los Ríos	1°16’32.2” S	79°25’32.4” W
29	La Poza - Ventanas	Los Ríos	1°51’27.6” S	79°22’33.8” W
30	La Huaquilla - Ventanas	Los Ríos	1°47’43.0” S	79°18’05.1” W
31	Montalvo	Los Ríos	1°40’26.7” S	79°38’54.3” W

32	SimónBolívar	Guayas	2°11'01.9" S	80°21'29.4" W
33	Babahoyo	Los Ríos	1°48'05.5" S	79°32'01.4" W
34	Rio Cayapas	Esmeraldas	0°56'14.7" N	79°40'01.4" W
35	La Mana	Cotopaxi	0°56' 18.5" S	79°13' 27.5" W
36	Santa Lucía	Guayas	1°2'45.1" S	79°46'7.4" W
37	Milagro	Guayas	2°6'11.3" S	79°34'18.7" W
38	Milagro	Guayas	2°6'11.3" S	79°34' 18.7" W
39	Milagro	Guayas	2°6'11.3" S	79°34'18.7" W
40	Milagro (Piñuelal)	Guayas	2°6'11.3" S	79°34'18.7" W
41	Milagro	Guayas	2°6'11.3" S	79°34'18.7" W
42	Milagro	Guayas	2°6'11.3" S	79°34'18.7" W
43	Milagro	Guayas	2°6'11.3" S	79°34'18.7" W
44	La Huaquilla - Montalvo	Los Ríos	1°47'31.7" S	79°17'5.5" W
45	Simón Bolívar	Guayas	1°59'48.3" S	79°28'52.3" W
46	Vinces	Los Ríos	1°33'35.4" S	79°44'58" W
47	Mapasingue Este (Guayaquil)	Guayas	2°9'5.8" S	79°55'15.2" W
48	Primavera (Durán)	Guayas	2°10'35.4" S	79°20'34.6" W
49	La Huaquilla - Montalvo	Los Ríos	1°47'31.6" S	79°17'5.5" W
50	Vinces	Los Ríos	1°33'35.4" S	79°44'58" W
51	El Coca	Orellana	0°27'52.6" S	76°59'4.2" W
52	Ricaurte	Los Ríos	1°34'0.3" S	79°28'1.2" W
53	La Revesa	Los Ríos	1°25'26.2" S	79°44'58.3" W
54	Caracol	Los Ríos	1°40'19.3" S	79°27'53.2" W
55	Daule - Rcto. Cascol	Guayas	1°53'52.9" S	79°56'11" W
56	Daule	Guayas	1°51'37.9" S	79°58'39.5" W
57	Salitre	Guayas	1°49'30.8" S	79°48'39.8" W
58	Salitre	Guayas	1°49'30.8" S	79°48'39.8" W
59	Rocafuerte	Manabí	0°55'17" S	80°26'55" W
60	La Ercilia - Ventanas	Los Ríos	1°26'29.5" S	79°27'43" W
61	Babahoyo - La Unión	Los Ríos	1°48'14.5" S	79°32'4.7" W
62	Babahoyo - La Unión	Los Ríos	1°48'14.5" S	79°32'4.7" W
63	Babahoyo - La Unión	Los Ríos	1°48'14.5" S	79°32'4.7" W
64	Echeandia - Sabanetilla	Bolívar	1°25'32.8" S	79°16'34" W

Conchological surveys since 1999 in Ecuador by the first author, also allow us to infer a clear decrease in the distribution and abundance of native species of *Pomacea*, which should be monitored in future with qualitative and quantitative studies specifically designed to take into account the presence of *P. canaliculata*. Urban growth and agricultural expansion in locations where we previously recorded native *Pomacea* spp. have had a particular impact on the coast (for example, Bodegas and Santa Rosa in the provinces of Guayas and Esmeraldas, respectively). Comparison with the historical references (Miller, 1879; Cousin, 1887; Jousseaume, 1887; Germain, 1910) reinforces this observation. A similar decline associated with human factors has been suggested for terrestrial and aquatic molluscs of the country in general (Correoso, 2008, unpublished; Lasso *et al*., 2016). The impact of urban expansion, intensive agriculture, fires, use of biocides and other toxic chemicals, and pollution on habitats and food resources are some of the main factors significantly decreasing plant and animal biodiversity globally; but so are invasive species. *Pomacea canaliculata* is such a species and its occurrence in locations where native species of *Pomacea* (and others) are known in Ecuador should be anticipated, investigated and managed. A better understanding of native species, their taxonomy, morphology, anatomy, biology and distributions is important for this purpose, as Ampuero (2013) discussed for Peru.

Like other molluscs for which there is no commercial interest, ampullariids have been largely ignored in the national zoological context. The finding of *P. canaliculata* in Ecuador and the environmental, ecological, social and economic threats that it poses have greatly modified this social perception, negatively. This negative perception of molluscs has been exacerbated by a series of malacological events, including the discovery of *Lissachatina fulica*, also an invasive species (Correoso, 2006; Correoso & Coello, 2009; Borrero *et al*., 2009), agricultural damage caused by certain species of slugs and the increasing density of *Cornu aspersum*, a legacy of attempts to rear it in the 2000s and which also appears to be affecting certain agricultural crops.

To a greater or lesser extent, the native species of *Pomacea* are used as food in Ecuador under the name “churos”, for example, dishes called “ceviches”, typical of the western coastal areas of the country (not to be confused with “churos terrestros” , mostly of the land snail *Naesiotus quitensis*). Other common names for apple snails in Ecuador are “caracol de laguna”, “caracol del Paraná”, “caracol gigante”, “caracol lunar”, “sacha”, “guarura” and“coroba o cuiba” (Molina Zamora, 2012). In the east, groups of natives and settlers in the Amazon region of Ecuador traditionally consumed species of *Pomacea* and also used them for ornamental, medicinal, ceremonial and ritual purposes, as in other regions of the Amazon, Orinoco and Paraná basins. Although consumption and other uses of these molluscs in the Neotropical Andean area (Ecuador, Peru and neighbouring countries) is ancient, they have been rarely referenced (Ramirez *et al*., 2003).

Jácome (2001a, b) discussed marketing, transfer of specimens and captive breeding of *Pomacea haustrum* (possibly misidentified; R.H. Cowie, pers. comm.) in small artisanal farms in communities of Puyo, Fátima and Hola Vida in Pastaza province). In Peru Ampuero (2013) and Padilla *et al*. (2004) discussed marketing and breeding, respectively, of *Pomacea* for consumption and medicinal use.

Ecological concerns regarding the pest and the genus *Pomacea* in Ecuador

The impacts of invasive alien species are immense, insidious and usually irreversible. The scope and cost of alien invasions are global and high. The threat to biodiversity and ecosystem services that invasive alien species pose may involve multiple contingencies (Costanza *et al*., 1997; Horgan *et al*., 2014b), including severely affecting native species and the structure and function of ecosystems, destruction and modification of habitat, predation, competition, disease transmission and human health as well as the economy (Comisión Europea, 2013).

The first reaction to the presence of *P. canaliculata* in Ecuador was alarm after seeing the damage that occurred in rice crops. Initial confusion regarding what to do was followed by a period in which responsible entities and agencies had to acknowledge existence of the problem and learn and implement management measures, destructive and/or palliative. As in other countries that had suffered invasion by *P. canaliculata*, the first responses were drastic and involved excessive spraying of molluscicides (see below) that did not discriminate between native and non-native species.

However, the situation in Ecuador has specific issues that do not exist in most regions of the world to which *P. canaliculata* has been introduced. Unlike other regions (such as Spain or Asian countries), Ecuador supports a rich fauna of species of the same genus (Correoso, 2008; Correoso, R.M., Martini, R., Narváez, G.M., Muzzio, J. & Orlando, N., unpublished). However, taxonomic studies of Ecuadorian non-marine molluscs are few and the conservation status and threats affecting only nine species have been evaluated for the IUCN *Red List of Threatened Species* (IUCN, 2016). Five of these are species of *Pomacea*: *P. aldersoni* listed as Least Concern; *P. chemnitzii*, *P. cousini* and *P. reyrei* as Data Deficient; and *P. quinindensis* as Vulnerable because it only occurs in one location and this location suffers pollution from urban and agriculture effluents, especially the high quantities of molluscicides applied to rice fields to control *Pomacea canaliculata* (Correoso, 2016). Most species, however, have not been catalogued or evaluated and they thus lack both specific legal protection in Ecuador and international awareness.

Although the Ecuadorian circumstances are certainly particular, the sequence of events caused by the pest in other countries should be illustrative and should be taken into account. In the Philippines, there are no native *Pomacea* species but there are other native ampullariids in the genus *Pila*. Populations of some species of this genus have experienced sharp declines perhaps as a result of extensive and indiscriminate application of pesticides to combat the introduced *P. canaliculata* (Anderson, 1993). Kwong *et al*. (2009) highlighted the predatory ability of *P. canaliculata* against various developmental stages of five species of freshwater snails in south China. In the USA, introduced *Pomacea maculata* are a potential threat to native *P. paludosa*, where their ranges overlap, as it has been suggested that they may have the potential to reduce the growth rate of the latter (Conner *et al*., 2008). The invasive species may not only compete for food with the native species but may also affect native scavengers (crabs, shrimp and fish) and aquatic vegetation, among other impacts. For example, in Thailand, damage by a species of *Pomacea* resulted in complete absence of aquatic macrophytes, high nutrient concentrations and high phytoplankton biomass (high turbidity), altering both biodiversity and the integrity and functioning of the ecosystem (Carlsson *et al*., 2004). Thus, the effects are not only on closely related or congeneric species but also on other evolutionarily distant species and on the natural balance of the invaded area in general.

Distinguishing harmless native species, perhaps belonging to the "*P. canaliculata* group" (Hayes *et al*., 2009b), from the invasive alien *P. canaliculata* in Ecuador is difficult. The great similarity in shell form and the sharing of similar habitats are factors and though there have been attempts to provide distinctive morphometric characters for distinguishing them, the fact is that the identification is difficult, often ambiguous (Ramirez *et al*., 2003; Ampuero, 2013) and may require detailed anatomical and molecular analyses (Hayes *et al*., 2012).

The above examples (there are many others) represent obvious and worrying concerns for the potential damage that *P. canaliculata* could cause in Ecuador, including possible effects on the already threatened native snails (declining as a result of human activities), but also on the flora and fauna in general and on the entire ecosystem of the invaded areas, with the threat of loss of biological diversity. According to our observations, the snail kite, *Rostrhamus sociabilis*, which is threatened and classified as vulnerable in

CITES Appendix II, has suffered a clear decline since 2009 in coastal regions because of increasing activity of farmers and excessive spraying of molluscicides and other poisons to control *P. canaliculata*. In this rice-growing area there is a complex mosaic of ecological relationships among the rice, indigenous and invasive snails, snail kites and pesticide application (MAE, 2011; Correoso, R.M., Martini, R., Narváez, G.M., Muzzio, J. & Orlando, N., unpublished) that should be monitored.

Analysis of the ecological impacts of the pest species on native ampullariids, particularly species of *Pomacea* in Ecuador, could be considered as a case study. As yet unassessed biotic and abiotic effects on ecosystems and their components (insects, fish, microorganisms and especially aquatic and terrestrial molluscs) and disruption of ecological functions (trophic relationships, inter- and intra-population dynamics, habitat alteration, etc.) will have to be investigated, as the short-term balance of these ecosystems, including rice, is at stake (cf. IUCN, 2000; Horgan *et al*., 2014b).

Agricultural implications of *P. canaliculata* in Ecuador and actions taken

Rice is one of the largest crops in Ecuador and the largest source of human calorie intake from cereals. It occupies more than a third of the area of agricultural production of the country, the largest area being near the coast, with small amounts also produced in the Andean foothills and in the Amazon (Troya Vera, 2013).

It is unknown how *P. canaliculata* invaded Ecuadorian rice fields, although a series of events coincided chronologically with its detection. These events included the rapid expansion of snail farming in Ecuador involving *Cornu aspersum*, the first national report of *Lissachatina fulica* by Correoso (2006), frequent heavy rainfall periods in the winter and certain inappropriate agricultural practices (Correoso, unpublished). Mere coincidences or not, they are striking, although other more normal causes of invasion are not ruled out, including trade, tourism, transportation, aquarium releases and aquaculture (IUCN, 2000; Rawlings *et al*., 2007; Ochoa Chumaña & García Onofre, 2012). The sociological phenomenon that seems to have emerged after the simultaneous occurrence of these adverse events associated with molluscs is equally striking and interesting: among part of the population an understandable rejection of these animals, almost a process of demonization, has arisen.

To reconstruct the time-frame of events that followed public acknowledgment of the existence of the pest in rice, official, scientific and non-scientific sources have been used.

The first apple snails were found in rice in 2005, specifically in the province of Guayas (Agricultural Cooperative in St. Mauricio, Canton El Triunfo) (MAE, 2011; Correoso, unpublished). After receiving the first reports of the pest, INIAP (National Autonomous Institute for Agricultural Research) began to get information about the invasive snails. However, it took four years until the existence of the pest became wider public knowledge, after reporting in the press, in 2009, its discovery in the province of Guayas (Daule and Colimes) in central-western Ecuador (Fig. 2). Gradually, reports appeared from other locations (MAE, 2011; Ochoa Chumaña & García Onofre, 2012). The public concern arising from these reports and the widespread alarm of rice farmers led to the authorities (Agrocalidad and INIAP) to assume official responsibility, begin official studies and adopt measures to manage the pest. Specifically, Agrocalidad was responsible for leading and coordinating both the institutions involved and the actions undertaken (MAE, 2011). These included training of technicians and farmers in the coastal area, disseminating information, monitoring, collecting and shipping samples for identification of other gastropod species with the potential to be considered as pests, developing control measures in laboratory experiments and looking for technical solutions for management of the pest in rice-growing areas (MAE, 2011).

However, this suite of measures has not had the hoped for results. Changes in management of rice crops did not eliminate the pest, although they did lessen its effects, but Agrocalidad was not successful in involving many small-scale farmers with limited capacity for economic response and consequent difficulty or impossibility of implementing the recommended measures. Neither did application of pesticides, including endosulfan, methomyl and their combinations (Ochoa Chumaña & García Onofre, 2012), copper sulphate or similar, succeed in eradicating the pest, making questionable its real effectiveness, especially because of the unwanted side effects (traditional pest resistance, elimination of other beneficial animals, phytosanitary issues and risks to human health) (Ochoa Chumaña & García Onofre, 2012). Many of these chemicals were used indiscriminately, despite being banned in many other countries because of their high levels of environmental impact (Ochoa Chumaña & García Onofre, 2012). Their use is no longer authorised in Ecuador. Some rice farmers have formed associations, aiming to gain greater influence in decision-making, and there are suggestions to change, at least temporarily, to crops other than rice (Ochoa Chumaña & García Onofre, 2012).

Since it was first reported, there has been a dramatic increase in the number of localities (and provinces) in which *P. canaliculata* has been found: two locations in 2008, eight in 2010 and 25 in 2012 (Fig. 3). Similarly, the affected rice paddy area increased from 200,000 ha in three provinces in 2010 to 414,000 ha in five provinces (48% of the area in cereal cultivation in the country) in early 2012 (Agrocalidad, unpublished data). These numbers indicate the severe impact that the pest is having on rice crops, which has resulted in 40-45% decreases in yields (Ochoa Chumaña & García Onofre, 2012), thereby increasing the price to the consumer.

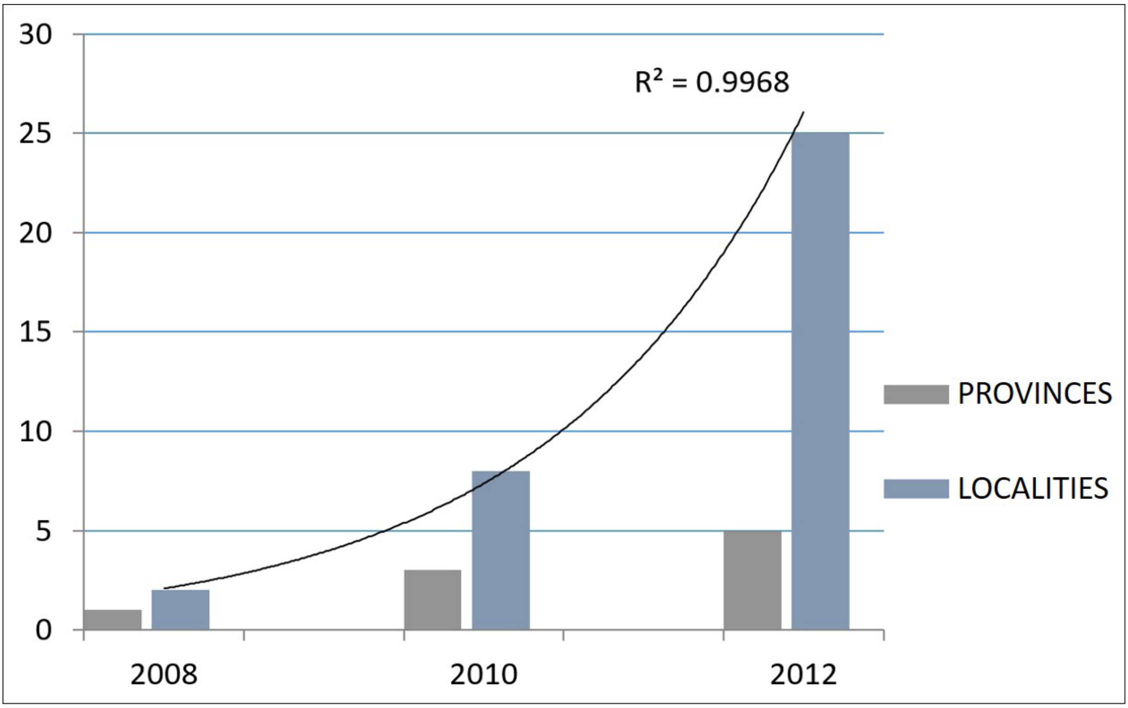

Fig. 3. The numbers of provinces and localities in which *P. canaliculata* was recorded from 2008 to 2012, showing an almost perfectly exponential increase.

Measures taken to eradicate, control or manage the pest have not stopped its spread, although they may have mitigated its effects. Consequently there is dissatisfaction among all stakeholders (including consumers) and a context of some urgency to improve the performance of pest management in the fight against the pest. Various articles in the Ecuadorian press have reported on the introduction of the pest, its expansion and its current status. They advocate modifying crop management to control the populations and learn to live with the snail as they feel it cannot be eliminated.

Epidemiological aspects associated with the genus *Pomacea* in Ecuador and its relation to *Lissachatina fulica*

Some species of native *Pomacea* are traditionally eaten in the Amazon and coastal regions of Ecuador, but there have been few or no epidemiological studies, despite their potential as vectors of human and animal parasites (as are many molluscs). In 2001, A. Jácome (pers. comm.) collected data for the management of the Amazonian snail *Pomacea haustrum* (tentative identification) and parasitological examinations were performed on several specimens of which the majority were infected with nematodes (unidentified) in the mantle cavity, suggesting that both *P. canaliculata* and native *Pomacea* species may also be naturally infected with nematodes, including the rat lungworm, *Angiostrongylus cantonensis* (Correoso, 2008, 2016).

Angiostrongylus cantonensis is a parasitic nematode, the life cycle of which involves rats as definitive hosts and snails as intermediate hosts (Muzzio Aroca, 2011; Cowie 2013). It is the main causative agent of human eosinophilic meningitis, resulting from ingesting infected intermediate hosts (Alicata, 1991; Muzzio Aroca, 2011). *Angiostrongylus cantonensis* was first discovered in southern China but has now been found in other parts of Southeast Asia, India, Sri Lanka, islands of the Pacific and Indian Oceans, Australia, Japan, Brasil, islands of the Caribbean, Africa, the southeastern USA and the Canary Islands (Cowie, 2013). The first cases of eosinophilic meningitis caused by *A. cantonensis* in Ecuador (and in South America), were reported in both children and adults in coastal regions in 2008, and the presence of the worm was demonstrated in *Rattus rattus* and *R. norvegicus* as well as in snails (Martini *et al*., 2008; Pincay *et al*., 2009).

It is important to determine how *A. cantonensis* became established and subsequently spread in Ecuador. The most likely hypothesis is that infected snails were imported by the national snail industry, for breeding and export, and when this business failed, the snails were dispersed to almost all the national territory. Rats ate the snails and became infected for the first time. The worms completed their life cycle in the rats, producing first stage larvae that are eliminated in the faeces, which were then eaten by native snails, initiating a natural focus, which then caused outbreaks in several areas simultaneously. As indicated above, the people of the affected areas have the habit of eating raw snails, a cultural practice that poses a risk now that the parasite is present in Ecuador.

Initially, human infection was thought to be associated with ingestion and/or handling of *Lissachatina fulica* (news that attracted wide media coverage), and later with *Pomacea* spp. The first cases were in patients with a common history of having consumed raw snails in the traditional Ecuadorian coastal dish called "ceviche" (Martini *et al*., 2008). *Pomacea* species and *L. fulica* are known to act as intermediate hosts of the nematode and as vehicles for transmission of the disease to humans (Kim *et al*., 2014).

The relationship between the disease and *Pomacea* spp. has been and continues to be surrounded by some controversy in Ecuador. The taxonomic complexity of the genus has hindered unequivocal determination of the species involved as hosts and in transmitting the nematode to humans. Martini *et al*. (2008) identified two specimens, verified as carriers of *A. cantonensis*, as *Pomacea lineata* (probably misidentified, as this is a species of eastern Brasil; R.H. Cowie, pers. comm.) from the area where people had become infected. Thus it is possible that at least two species of *Pomacea* in Ecuador can act as hosts.

Laboratory screening by Muzzio & Martini (unpublished) show that the proportion of infected individuals of *Pomacea* spp. (28 of 463 individuals screened, 6.1%) is lower than that of *L. fulica* (123 of 814 screened, 15.1%) (Correoso *et al*., 2015). Combining the results for both species, the provinces of Los Ríos, Guayas and Manabi have the highest levels of infection and thus appear to present the highest risk.

Native *Pomacea* species have been eaten both in the coastal and Amazonian areas of Ecuador for a long time without evidence of the disease, despite the fact that various species of snails and slugs can act as vectors of *A. cantonensis* in the region (Martini, 2008; Correoso, unpublished.). The coincidence of the time when human cases began to be detected and the arrival of *A. fulica* and *P. canaliculata* suggests that one or both of these species, both well known internationally as hosts of the parasite and as causing human infection (Lv *et al*., 2008, 2011; Cowie, 2013), was the vector that introduced the nematode. Following introduction to Ecuador, *L. fulica* has expanded its distribution more rapidly than *P. canaliculata* (Correoso & Coello, 2009, Muzzio Aroca, 2011). Nonetheless, it is of concern that as the distribution of *P. canaliculata* increases, other native *Pomacea* species may become infected. Samples taken in the provinces where both invasive species currently coexist with native *Pomacea* species (i.e. Los Ríos, Guayas, Manabi and Santo Domingo de los Tsáchilas) have tested positive for *A. cantonensis* (Muzzio Aroca, 2011).

In this context, the African species perhaps being the more important vector of the disease in Ecuador (as suggested by the quantitative data above), it is logical to think that it would have been responsible for bringing *A. cantonensis* to the country and, among other considerations, to lead to infection of *Pomacea* spp., which would not be surprising given that *P. canaliculata* is a known host of the parasite. *Pomacea canaliculata* can be infected from rats and other sources, including faeces of *Lissachatina fulica*, or vice-versa. This would have happened regardless of which of the two species, *P. canaliculata* or *L. fulica*, reached Ecuador first. Alternatively, introduced specimens of both species may have been parasitized on arrival.

Measures and proposals for action against *P. canaliculata*

Before discussing what measures should be applied and what has been done in Ecuador to combat *P. canaliculata* it is important to have a vision, even a general one, of the current situation and achievements in the fight against this invasive species worldwide.

Distinguishing the two main invasive species, *P. canaliculata* and *P. maculata* (formerly known as *P. insularum*), based on shell morphology is unreliable; anatomical study and molecular analyses are the only certain ways to distinguish them (Matsukura *et al*., 2008; Hayes *et al*., 2012). There is a clear need for information on both species to permit a full understanding of their potential ecological and economic impacts (Barnes *et al*., 2008). Ecosystem impacts have been addressed in Florida (USA) and in Asia and appear complex (Hayes *et al*., 2015). Total costs related to rice in the Philippines, one of the Asian countries most affected by the pest, amounted to $28-45 million in 1990 (Naylor, 1996; Heiler *et al*., 2008). Despite numerous studies, reviewed by Cowie (2002), Joshi & Sebastian (2006) and Hayes *et al*. (2015), knowledge gaps remain and the pest still cannot be adequately controlled in many regions (e.g. Levin, 2006).

Often, detection of the pest generates an initial response of uncertainty in how to deal with it, major economic investment but failure to eradicate it (Levin, 2006; Martin *et al*., 2012). The list of control methods that have been tried is long and includes chemical, biological, cultural and mechanical methods in addition to educational programmes, legislation and combinations of these approaches, reviewed by Cowie (2002), Joshi & Sebastian (2006) and Levin (2006). The IUCN Invasive Species Specialist Group (Cowie, 2005) considered it probably impossible to eradicate established populations of *P. canaliculata*, despite the numerous measures that had been adopted in the fight against the pest, none of which had been demonstrated to be safe, effective and economically viable.

Involvement of USDA-APHIS and other international agencies

Having assisted in identification of the new pest in Ecuador, the next APHIS activity was to support the participation of two Agrocalidad agricultural technicians to attend the workshop "Identification, Trapping, Collection and Eradication of Mollusk Pests" that took place in Zamorano, Honduras, organised by APHIS and OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria) for Central American officials.

Following this training, during 2011 Agrocalidad with the support of APHIS and the Inter-American Institute for Cooperation on Agriculture (IICA) began to collect informationregarding field practices and activities performed by non-governmental agencies, farmers and universities to control rice apple snails and other molluscs. Additionally, APHIS, IICA and Agrocalidad organised two workshops to discuss development of an official control programme for molluscs and to unify approaches of the various institutions that were working with the apple snails.

At the end of 2011, APHIS, IICA and Agrocalidad organised a mollusc regional workshop for the Andean countries, led by Dr. David Robinson, APHIS malacologist. During this workshop, field training in collection of apple snails was organised for Agrocalidad surveillance technicians. Since then, APHIS has provided Agrocalidad with identification support through the USDA-APHIS National Malacology Laboratory.

During 2012 APHIS and IICA supported Agrocalidad in the development of publicity and technical material for farmers to promote awareness of field practices for reducing the presence of apple snails in rice fields, as the pest had reached unexpected abundance causing severe production loss.

Additionally, in an interagency effort involving APHIS, IICA, Food for Peace (PL480), Agrocalidad and the Gesellschaft für Technische Zusammenarbeit (GTZ), Dr. Ravindra C. Joshi, then of the Koronivia Research Station, Ministry of Primary Industries, Department of Agriculture, Fiji, was brought in as a consultant on "Rice Apple Snail (*Pomacea canaliculata*) in Ecuador: Current Status and Management with Non-Chemical Methods in Transplanted Rice Fields".

APHIS, PL480 and IICA continue supporting Agrocalidad in their efforts to educate the rice producers and train officials in activities to control and reduce the abundance of rice apple snails in Ecuador.

Measures taken and proposed in Ecuador

The initial reactions to the appearance of *P. canaliculata* in Ecuador were similar not only to what happened in other countries, but also with other invasive alien species. Often, in the absence of preparation and knowledge, the responses adopted were piecemeal, too late and ineffective. At an international level useful information has not always been widely shared or not made available in appropriate formats. To remedy this situation, international organisations should publish basic recommendations to combat invasive alien species (e.g. IUCN, 2000) that are generally useful against *P. canaliculata* in Ecuador. The invasion of *P.canaliculata* is not simply of concern to individual nations but a serious international problem. The integration of Ecuador in a framework of international cooperation to combat this (and other) pests is essential.

As an indispensable foundation before any invasive species can be controlled or managed, it is necessary to undertake exhaustive monitoring to address the species' correct identification, origin of the introduction and exact locations of the infestation. Some of these steps have happened in Ecuador regarding *P. canaliculata*, but others are still in progress.

It is important to create a legal and institutional framework to respond effectively to the threat of the apple snails. Ideally it should be integrated in a global system of action to prevent, minimize and mitigate adverse effects (social, economic, biodiversity, ecosystem) of any potential invasive alien species. In 2013, the European Commission drafted the first comprehensive legislation to protect biodiversity from invasive species (Comisión Europea, 2013).

Awareness, information and training are key factors in dealing with the pest. It is necessary to ensure that all citizens, key sectors in the country and the government itself, have a real appreciation of the magnitude of the problem and the costs involved. In Asia and elsewhere, in spite of knowing the devastating effects of *P. canaliculata*, it continued to be deliberately introduced to new locations by people thinking about its potential yet dubious benefits (e.g. food, biological control of aquatic weeds, etc.) (Lach *et al*., 2000). The ability to respond appropriately to deal with an infestation increases in relation to public awareness, information dissemination, education and training. Certainly, the support of local communities affected by the pest is essential, as is employment of all the informational mechanisms available. Involvement of society and knowledge of the relationships of the population with these snails (cultural, gastronomic or any other kind) are critical to decision making. National coordination is necessary to avoid piecemeal responses.

Increasing the effort in research is essential to generate knowledge of the snails' distribution, biology, invasive characteristics, impacts and relations with the native molluscan biodiversity, as well as to assess methods of detection, eradication and control, shared with other countries (e.g. IUCN, 2000; Cowie, 2002; Correoso *et al*., 2011, 2015; Lasso *et al*., 2016; Correoso, unpublished).

Due to the close relationship between *P. canaliculata* and native species of *Pomacea* in Ecuador and their morphological similarity, nonspecific eradication measures could generate serious ecological problems that affect the poorly known molluscan diversity of continentalEcuador. This therefore provides an excellent justification for planning and implementing faunistic and taxonomic studies of this fauna, which would fill one of the most significant gaps in zoological knowledge of the country, with multiple benefits.

Another challenge is to determine if there is more than one species affecting the Ecuadorian rice paddies and if so, characterize them morphologically, anatomically, genetically and biogeographically, and design different management strategies for each of them, as necessary. The erroneous identification of the pest species of *Pomacea* could cause the adoption of ineffective management measures (Martin *et al*., 2012). Rawlings *et al*. (2007) and Martin *et al*. (2012) demonstrated by genetic analysis, that most of the invasive apple snails in Florida and Alabama were *P. maculata* and not *P. canaliculata*, as had been previously thought. Hayes *et al*. (2008) also demonstrated by genetic analysis that the main invasive species in Asia, generally considered formerly to be *P. canaliculata*, was in fact two species, *P. canaliculata* and *P. maculata*.

Undoubtedly, much basic knowledge of the biology of *P. canaliculata* and of *Pomacea* in general (e.g. population dynamics, habitat preferences, behaviour, fecundity, feeding habits and preferences) remains to be investigated, although some aspects, at least for *P. canaliculata*, are already quite well understood (Hayes *et al*. 2015). Specialists and modern technology, especially for molecular approaches, may be key (Dong *et al*., 2011; Matsukura *et al*., 2013). Studies should be undertaken in Ecuador, to evaluate possible variation specific to the country, something that has hardly begun, with the exception of the basic studies of Troya Vera (2013). If *ex situ* breeding of apple snails is necessary for such studies, it should be legislatively controlled. The ecological-environmental impacts of *Pomacea* species have been reported by different authors in various parts of the world (e.g. Carlsson *et al*., 2004; Kwong *et al*., 2009; Horgan *et al*., 2014b; Wilcox & Fletcher, 2016). In addition to being aware of such impacts, it would be important to monitor and limit them in strategically chosen places.

Measures and strategies for managing the pest should continue to be investigated, including physical, chemical and/or biological techniques, implementation of which would alleviate the adverse impacts where *P. canaliculata* is already present. However, early eradication following initial detection in a new locality is the best approach. Therefore, it is essential to develop and implement campaigns and programmes of surveillance, prevention, control and eradication that increase the effectiveness of measures currently in place. But new approaches must also be investigated, for instance extending lines of investigation intobioproducts present in plants that are toxic to snails and their use as pesticides, as has been done in various parts of the world and with which some success against *P. canaliculata* has been achieved in Ecuador (Ochoa Chumaña & García Onofre, 2012). Techniques developed should aim to cause the least possible impact on the environment and in particular to the indigenous *Pomacea* species. The methods of control should be socially, culturally and ethically acceptable, efficient, non-polluting and not adversely affecting the native flora and fauna, human health and welfare of domestic animals and crops (IUCN, 2000). While acknowledging that it is difficult to meet all these criteria, they should be adopted as appropriate goals within the need to find a balance between the costs and benefits of control and the desired results. Ecological and biodiversity studies should include assessment of the potential impact of the pest on native species of molluscs.

It is important to establish some well-defined, published monitoring strategies. Sometimes biological control agents may be the preferred option when compared to physical and chemical methods of control, but require rigorous analysis before implementation and subsequent monitoring, especially regarding their non-target impacts. Certain biocontrol efforts, for example with fish, have been successful in other countries (Ochoa Chumaña & García Onofre, 2012) and their investigation in Ecuador has begun (Molina Zamora, 2012). Physical removal can be an effective alternative, especially if implemented based on published data on the biology and behaviour of the snails (Heiler *et al*., 2008). Chemicals used should be as specific as possible, and non-persistent and non-accumulating in the food chain. Persistent organic pollutants, including organochlorines, should not be used.

Based on data that are obtained and published by researchers who are studying the pest, we must establish priority areas for action that depend on the level of exposure to risk and the quality or natural value of the areas. A formal control strategy that focuses on these areas should then be designed to incorporate monitoring, management methodology and timelines. There should be better exchange of information between national and international scientific and management agencies, not only regarding the identities of invasive species but especially dealing with methods of control.

Crop management measures are important to contain and reduce pest damage and basically involve techniques that hinder the snail's attacks and expansion of its distribution. While some methods are known to fail, such as drying down of crop fields because the snails bury into the mud and survive (Ochoa Chumaña & García Onofre, 2012), other methods,based on global knowledge of the pest should continue to be evaluated (Joshi & Sebastian, 2006; Ochoa Chumaña & García Onofre, 2012).

In addition to reactive measures, prevention, surveillance and monitoring of at risk ecosystems to prevent introductions or detect new invasions are important, as suggested by Martin *et al*. (2012) for *P. maculata* in the USA. A system of communication and alerts could be extended through all rice growing parts of the country. Early detection is crucial in order that predetermined and strong responses can be implemented to prevent entry or eradicate the pest before it can become established. Participation of informed citizens would facilitate detection (IUCN, 2000).

In terms of prevention, efforts should focus on the usual means of pest entry, considering that many alien species are introduced unintentionally. The spread of the snail by human practices such as the buying and selling of rice seedlings for transplantation, rental of machinery for tillage, or sale of other aquatic plants (e.g. azolla) harbouring small and easily overlooked hatchlings and small juveniles are to be avoided (Ochoa Chumaña & García Onofre, 2012). Before moving machinery from an affected or possibly affected area to a new location, it should be systematically cleaned, and even certified. Regarding aquaculture, which seems to have been the cause of the arrival of *P. canaliculata* in Ecuador, and because improper aquaculture practices have resulted in problems in Asian countries, any aquaculture activity should take into account consideration of responsible action as recommended by Hewitt *et al*. (2006).

Comprehensive epidemiological studies incorporating *L. fulica*, *P. canaliculata*, other *Pomacea* species and other native molluscs in relation to possible transmission of *A. cantonensis* in Ecuador are also recommended.

Conclusions

The pest status of *Pomacea canaliculata* in Ecuador is complex not only because of the agricultural impacts identified in this review but also because of its epidemiological role as a host of the parasite *Angiostrongylus cantonensis*, a major cause of eosinophilic meningitis, and its direct ecological impact.

In addition, non-selective and intensive application of pesticides and other hasty measures and policies adopted, on top of other human-caused environmental problems in general, will result in indirect impacts, the first and most obvious casualty probably being the nativespecies of *Pomacea*. This impact on native molluscan biodiversity (poorly studied in Ecuador) has alarming consequences in a country considered one of the most biodiverse in the world. If such ecological impacts continue they could become a serious threat to Ecuador's biota in general, its ecosystems and its people, much more broadly than just the currently perceived threats to rice plantations. Invasive *Pomacea* alter ecosystems at different levels and compromise their ability to provide ecosystem services.

Given the sensitive national situation concerning invasive *Pomacea* and the previous experience of other countries, we suggest quickly establishing a comprehensive research programme investigating all relevant aspects of the pest and its management (short, medium and long term). These should include, but not be limited to, comprehensive epidemiological studies regarding infection of *Pomacea canaliculata*, *Lissachatina fulica* and native *Pomacea* species by *Angiostrongylus cantonensis* and its natural transmission in Ecuador, and ecological research involving aquatic and terrestrial biodiversity potentially impacted by the pest, and including native species of molluscs and other invertebrates and vertebrates.

A broad educational awareness campaign regarding mollusc pests should be initiated at study centres in the affected areas. National academic activities on the conservation of native aquatic molluscs and related aquatic biodiversity should be undertaken. Ecuador should continue to generate published resources and infrastructure to combat the pest in conjunction with other affected countries, developing consistently and internationally integrated actions that avoid repeating steps already taken elsewhere (IUCN, 2000).

In conclusion we suggest implementation of integrated agricultural, epidemiological and malacological research that will help achieve a comprehensive understanding of the situation and the particular pest, disseminating scientific and informational materials about the issue nationally and organising symposia and workshops in order to permit concerted implementation of activities to control, mitigate or live with the pest in Ecuador, sustainably with the environment.

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References

Alicata, J.E. 1991. The discovery of *Angiostrongylus cantonensis* as a cause of human eosinophilic meningitis. *Parasitology Today* 7: 151-153.

Ampuero, A.A. 2013. Diferenciación Morfológica de las Especies de "Churo" (Mollusca, Caenogastropoda: *Pomacea* spp.) Comercializados en Iquitos. Tesis para optar al título de biólogo. Universidad Nacional Mayor de San Marcos.

Anderson, B. 1993. The Philippine snail disaster. *The Ecologist* 23: 70-72.

Ardila V, 2008. Lista de los géneros de moluscos terrestres de Colombia (Mollusca: Gastropoda: Prosobranchia: Mesogastropoda y Pulmonata: Stylommatophora). *Biota Colombiana* 9(1): 39-62.

Baker, G.H. 1998. The golden apple snail, *Pomacea canaliculata* (Lamarck) (Mollusca: Ampullariidae), a potential invader of fresh water habitats in Australia. In: *Proceedings of the sixth Australasian applied entomological research conference, Volume 2* (Zalucki, M.P., Drew, R.A.I. & White, G.G., ed.), p. 21-26. University of Queensland, Brisbane.

Barnes, M.A., Fordham, R.K. & Burks, R.L. 2008. Fecundity of the exotic applesnail, *Pomacea insularum*. *Journal of the North American Benthological Society* 27: 738-745.

Borrero, F.J., Breure, A.S.H., Christensen, C.C., Correoso, M. & Mogollón Ávila, V.

2009. Into the Andes: three new introductions of *Lissachatina fulica* (Gastropoda, Achatinidae) and its potential distribution in South America. *Tentacle* 17: 6-8.

Carlsson, N.O.L, Brönmark, C. & Hansson, L.-A. 2004. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85(6): 1575-1580.

Carlsson, N.O.L, Sarnelle, O. & Strayer, D.L. 2009. Native predators and exotic prey - an acquired taste? *Frontiers in Ecology and the Environment* 7(10): 525-532.

Comisión Europea. 2013. *Propuesta de Reglamento del Parlamento Europeo y del Consejo Sobre la Prevención y la Gestión de la Introducción y Propagación de Especies Exóticas Invasoras*. Comisión Europea, Bruselas [Brussels]. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=SWD:2013:0322:FIN:ES:PDF

Conner, S.L., Pomory, C.M. & Darby, P.C. 2008. Density effects of native and exotic snails on growth in juvenile apple snails *Pomacea paludosa* (Gastropoda: Ampullariidae): a laboratory experiment. *Journal of Molluscan Studies* 74: 355-362.Correoso, R.M. 2002. Caracterización y distribución preliminar de los moluscos terrestres y fluviales del Ecuador Continental. *Ciencia* 5(2): 147-154.

Correoso, R.M. 2006. Estrategia preliminar para evaluar y erradicar *Achatina fulica* Gastropoda: Achatineaceae) en Ecuador. *Boletín Técnico IASA, Serie Zoológica* 2: 45-53.

Correoso, R.M. 2008. *Los Moluscos Terrestres y Fluviales del Ecuador Continental*. Editorial SIMBIOE, Quito.

Correoso, R.M . 2016. *Pomacea quinindensis*. *The IUCN Red List of Threatened Species 2016*: e.T189606A65493893. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T189606A65493893.en. Accessed 9 March 2017.

Correoso, M. & Coello, M. 2009. Modelación y distribución de *Lissachatina fulica* (Gastropoda, Achatinidae) en Ecuador. Potenciales impactos ambientales y sanitarios. *Geospacial* 6: 79-90.

Correoso R.M, Martini, R., Narváez, G.M., Muzzio, J. & Orlando, N. 2011. Distribución y hospederos de *Angiostrongylus cantonensis* en Ecuador. Presentado en Concurso Científico, Anual Externo INHMT "Leopoldo Izquieta Pérez". Unpublished.

Correoso, R.M., Coello, G.M.R., Arrebola Burgos, J. & Martini, L.R. 2015. *Pomacea canaliculata plaga del arroz en Ecuador*. Primera edición electrónica. Universidad de las Fuerzas Armadas – ESPE, Quito.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. & van den Belt, M.

1997. The value of the world's ecosystem services and natural capital. *Nature* 387: 253-260.

Cousin, A. 1887. Faune malacologique de la République de l'Équateur. *Bulletin de la Société Zooologique de France* 12: 187-287, pl. 4.

Cowie, R.H. 1997. *Pila* Röding, 1798 and *Pomacea* Perry, 1810 (Mollusca, Gastropoda): proposed placement on the Official List, and Ampullariidae Gray, 1824: proposed confirmation as the nomenclaturally valid synonym of Pilidae Preston, 1915. *Bulletin of Zoological Nomenclature* 54: 83-88.

Cowie, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts, and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CABI Publishing, Wallingford.

Cowie, R.H. 2005. *Global Invasive Species Database Species profile: Pomacea canaliculata.* http://www.iucngisd.org/gisd/species.php?sc=135. Accessed 12 September 2013. Cowie, R.H. 2013. Biology, systematics, life cycle, and distribution of *Angiostrongylus cantonensis*, the cause of rat lungworm disease. *Hawai'i Journal of Medicine and Public Health* 72 Supplement 2: 6-9.

Cowie, R.H. & Héros, V. 2012. Annotated catalogue of the types of Ampullariidae (Mollusca, Gastropoda) in the Muséum national d'Histoire naturelle, Paris, with lectotype designations. *Zoosystema* 34: 793-824.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastropoda: Ampullariidae: *Asolene*, *Felipponea*, *Marisa*, *Pomacea*, *Pomella*): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-24. Philippine Rice Research Institute, Nueva Ecija.

Dong, S., Shentu, X., Pan, Y., Bai, X., Yu, X. & Wang, H. 2011. Evaluation of genetic diversity in the golden apple snail, *Pomacea canaliculata* (Lamarck), from different geographical populations in China by inter simple sequence repeat (ISSR). *African Journal of Biotechnology* 10(10): 1777-1783.

EFSA [European Food Safety Authority, Panel on Plant Health]. 2012. Statement on the identity of apple snails. *EFSA Journal* 10(4): 2645 [11 p.].

Germain L. 1910. Étude sur les mollusques terrestres et fluviatiles recueillis par M. le Dr Rivet. *Mission du Service Géographique de l'Armée pour la Mesure d'un Arc de*

Méridien Équatorial en Amérique du Sud sous le Contrôle Scientifique de L'académie des Sciences 1899-1906 9(3): 1-[78], pls. 1-4.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H., Jørgensen, A., Schultheiß, R., Albrecht, C. & Thiengo, S.C. 2009a. Molluscan models in evolutionary biology: apple snails (Gastropoda: Ampullariidae) as a system for addressing fundamental questions. *American Malacological Bulletin* 27: 47-58.

Hayes, K.A., Cowie, R.H. & Thiengo, S.C. 2009b. A global phylogeny of apple snails: Godwanan origin, generic relationships, and the influence of outgroup choice (Caenogastropoda: Ampullariidae). *Biological Journal of the Linnaean Society, 98*: 61-76. Hayes K.A., Cowie R.H., Thiengo S.C. & Strong E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Heiler, K.C.M., von Oheimb, P.V., Ekschmitt, K. & Albrecht, C. 2008. Studies on the temperature dependence of activity and on the diurnal activity rhythm of the invasive *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Mollusca* 26(1): 73-81.

Hewitt, C.L., Campbell, M.L. & Gollasch, S. 2006. *Alien Species in Aquaculture. Considerations for Responsible Use*. IUCN, Gland and Cambridge.

Hidalgo, J.G. 1866 Description d'espèces nouvelles de la République de l'Équateur. *Journal de Conchyliologie* 14: 343-344.

Hollingsworth, R.G. & Cowie, R.H. 2006. Apple snails as disease vectors. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 121-132. Philippine Rice Research Institute, Nueva Ecija.

Horgan, F.G., Felix, M.I, Portalanza, D.E., Sánchez, L., Moya Rios, W.M., Farah, S.E., Wither, J.A., Andrade, C.I. & Espin, E.B. 2014a. Responses by farmers to the apple snail invasion of Ecuador's rice fields and attitudes toward predatory snail kites. *Crop Protection* 62: 135-143.

Horgan, F.G., Stuart, A.M. & Kudavidanage, E.P. 2014b. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica* 54: 90-100.

Hylton Scott, M.I. 1958. Estudio morfologico y taxonomico de los ampullaridos de la Republica Argentina. *Revista Argentino de Ciencias Naturales "Bernardino Rivadavia" e Instituto Nacional de Investigacion de las Ciencias Naturales. Ciencias Zoológicas* 3: [2] p. + 233-333, pls. 1-23.

IUCN, 2000. *Guías para la Prevención de Pérdidas de Diversidad Biológica Ocasionadas por Especies Exóticas Invasoras*. http://data.iucn.org/dbtw-wpd/edocs/Rep-2000-052-Es.pdf

IUCN 2016. *IUCN Red List of Threatened Species*. Version 2016-3. www.iucnredlist.org. Accessed 9 March 2017.

Jácome, I. 2001a. Aspectos básicos del manejo del caracol amazónico (*Pomacea haustrum*), en la Estación piscícola Acuatilsa, Mera, Pastaza. In: Memorias del Curso de Manejo de Especies Acuáticas en la Amazonia 15 y 16 agosto 2001 (no editor). Unpublished.

Jácome, I. 2001b. Informe preliminar sobre el Manejo de caracol amazónico *Pomacea haustrom*, como alimento humano (no editor). Unpublished.

Jackson, D. & Jackson, D. 2009. Registro de *Pomacea canaliculata* (Lamarck, 1822) (Ampullariidae), molusco exotico para el norte de Chile. *Gayana* 73(1): 40-44.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija.

Jousseaume, F. 1887. Mollusques nouveaux de la république de L'Equateur. *Bulletin de la Société Zoologique de France*. 12: 165-186, pl. 3.

Jousseaume, [F.], 1894, Description d'un mollusque nouveau. *Le Naturaliste* (2) 8(173): 120-121.

Kim, J.R., Hayes, K.A., Yeung, N.W. & Cowie, R.H. 2014. Diverse gastropod hosts of *Angiostrongylus cantonensis*, the rat lungworm, globally and with a focus on the Hawaiian Islands. *PLoS ONE* 9(5): e94969.

Köhler, F. & Glaubrecht, M. 2006. The types of Ampullariidae Gray, 1824 (Mollusca, Gastropoda) in the malacological collection of the Natural History Museum, Berlin: an annotated catalogue with lectotype designations. *Mitteilungen aus dem Museum* *für Naturkunde in Berlin, Zoologische Reihe* 82: 198-215.

Kwong, K.-L., Chan, R.K.Y. & Qiu, J.-W. 2009. The potential of invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia* 51(2): 343-356.

Lach, L., Britton, D.K., Rundell, R.J. & Cowie, R.H. 2000. Food preference and reproductive plasticity in an invasive freshwater snail. *Biological Invasions* 2: 279-288.

Lasso, C.A, Correoso., [R.]M., Lopes-Lima, M., Ramírez, R. & Tognelli, M. 2016. Estado de conservación y distribución de los moluscos de agua dulce de los Andes tropicales. In: *Estado de Conservación y Distribución de la Biodiversidad de Agua Dulce en los Andes Tropicales* (Tognelli, M.F., Lasso, C.A., Bota-Sierra, C.A., Jiménez-Segura, L.F. & Cox, N.A., ed.), p. 57-66. IUCN, Gland, Cambridge, Arlington.

Letelier, S. & Soto-Acuña, S. 2008. Registro de *Pomacea* sp. (Gastropoda: Ampullaridae) en Chile. *Amici Molluscarum* 16: 6-13.

Levin, P. 2006. *Statewide Strategic Control Plan for Apple Snail (Pomacea canaliculata) in Hawai'i*. The Hawai'i Land Restoration Institute, Wailuku.

Linares, E.L. & Vera. M.L. 2012. *Catálogo de los Moluscos Continentales de Colombia*. Biblioteca José Jerónimo Triana No 23. Universidad Nacional de Colombia, Facultad de Ciencias, Instituto de Ciencias Naturales, Bogotá.

López, M.A., Altaba, C.R., Andree, K.B. & López, V. 2010. First invasion of the apple snail *Pomacea insularum* in Europe. *Tentacle* 18: 26-28.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species Database*. Invasive Species Specialist Group, Auckland.

Lv, S., Zhang, Y., Steinmann, P. & Zhou, X.N. 2008. Emerging angiostrongyliasis in mainland China. *Emerging Infectious Diseases* 14: 161-164.

Lv, S., Zhang, Y., Steinmann, P., Yang, G.-J., Yang, K., Zhou, X.-N. & J. Utzinger. 2011. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biol ogy* 56: 717-734.

Martin, C.H., Bayha, K.M. & J.F. Valentine. 2012. Establishment of the invasive island apple snail *Pomacea insularum* (Gastropoda: Ampullariidae) and eradication efforts in Mobile, Alabama, USA. *Gulf of Mexico Science* 2012(1-2): 30-38.

Martini, R.L., Gómez, E.L., Muzzio, J.K. & Solorzano, L. 2008. Descripción del primer foco de transmisión natural del *Angiostrongylus cantonensis* en Ecuador. *Revista Ecuatoriana de Higiene y Medicina Tropical* 45(2): 26-36.

Matsukura, K., Okuda, M., Kubota, K. & Wada, T. 2008. Genetic divergence of the ge nus *Pomacea* (Gastropoda: Ampullariidae) distributed in Japan, and a simple molecular method to distinguish *P. canaliculata* and *P. insularum*. *Applied Entomology and Zoology* 43(4): 535-540.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

MMAMA [Ministerio de Agricultura, Alimentación y Medio Ambiente]. 2015. *National Contingency Plan for* Pomacea *spp*. MMAMA, Spain. http://www.mapama.gob.es/es/agricultura/temas/sanidad-vegetal/plannacionalcontingencia_ingles_julio2015_tcm7-390043.pdf

MAE, 2011. *Lista Preliminar de Especies Exóticas Introducidas e Invasoras en el Ecuador Continental*. Ministerio del Medio Ambiente, Quito.

Miller, K. 1879. Die Binnenmollusken von Ecuador (Schluss.). *Malakozoologische Blätter* (Neue Folge) 1: 117-203, pls. 4-15.

Molina Zamora, V.S. 2012. Evaluación del Uso del Sábalo (*Brycon*, sp.) Para el Bio Control de la Población de Caracol Manzana (*Pomacea* sp.). Tesis. Universidad Estatal Amazónica. Escuela de Ingeniería Agropecuaria. Santa Clara.

Muzzio Aroca, J.K. 2011. Moluscos Hospederos Intermediarios de *Angiostrongylus cantonensis* en dos Provincias de Ecuador. Masters thesis. Instituto de Medicina Tropical "Pedro Kouri", Havana, Cuba. http://repositorio.educacionsuperior.gob.ec/bitstream/28000/777/1/T-SENESCYT-0362.pdf

Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Ochoa Chumaña, L.A. & García Onofre, J.A. 2012. Determinación de la actividad molusquicida de dos extractos vegetales sobre caracol manzana (*Pomacea canaliculata*) y su impacto en la diversidad de artrópodos. Tesis de grado ingenieros Agrícolas y biológicos. Escuela Superior Politécnica del Litoral, Guayaquil. Ecuador. http://www.dspace.espol.edu.ec/handle/123456789/21123.

Padilla, P., Alcántara, F., Tello, S., Ismiño, R. & Schulze, J.C. 2004. Cultivo y aprove chamiento de moluscos acuáticos en la comunidad indígena Cocama-Cocamilla

-lecciones aprendidas. In: *Memorias: Manejo de Fauna Silvestre en Amazonia y Latinoamérica*, p. 592-597. Instituto de Investigaciones de la Amazonía Peruana, IIAP-PEA, Iquitos.

Pain T. 1946. Two new species of Pila (=*Ampullaria*) from South America. *Proceedings of the Malacological Society of London* 26: 180-181, pl.6.

Pain, T. 1960. *Pomacea* (Ampullariidae) of the Amazon River system. *Journal of Conchology* 24: 421-432.

Pain, T. 1964. The *Pomacea flagellata* complex in Central America. *Journal of Conchology* 25: 224-231, pl. 13.

Pincay, T., García, L., Narváez, E., Decker, O., Martini, L. & Moreira, J.M. 2009. Angiostrongyliasis due to *Parastrongylus* (*Angiostrongylus*) *cantonensis* in Ecuador. First report in South America. *Tropical Medicine and International Health* 14 supplement 2: 37.

Ramírez, R., Paredes, C. & Arenas, J. 2003. Moluscos del Perú. *Revista de Biología Tropical* 51(3): 225-284.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. BMC *Evolutionary Biology* 7: 97.

Rosario, J. & Moquete, C. 2006. The aquatic snail *Ampullaria canaliculata* L. - plague of irrigated lowland rice in the Dominican Republic. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 514-515. Philippine Rice Research Institute, Nueva Ecija.

Rumi, A., Gutiérrez, D.E., Núñez, V. & Darrigran, G.A. 2008. Malacología Latinoamericana. Moluscos de agua dulce de Argentina. *Revista de Biología Tropical* 56(1): 77-111.

Simone, L.R.L. 2006. *Land and Freshwater Molluscs of Brazil*. EGB, Fapesp, São Paulo.

Troya Vera F.D. 2013. Determinación del ciclo biológico del caracol *Pomacea canaliculata* utilizando hojas de arroz como base alimenticia, bajo condiciones de invernadero. Tesis de Grado. Universidad Técnica de Babahoyo. Babahoyo.

Vázquez Perera, A.A. & Perera Valderrama, S. 2010. Endemic freshwater molluscs of Cuba and their conservation status. *Tropical Conservation Science* 3: 190-199.

Vázquez-Silva, G., Castro-Barrera, T., Castro-Mejía, J. & Mendoza-Martínez, D. 2011. Los caracoles del género *Pomacea* (Perry, 1810) y su importancia ecológica y socioeconómica. *ContactoS* 81: 28-33.

Wada, T. 2004. Strategies for controlling the apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) in Japanese direct-sown paddy fields. *Japan Agricultural Research Quarterly* 38 (2): 75-80.

Wikipedia contributors. 2017. List of Non-marine Molluscs of Venezuela. *Wikipedia, The Free Encyclopedia* (18 January 2017). https://en.wikipedia.org/wiki/List_of_non-marine_molluscs_of_Venezuela. Accessed 9 May 2017.

Wilcox, R.C. & Fletcher, R.J., Jr. 2016. Experimental test of preferences for an invasive prey by an endangered predator: implications for conservation. *PLoS ONE* 11(11): e0165427.

Identity, reproductive potential, distribution, ecology and management of invasive *Pomacea maculata* in the southern United States

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Abstract

Established populations of introduced *Pomacea maculata*, a highly fecund, large species of apple snail native to South America, now occur throughout southeast Asia, in Spain and extensively across the southern United States. Substantial research on non-native apple snails takes place in Southeast Asia and has frequently identified apple snails as *P. canaliculata*. That these Asian populations represent at least two *Pomacea* species, *P. canaliculata* and *P. maculata*, has been confirmed through anatomical and genetic evidence. However, the two species are often still confused because of their similar shell morphologies and life history traits. This contribution reviews the distribution, life history, ecology and management of *P. maculata* introduced to the southern USA. So far the agricultural impacts of *P. maculata* in the USA fail to match those of non-native applesnails elsewhere, but the invasion of wetlands by this species suggests the need for increased vigilance to prevent further spread and avoid the ecological impacts that have been associated elsewhere with *P. canaliculata*.

Additional keywords: Ampullariidae, apple snail, Gastropoda, introduced, spread

Introduction

Apple snails in the genus *Pomacea* (family Ampullariidae) span the aquatic-terrestrial interface (Fig. 1; Hayes *et al*., 2009a, b), reach a considerable size (Youens & Burks, 2008), and tend to act as true herbivores rather than only as algal grazers (Hayes *et al*., 2015). Due to the damage caused to rice (Joshi & Sebastian, 2006), *Pomacea canaliculata* has been listed among 100 of the world's worst invasive species (Lowe *et al*., 2004) and is the only freshwater snail on the list. However, some of the studies that prompted the inclusion of *P. canaliculata* among the world's worst invasives probably involved *P. maculata* or a mixture of the two species, as the similar morphologies and reproductive behaviours of *P. canaliculata* and *P. maculata* have resulted in substantial taxonomic confusion (Cowie *et al*., 2006; Hayes *et al*., 2012). This confusion has indirectly slowed the pace of rigorous research and awareness of the threats posed by *P. maculata* (senior synonym of *P. insularum*; Hayes *et al*., 2012) as a separate species from *P. canaliculata* (Burks *et al*., 2016).

Howells *et al*. (2006) were the first to review the state of knowledge of non-native apple snails in the USA at a time when there was substantial concern about the potential impact of non-native *Pomacea* species on rice crops. Fortunately, this anticipated impact has not yet happened and *P. maculata* populations are largely limited to ponds and lakes with aquatic vegetation on which they lay their clutches (Fig. 1). In Texas, the timing and methods of rice farming probably minimised the impacts of apple snails compared to the impacts in other invaded areas such as Southeast Asia (Burlakova *et al*., 2010). To our knowledge, no other studies have examined agricultural impacts of *P. maculata* in the continental USA. However, we caution against extrapolating the absence of a quantifiable economic impact on rice in Texas to susceptible agricultural areas that have longer periods of standing water, such as rice fields used for waterfowl hunting or crayfish farming. In this review, we focus on the identity, reproductive potential, distribution, ecology and management of *P. maculata* in the southern USA and reference studies reviewed in more detail elsewhere (Burks *et al*., 2016). There are populations of *P. canaliculata* in California, Arizona, Florida (Rawlings *et al*., 2007) and Hawaii (Tran *et al*., 2008), but none of these populations has expanded to the extent that *P. maculata* has across the southern USA (Byers *et al*., 2013).

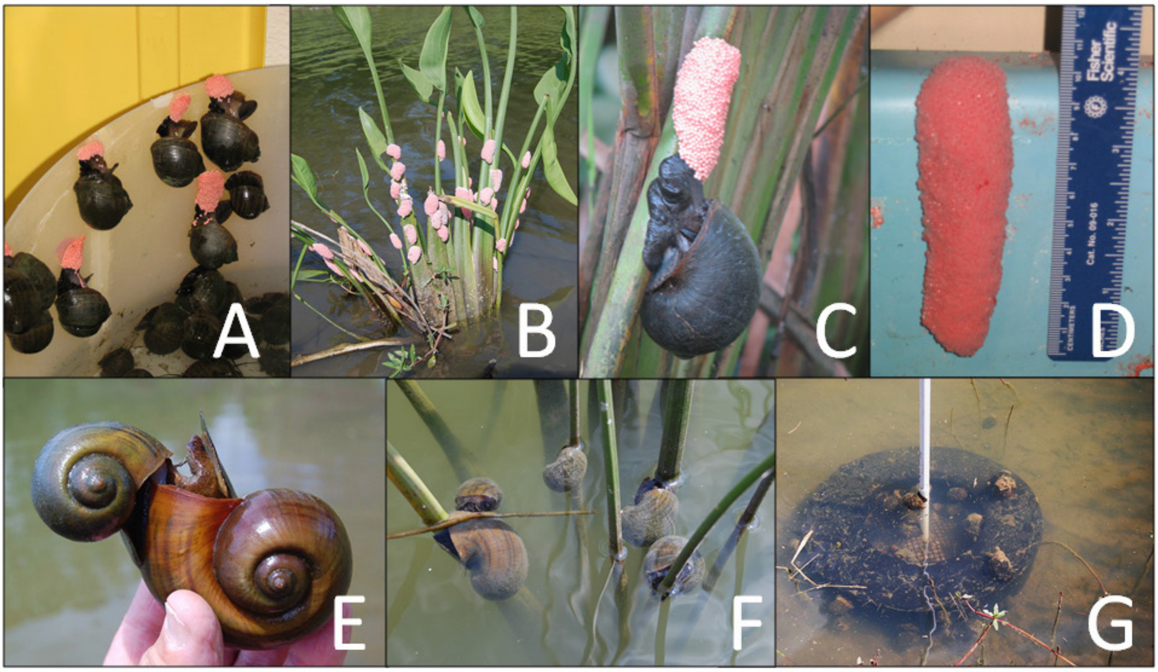

Fig. 1. *Pomacea maculata* and its environment. A: moderately sized (30-50 g) *P. maculata* females depositing clutches following collection. B: abundant egg clutches deposited on emergent vegetation in Florida. C: female laying a large clutch in Armand Bayou, Texas (it took about 45 minutes). D: nearly 10-cm long clutch deposited by *P. maculata* in the laboratory (Southwestern University, Georgetown, Texas). E: mating pair collected from Florida that illustrates the size difference between the sexes (smaller snail is the male). F: adult *P. maculata* stripping vegetation in Wellman Pond, Florida. G: snail trap (design by J. van Dyke) deployed in shallow water of Wellman Pond, Florida.

Identity

Management responses to invasion by *P. canaliculata* and *P. maculata* may be similar, but their potential for spread, ecological impacts, susceptibility to predation and rate of population growth may differ. For example, it remains unclear why *P. maculata* has expanded across the southern USA while *P. canaliculata* has not, despite both species occurring in Florida (Rawlings *et al*., 2007; Bernatis *et al*., 2016). We recommend that the research community and the public use the scientific names *P. canaliculata* and *P. maculata* for the two most widespread invasive apple snails. Consistent, clear communication will help stop the conflation of these two species that is often found in the literature, websites and social media. Readers should recognise that accounts of apple snail impacts, especially in the popular press, may mix information on multiple congeneric species by relying on common names rather than the scientific names (Hayes *et al*., 2009a; 2012).

Taxonomy

Hayes *et al*. (2012) recognised the species name *Pomacea maculata* as the senior synonym of a number of other *Pomacea* species, including *P. insularum*, the name formerly used for it in North America (e.g. Rawlings *et al*., 2007) as well as in Asia (Hayes *et al*., 2008). Rawlings *et al*. (2007) and Hayes *et al*. (2012) included several taxonomic and nomenclatural changes relevant to the non-native ampullariid fauna of the USA since the review of Howells *et al.* (2006) was published. These taxonomic developments resulted from years of investigation, a comprehensive and ongoing review of the genus and family (Cowie & Thiengo, 2003; Hayes *et al*., 2008, 2009a, b, 2015; Cowie & Héros, 2012; Cowie, 2015; Cowie *et al*., 2015, 2017) and a targeted comparison of morphological, reproductive and genetic traits of *P. canaliculata* and *P. maculata* (Hayes *et al*., 2012).

By designating the same specimen as the neotype of *P. maculata* and the lectotype of *P. insularum*, as well as the neotype of *P. gigas* (a name that has also been used in the invasive apple snail literature in Asia), Hayes *et al*. (2012) rendered them objective synonyms. The names *P. insularum* and *P. gigas* are no longer valid names for the species and should not be used. Although the common name ‘island apple snail’ still appears in some recent publications (e.g. Meza-Lopez & Siemann, 2015; Smith *et al*., 2015), it also lacks meaning as it reflects the no longer valid name ‘*insularum*’, which means ‘of the islands’.The correct name, *P. maculata*, should instead be used in all studies from this point forward (Burks *et al*., 2016). Furthermore, because of past difficulty of distinguishing *P. maculata* from *P. canaliculata* and of distinguishing both of these species from other *Pomacea* species, it is important that any study deposit voucher specimens in a publicly accessible museum collection so that future researchers can confirm the identity of the material.

Perhaps predictably, the question of an accurate common name often arises in work associated with apple snails. The name '*maculata*' (in English 'maculate') means spotted or stained, but unfortunately that translation does not apply to a consistent morphological feature of *P. maculata*. Therefore, use of the terms spotted or stained as common names does not inform identification of the species and risks the likelihood that someone will substitute other terms in the future, unleashing more ambiguity and amplifying the taxonomic confusion. For example, use of "Amazonian apple snail" does not make scientific sense because many species of apple snails occur within the Amazon basin (Hayes *et al*., 2009a). Due to the extensive diversity in the genus *Pomacea* (Hayes *et al*., 2009a), *P. maculata* may be confused with other large apple snails with channelled sutures. Some agencies employ the common names 'giant apple snail' or 'channeled [USA spelling] apple snail' but these common names have just created more problems, especially as 'channeled' refers to the channelled sutures from which *P. canaliculata* gets its scientific name.

In addition, the abbreviation of giant apple snail to 'GAS' creates the potential for confusion with the name 'golden apple snail', also referred to as 'GAS', and which has been widely used for the invasive species in Asia. The term 'golden apple snail' was thought originally to be only *P. canaliculata,* but we now know it has also been used for *P. maculata* (Hayes *et al*., 2012). The acronym GAS may also cause confusion, as it is also used for the giant African snail, *Lissachatina fulica* (previously known as *Achatina fulica*), itself a problematic species (e.g. Fontanilla *et al*., 2014; Iwanowicz *et al*., 2015). Therefore, in this contribution, we do not use a common name for *P. maculata* nor do we recommend one.

Taxonomic confusion among apple snails also extends into the aquarium trade in the USA. *Pomacea maculata* differs substantially from *Pomacea diffusa*, the scientific name of the so-called 'spike-topped' apple snail found in the aquarium trade (Rawlings *et al*., 2007). Howells *et al*. (2006) treated it as *P. bridgesii*, of which *P. diffusa* was then considered a subspecies (Cowie & Thiengo, 2003), but Rawlings *et al*. (2007) recognised *P. diffusa* as a valid species, with 'spike-topped' apple snail as its common name. *Pomacea diffusa* is still often misidentified as *P. bridgesii* (often mis-spelled '*bridgesi*') in the aquarium trade, or it is frequently incorrectly labelled as a generic 'mystery' snail (Rawlings *et al*., 2007; Hayes *et al*., 2008). We provide this update as some proportion of *P. maculata* introductions probably occur from aquarium releases by consumers mistakenly sold the wrong species (Karatayev *et al*., 2009).

Genetics and hybridisation

Less genetic research has focused specifically on *P. maculata* than on *P. canaliculata*. Using the COI gene for barcoding, Rawlings *et al.* (2007) confirmed *P. maculata* as the identity of several populations of non-native apple snails in Florida, Georgia and Texas, and identified four mitochondrial haplotypes. Martin *et al.* (2012) later documented *P. maculata* with a haplotype shared by snails in Florida and Georgia. To our knowledge, all the non-native ampullariid populations across the southern USA that have been genetically characterised consist of *P. maculata* (Teem *et al*., 2013; Deaton *et al*., 2016), with a few notable exceptions in Florida of *Marisa cornuarietis*, plus a species identified incorrectly as *P. haustrum*, and a few populations of *P. canaliculata*, which also occurs in Arizona and California (Rawlings *et al*., 2007). Only a limited number of haplotypes occur within the populations of *P. maculata* (Burks & C. Savrick, unpublished). Recently, Matsukura *et al.* (2016a) published a set of microsatellites for both *P. maculata* and *P. canaliculata* that will be useful in future genetic studies of introduced populations.

While Hayes *et al*. (2012) showed that *P. canaliculata* and *P. maculata* are distinct species, they also speculated about the possibility of hybridisation or incomplete lineage sorting. Research in both the non-native (Asia) and native (South America) ranges implies that hybridisation does occur (Matsukura *et al*., 2013, 2016b; Yoshida *et al*., 2014), but these results cannot yet distinguish the mechanisms underlying genetic exchange or answer whether hybridisation happened before or after invasion in non-native ranges. Matsukura *et al*. (2016b) suggested that hybridisation has the potential to alter environmental tolerances and facilitate invasion in non-native ranges. In the USA, documented overlapping populations of *P. canaliculata* and *P. maculata* only occur in Florida (Rawlings *et al*., 2007), but no studies of hybridisation in these populations have been published.

Life history and reproductive potential

Clutch and egg size

Clutches of bright pink *P. maculata* eggs are laid on emergent substrates above the water line, generally during dawn and dusk, and deposition of a large clutch can take 30 minutes or more (Kyle *et al*., 2011). The number of eggs in a clutch averages around

1500-2100 but varies considerably up to a recorded maximum of 4751 (Barnes *et al*., 2008; Burks *et al*., 2010; Kyle *et al*., 2011; Hayes *et al*., 2012). These numbers contrast with those for *P. canaliculata* clutches, which contain on average 200-300 eggs (Hayes *et al*., 2012). The diameter of eggs of *P. maculata* is smaller (~2 mm) than that of *P. canaliculata* (~3 mm) (Barnes *et al*., 2008; Hayes *et al*., 2012). For the untrained eye, it may still be difficult to assess the differences in egg size in the field. Consequently, genetic confirmation should still accompany any identification of a newly established population. The relative threat potential of *P. maculata* may be assessed, in part, by comparing its overall fecundity to that of other species. Based on the average number of eggs in a clutch, *P. maculata* may be even more likely to become a nuisance species than the already destructive *P. canaliculata* (Barnes *et al*., 2008).

Hatching rates and clutch submersion

Pomacea maculata lays its eggs in clutches deposited on hard surfaces above the waterline. They typically take 10-14 days to hatch completely and can yield hundreds of hatchlings (Barnes *et al*., 2008). Three mechanisms may explain when low hatching efficiency or total clutch failure occurs, i.e. non-fertilisation, female age or water damage, all of which warrant additional investigation. In one Florida culture, older *P. maculata* (> 3 years) appeared to have less successful hatch rates than younger snails (Bernatis, unpublished). As estimates vary widely, more studies need to examine the trade-offs between time to reproduction, female size or age, and hatching efficiency, as has been done for *P. canaliculata* (Tamburi & Martín, 2009, 2011). To test the effects of freshwater inundation, Martin & Valentine (2014) used subsections of clutches (less than 1 week old), but did not find any statistical differences among treatments. However, their reported hatching rates were considerably lower (0-17 %) compared to studies with *P. canaliculata* (Pizani *et al*., 2005). Horn *et al*. (2008) sectionedlarge clutches into three parts and found more hatchlings emerged from clutch segments above water than from submerged segments. To date, all studies that have examined hatching efficiency of *P. maculata* have focused on fresh water, but submergence in salt water should also be considered.

Eggs need to remain dry and out of the water for a minimal amount of time (i.e. 6-9 days) to maximize hatching success. In a laboratory study (Burks & M. K. Trawick, unpublished), clutches of different ages attached to vertical plant stems were submerged for 8 or 24 h per day, while control clutches were completely above the water. Hatching efficiency in control clutches was 83 %, whereas submergence generally reduced hatching efficiency compared to controls (Fig. 2). Clutch age at first submergence offset the impact of submergence as 9 day old clutches still exhibited 38-87 % overall hatching efficiency, whereas clutches that were 3 and 6 days old at the start of the experiment essentially failed to hatch when submerged completely for 24 h per day and exhibited reduced hatching success when submerged for 8 h per day (Fig. 2). Horn *et al*. (2008) suggested that management efforts might use the negative effects of immersion on clutches by flooding areas with high abundances of clutches. For clutches immersed for a brief period of time, egg development may still occur. More mature clutches that become only partially submerged, or submerged for shorter periods of time, may still yield substantial numbers of hatchlings (Burks & M. K. Trawick, unpublished). No research to date has examined how flooding would affect dispersal of eggs and subsequent long-term survivorship of hatchlings, the costs of which must be considered when balanced against the benefits of reducing hatching success with flooding.

Maturation time, longevity and overall fecundity

Life history traits of *P. maculata*, including life span, age-specific growth rates, age and size at maturity, size-dependent fecundity and total longevity, remain largely undocumented. In outdoor mesocosms (Bernatis, 2014), *P. maculata* first copulated when 3-4 months old (shell length 30-33 mm), a similar age to that implied by Yoshida *et al*. (2014) and Matsukura *et al*. (2016b). Although not yet confirmed by life history studies, stable isotope analysis of a limited number of *P. maculata* shells indicated a lifespan of 1-3 years (Arnold *et al*., 2014), which seems noteworthy given the size this species can reach as adults. It is worth considering the potential outcomes of a three-year lifespan. If females start to produce average sized clutches (~1500 eggs; Barnes *et al*., 2008) during their first year, live for one or two more years, and produce clutches once a week for three summer months (probably an underestimate of their reproductive season in the southern USA) during two or three summers, then one female could contribute 36,000 (two summers) to 54,000 (three summers) eggs in her lifetime. Even if just 0.01 % of those eggs survived to reproductive age (1 in 10,000), a single female would have replaced herself with three to five offspring.

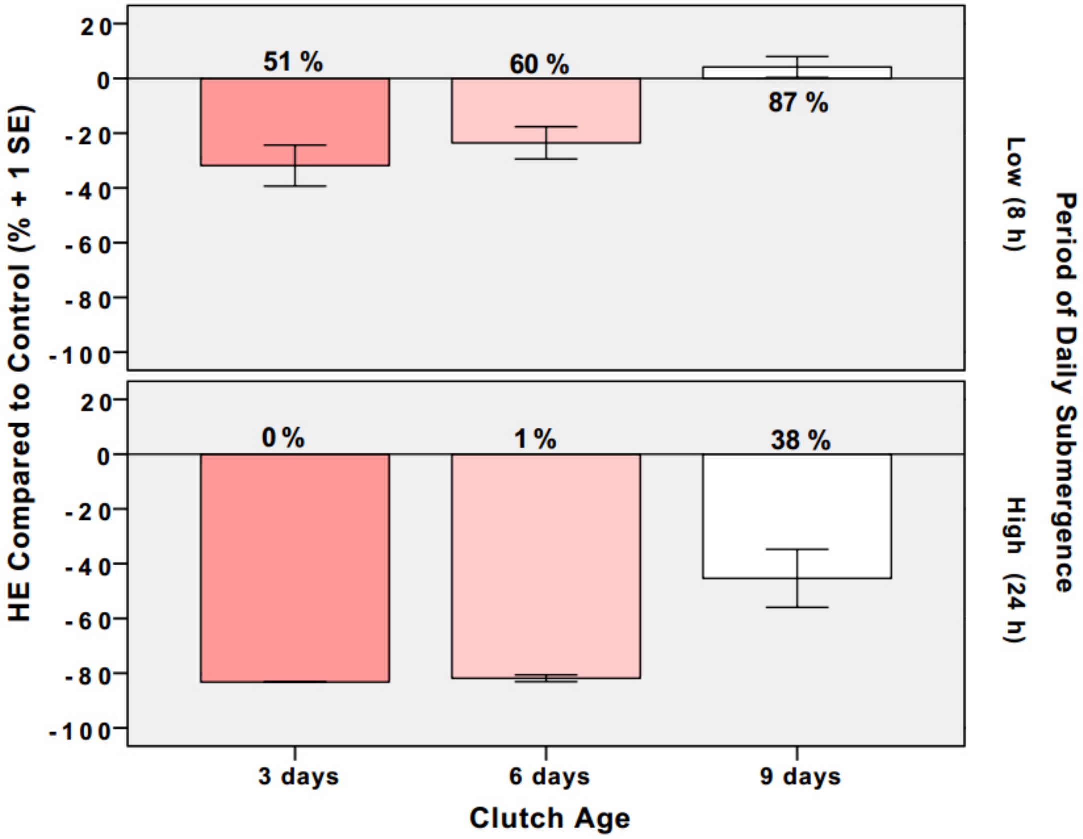

Fig. 2. Hatching efficiency (HE) of clutches of different ages (3, 6 and 9 days old) in response to different periods of full submergence each day (8 h or 24 h), compared to control clutches that were never submerged. Control clutches achieved 83 % HE and submergence reduced HE of most treatments as represented by bars below the 0 % comparison line. Percentages associated with each bar indicate HEs. At the one extreme, immature clutches (3 and 6 days old) failed to hatch when fully submerged for 24 h per day. At the other extreme, 9 day old clutches submerged for 8 h per day hatched at a rate not significantly different from control clutches.

Distribution and abundance

The native range of *Pomacea maculata* includes much of Brasil and extends to Uruguay and parts of Argentina (Hayes *et al*., 2012). Genetic evidence suggests that *P. maculata* in the southern USA came from either Argentina or Brasil (Rawlings *et al*., 2007). Given the propensity for the aquarium trade to include *Pomacea* species (Karatayev *et al*., 2009), we speculate that the current distribution of *P. maculata* in the southern USA resulted from repeated introductions and subsequent expansion of the populations. *Pomacea maculata* has spread rapidly from its initial introduced populations in Texas (1989) and Florida (1991) and now occurs throughout the coastal plain of seven southern states (Texas, Louisiana, Mississippi, Alabama, Georgia, Florida and South Carolina; Rawlings *et al*., 2007; Byers *et al*., 2013). Fortunately, not every introduction of apple snails persists. In 2009, a few *P. maculata* egg masses in a park in Louisiana were discovered early enough in the invasion process to destroy them and no further egg masses have been seen at that location (Carter, unpublished). Early hand removal efforts (Bernatis & Warren, 2014) may help prevent establishment.

While some introductions fail to establish, others do take hold. In southern Louisiana several isolated introductions have led to range expansions into bayous that lead to intersections with the Gulf Intracoastal Waterway, a canal that connects wetlands from Texas to Florida. Isolated colonies have also been reported in separate sections of the Atchafalaya Basin, the largest bottomland hardwood swamp in the USA (Carter & G. LaFluer, unpublished). This westerly range expansion across Louisiana has led to concern that the snails may begin to affect rice and crayfish cultivation in southwestern regions of the state. The separate, isolated colonies have led to speculation that some of them originated from local residents releasing snails purposely to control overgrowth of plants in their own private ponds or to attract wetland birds, or as accidental bait release (Carter, unpublished).

Few publications provide density or abundance data for *P. maculata*. In southeastern Texas, in permanent habitats such as ponds and lakes, Burlakova *et al*. (2010) documented low densities (< 2 snails/m^2) and stable populations, with higher densities in the macrophyte-dominated zone than in open water. Juveniles were scarce despite an abundance of egg clutches. In ephemeral ponds in Florida, Smith *et al*. (2015) found a negative correlation between *P. maculata* abundance and frequency of preferred plant species, but no relationship with any other variable. In flooded ephemeral agricultural habitats, Burlakova *et al*. (2010) found extremely high densities (> 130 snails/m^2) and speculated that differential survivorship of hatchlings and young snails drove the differences in population dynamics in these habitats compared to the permanent habitats noted above. Kyle *et al.* (2009) also reported dominance of large-sized snails in another population, but with limited evidence of recruitment over a three-year period. Even 1 % successful recruitment might produce devastating effects given the reproductive potential of *P. maculata* (Kyle *et al*., 2011).

Influence of abiotic conditions on distribution

The characteristics of the habitats into which organisms invade play a key role in determining colonisation success (Lockwood *et al*., 2013). Abiotic conditions of a system may cause the immediate death of an organism, allow it to thrive, reproduce and become a problem, or any level of success between these extremes. For aquatic snails, the key abiotic variables are likely to be availability of calcium carbonate, levels of pH, dissolved oxygen, salinity and temperature, and the frequency at which habitats dry out.

Calcium carbonate

As calcium is important for shell formation, regeneration and growth (White *et al*., 2007), calcium carbonate availability is one factor that generally limits the distributions of freshwater molluscs (Lodge *et al*., 1987). A substantial proportion of aquatic systems in the southern USA have calcium carbonate levels well above the limiting threshold of 29.8 mg/L reported for *P. canaliculata* by Martín *et al*. (2001); in fact, in some it is > 61 mg/L (Scott *et al*., 2002; Perlman, 2016). Therefore, in the southern USA, calcium carbonate levels probably play a minor role in limiting establishment of *P. maculata*, except in combination with pH.

pH

Many water bodies on the coastal plain of the southern USA have low pH because of the large amounts of decaying organic matter and slow moving water. Low pH makes it extremely difficult for snails to construct shells made of calcium carbonate (Batzer *et al*., 2005). As a result, water bodies like the Okefenokee Swamp in southern Georgiahave no shelled mollusc species (Kratzer & Batzer, 2007). Water bodies with a pH of 4 or lower may be a barrier to apple snails (Ramakrishnan, 2007) and pH of 5.5 or lower is lethal to *P. maculata* hatchlings (Bernatis *et al*., 2016). The Okefenokee Swamp has a low enough pH to preclude *P. maculata* from invading and establishing a permanent population. Although available pH data capture general regional patterns (Byers *et al*., 2013), there can be small areas with more neutral pH among these systems, particularly in flowing water, that may be able to support apple snails in otherwise low pH areas. This may be the explanation for two populations that appear to exist in low pH areas (i.e. pH 5.5) in Georgia (Byers *et al*., 2013). Overall, the majority of the inner coastal plain of

North Carolina appears inhospitable because of large areas with low pH within areas with favourable climate (Byers *et al.,* 2013).

Although apple snails may be able to tolerate relatively low pH environments, reduced growth rates may negatively impact reproduction and changes in shell strength may increase susceptibility to predation and accidental damage. For both *P. maculata* and *P. canaliculata* (Bernatis, 2014), erosion and brittleness of shells was higher in experimental treatments at pH 5.5 than at higher pH values. Given its influence on survival and reproduction, the full extent of the direct and indirect effects of pH on *P. maculata* warrants additional investigation.

Oxygen

Dissolved oxygen levels represent another obvious and potentially limiting factor for aquatic snails. However, given the amphibious nature of *Pomacea* species (Hayes *et al*. 2009b, 2015), their possession of both a lung and a gill lessens the likelihood that dissolved oxygen will influence their distributions (Seuffert & Martín, 2009a). Several studies have looked at the influence of oxygen during different life stages of *Pomacea* spp., and in particular, during aestivation or aerial exposure. In general, *Pomacea* spp. obtain oxygen either aerially or from the water and may rely on aerial respiration to compensate for less than adequate dissolved oxygen in the water (Seuffert & Martín, 2009a, b).

Salinity

Exposure to periodic salinity may play an important role in determining the distribution of freshwater organisms that are exposed to estuarine conditions (Jordan & Deaton, 1999). In many coastal environments, normal periodic changes in salinity occur but occasional extreme conditions may also occur after an environmental disturbance (e.g. flooding). Changes in salinity can cause osmotic stress, which may result in a variety of responses, both behavioural and physiological (McMahon, 1983). Given their ability to take refuge in a shell, several species of non-native, invasive freshwater molluscs show variable degrees of tolerance to salinity (Verbrugge *et al.,* 2012).

Martin & Valentine (2014) measured salinity tolerance and growth of 10 day old *P. maculata* hatchlings and found greater than 40 % survival rates up to 15 ppt. Both Ramakrishnan (2007) and Bernatis *et al.* (2016) investigated salinity tolerances of juvenile and adult *P. maculata* and reached similar conclusions. For example, *P. maculata* tolerated chronic salinity exposure of 6.8-8.0 ppt for 28 days (Bernatis *et al*., 2016) and multiple *P. maculata* populations occur within 10 km of the mouth of the St. John's River (Florida) where salinity reaches 4 ppt. Consequently, weakly tidally influenced systems, i.e. with salinity of < 8 ppt, may serve as acceptable habitat for *P. maculata* to thrive (Martin & Valentine, 2014).

Temperature

Temperature regimes limit distributions, alter metabolic rates and influence behaviour of many organisms, including apple snails (Byers *et al*., 2013). Both low and high temperatures critically influence life history traits of apple snails and hence their distributions. Deaton *et al*. (2016) found that *P. maculata* collected in Louisiana, and other snails from a laboratory population, experienced no mortality and remained active at 15 °C after 10 days. After 10 days, temperatures of 10 °C and 5 °C reduced activity and resulted in 50 and 60 % mortality, respectively. For snails at 0 °C, 100 % mortality occurred after only 5 days.

Buenos Aires, with a mean minimum temperature of the coldest month of 7.6 °C, is possibly the coldest area in the native range of *P. maculata*. Bernatis (2014) reported that adult *P. maculata* survived in water temperatures as low as 2 °C in outdoor mesocosms during winter in Florida. Despite the presence of a thin layer of ice on the water surface, some snails occasionally moved around and fed. In addition, occasional freezing temperatures have not eliminated apple snails from the Ebro River delta in Spain, which is the most northerly non-native location where *P. maculata* occurs (Andree & López, 2013). Invasive success in other non-native ranges indicates the need for more research to determine the extent to which *P. maculata* can spread further north in the USA.

In contrast to cold tolerance, in aquatic systems that periodically dry up, animals unable to retreat to pools of water have evolved several coping techniques, including aestivation during hot months (Darby *et al*., 2008; Hayes *et al*., 2015). Regarding upper thermal limits, *P. maculata* tolerated experimental temperatures of 15.2-36.6 °C (Ramakrishnan, 2007), and growth occurred without mortality at both 15 °C and 35 °C (Gettys *et al*., 2008). To examine the combined influence of temperature and relative humidity (RH), Ramakrishnan (2007) tested how long juvenile and adult *P. maculata* could survive without immersion in water by using small chambers with a platform situated above different salt solutions. Adult *P. maculata* survived for 70 days at 30 °C and > 95 % RH and at least 308 days at 20-25 °C in 75-95 % RH, with smaller snails suffering proportionately greater water loss (Ramakrishnan, 2007). Bernatis *et al.* (2016) reported similar results for both *P. maculata* and *P. canaliculata*. As RH increased above 80 %, adults of both species survived 365 days on moist sand, while surviving only 22 weeks in lower RH (< 60 %) and on a dry sand substrate.

Modelling future distributions

Newly introduced species take time to expand throughout invaded ranges and reach an equilibrium population density. Consequently, estimating even their current distributions can often be difficult. Even when small, new populations can serve as a source of propagules for range expansion (Ackleh *et al*., 2007; Lockwood *et al*., 2013). Niche modelling is a tool to estimate the areas that are environmentally suitable for a given non-native species. Non-native species might come to occupy all suitable areas assuming sufficient time and no barriers to dispersal (Guisan *et al*., 2013). Accordingly, one can predict areas where the species might occur given enough time to spread and reach spatial equilibrium. Byers *et al.* (2013) modelled the projected distribution of *P. maculata* in the southeastern USA. Their MaxEnt model identified minimum temperature in the coldest month and precipitation in the warmest quarter as the most important climatic determinants of the distribution of *P. maculata* and indicated that it could spread throughout the coastal plain of the southeastern USA, both on the Atlantic and Gulf coasts.

Ecological interactions

When *P. maculata* invades wetlands, it may modify the structure and function of food webs indirectly by competing with native species (Connor *et al*., 2008; Posch *et al*., 2013) or directly by consuming plants (Morrison & Hay, 2011a; Smith *et al*., 2015) and providing novel prey for native predators (Cattau *et al*., 2016) (Fig. 3). Such invasions might also lead to shifts in feeding patterns among other organisms or provide a new host for parasites, including the rat lungworm, *Angiostrongylus cantonensis* (see section below on *P. maculata* as a parasite vector). Through consumption of macrophytes, certain invasive *Pomacea* species can alter whole ecosystems by causing shifts between alternative stable states (Carlsson *et al*., 2004a). Such shifts, from clear to turbid water, have yet to be reported in areas of the USA invaded by *P. maculata*. However,

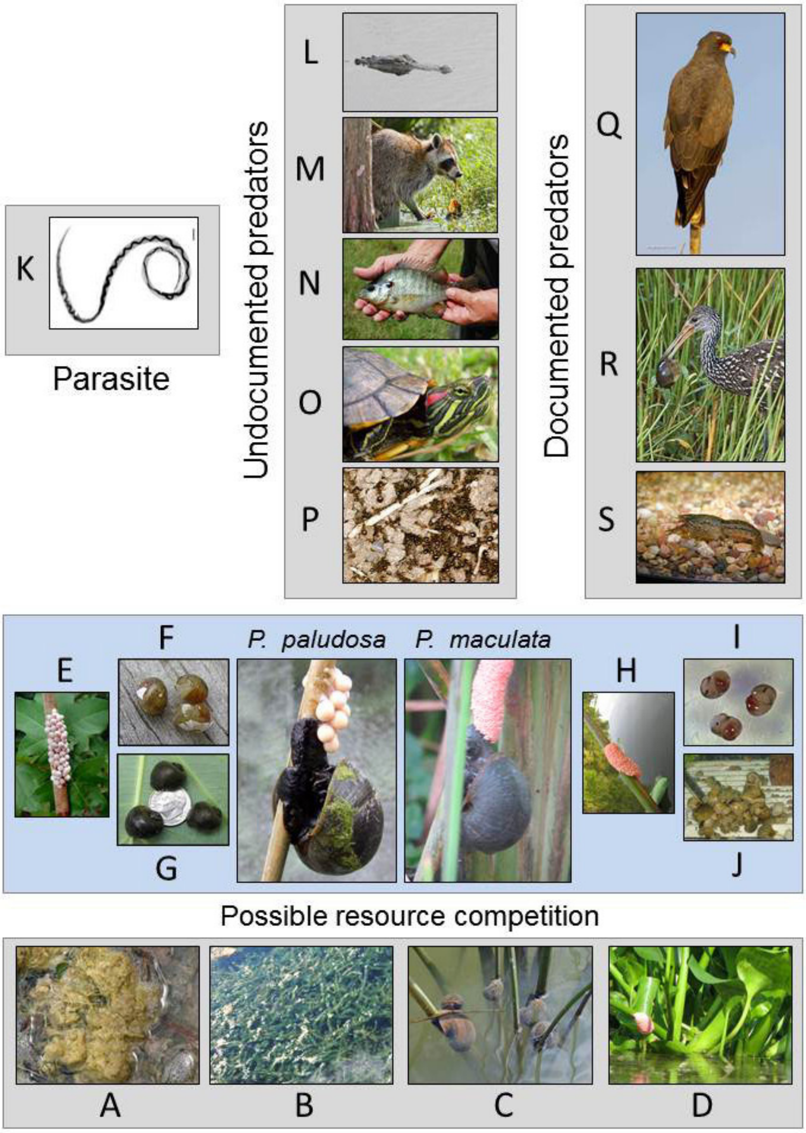

Fig. 3. Simplified three-tiered figure emphasising the intermediate positions of *Pomacea maculata* and *Pomacea paludosa* in aquatic communities in the southern USA. The ranges of these two species overlap in Florida and Georgia. The bottom tier includes primary producers consumed by *Pomacea* species to different extents: A, periphyton; B, submerged macrophytes, C, emergent macrophytes; D, freely-floating macrophytes. Competition for these resources could occur between overlapping *Pomacea* species (Conner *et al*., 2008; Posch *et al*., 2013). The middle tier includes life history stages: for *P. paludosa*: E, eggs; F, hatchlings; G, juveniles. *Pomacea maculata* deposit much smaller eggs (I) that yield smaller hatchings (I) but which rapidly grow to the juvenile stage (J). Total annual fecundity of *P. maculata* may exceed that of *P. paludosa* by an order of magnitude. The pink colour of *P. maculata* eggs (H) provides a warning to potential consumers that the eggs possess a neurotoxin. Apple snails in the southern USA may provide a reservoir for a medically important parasite, *Angiostronglyus cantonensis* (K) that has been found in Florida. As well as a representative parasite, the top tier also depicts undocumented and documented predators. Referring only to *P. maculata*, the centre pictures show possible predators not yet documented in the literature, including: L, alligators; M, raccoons; N, redear sunfish; O, turtles; and P, fire ants; with the last three as potential egg predators. The right side of the top tier emphasizes avian apex predators: Q, limpkins; and R, snail kites; that forage on similar-sized snails regardless of species (Cattau *et al*., 2016). The last photo shows a freshwater crayfish (S), which may consume apple snail hatchlings of different species at different rates (Dorn & Hafsadi, 2016).

a comparison of feeding rates among various species of *Pomacea* indicates that feeding of *P. maculata* equals or surpasses that of other invasive *Pomacea* species (including *P. canaliculata*), which implies the potential for similar negative effects (Morrison & Hay, 2011a).

Pomacea maculata *as a competitor and consumer*

In laboratory experiments, *P. maculata* suffers from intraspecific competition at high densities (Connor *et al*., 2008; Posch *et al*., 2013), but the implications of such competition for population dynamics in natural habitats remain largely unknown. Also, when kept in culture, especially with limited access to additional calcium, adult snails will eat the shells of conspecifics (Burks, personal observation), but no study to date has quantified shell condition of snails collected from populations across a density or pH gradient. More attention has been directed at investigating interspecific than intraspecific competition and examining feeding preferences.

Populations of *P. maculata* started to establish in Florida in the late 1990s and now substantially overlap with populations of the native Florida apple snail, *P. paludosa* (Rawlings *et al*., 2007; Dorn & Hafsadi, 2016; Cattau *et al*., 2016). Many reports indicate that periphyton is a main part of the diet of *P. paludosa* (*Pomacea* Project, 2016), but only a few studies have measured it (Shuford *et al.,* 2005). Instead of depending primarily on periphyton, *P. paludosa* may thrive on a combination of periphyton and vascular plants as demonstrated by an experiment in which adults grew significantly more when consuming a complex of *Utricularia* sp. and periphyton than loosely attached benthic algae (Sharfstein & Steinman, 2001). *Pomacea maculata*, perceived as a voracious herbivore, feeds on different types of aquatic macrophytes (Burlakova *et al*., 2009; Burks *et al*., 2011; Morrison & Hay, 2011a; Fig. 3) but also on periphyton. In aquaria, *P. maculata*, particularly small individuals, grazed periphyton growing on the shells of conspecifics (Howells *et al*., 2006), and periphyton added to reconstituted *Myriophyllum spicatum* increased consumption (Boland *et al*., 2008). Consumption by both apple snail species might include periphyton, but also vascular tissue depending on the host plant's structural complexity. Consequently, if food is limiting, competition may occur between native and non-native apple snails (Fig. 3) when *P. paludosa* feeds on vascular plants (Morrison & Hay, 2011a).

Depending on the plant species, *P. paludosa* may feed at a slower rate than *P. maculata*. For example, the feeding rate of *P. maculata* on *Vallisneria americana* exceeded that of *P. paludosa* nearly 10-fold (Monette *et al*., 2016). Monette *et al*. (2016) also found that *P. paludosa* ate around the edges of the leaves of *V. americana*, whereas *P. maculata* typically cut the strands into fragments, causing more damage. In experiments, when the two species are placed together, the presence of the larger and more fecund *P. maculata* results directly in negative impacts on growth and survival of *P. paludosa* (Fig. 3; Connor *et al*., 2008; Posch *et al*., 2013).

Numerous experimental laboratory studies have assessed feeding preferences of *P. maculata* among diverse macrophyte species (Burlakova *et al*., 2009; Baker *et al*., 2010; Morrison & Hay, 2011a, b; Burks *et al*., 2011; Bernatis, 2014). Most studies found clear preferences and host plant consumption patterns generally agreed for similar plant species, although there were some discrepancies among studies. Such discrepancies are probably explained by the different methodological approaches, different experimental durations and use of different suites of plant species. For example, among all the plant species tested, the greatest differences in consumption tendencies involved wild taro (*Colocasia esculenta*) and water hyacinth (*Eichhornia crassipes*). In none of the studies did *P. maculata* exhibit a strong preference for these two cosmopolitan macrophytes, but the extent to which they did consume them varied substantially. Nonetheless, it is clear that *P. maculata*, despite its preferences, will consume a wide variety of aquatic macrophytes, and given the differences in feeding patterns among the studies, it should never be used for biocontrol of aquatic weeds (see Control section).

Interested in differential herbivory on plant structures, Bernatis (2014) compared consumption patterns between *P. maculata* and *P. canaliculata* and specifically looked at preferences among different parts of the plants. Neither species restricted feeding to just stems, blades or leaves, but also consumed the roots and root structures (e.g. bulbs, rhizomes) of many species. In the case of *C. esculenta*, and to some extent, the water lily *Nuphar advena*, apple snails consumed the root structures even in the presence of other preferred foods. Snails readily fed on new growth, but once stems reached above the water line, there was minimal foraging. In contrast to these two plant species, apple snails seldom fed on new growth of *V. americana*, even when no other food was available. This result using *V. americana* conflicts with the herbivory rate found by Monette *et al.* (2016). Plants may alter their composition to limit palatability in the presence of herbivores (Qiu & Kwong, 2009), so it is possible that some preference patterns or differences between studies may be related to plant chemical defences, which may be influenced by local conditions. However, no studies have measured and isolated specific plant compounds that may deter apple snail grazing through inducible defences, a possible explanation for the discrepancies in feeding rates among previous studies (Burlakova *et al*., 2009; Baker *et al*., 2010).

Most studies on feeding of *P. maculata* have involved laboratory experiments under controlled conditions and a number of questions are open for investigation. For example, the possibility exists that *P. maculata* may not be strictly herbivorous. *Pomacea canaliculata* consumes amphibian eggs (Karraker & Dudgeon 2014) and preliminary food trials indicated that *P. maculata* ate raw meat when provided (Carter, unpublished). Following the example of studies with *P. canaliculata*, ecologists and managers must gather more ecologically relevant measures of the consumptive impact of *P. maculata* by undertaking more field measurements and experiments (Horgan *et al*., 2014). At the same time, such future studies must exercise extreme caution to prevent introduction of snails to novel habitats and must heed regulations that seek to limit their spread.

Pomacea maculata *as a prey resource*

Pomacea maculata occupies an intermediate position in the food web (Fig. 3) and may serve as a food source for a number of predators similar to those known for *P. canaliculata* (Yusa 2006; Yusa *et al*., 2006; Hayes *et al*., 2015). However, with the exception of studies on snail kites, there are fewer published studies of predation on *P. maculata* than *P. canaliculata*. Swamp crayfish (*Procambarus clarkii*) and red-eared slider turtles (*Trachemys elegans scripta*) both consumed egg clutches in laboratory settings (Burks & A.L. Plantz, unpublished), but the extent to which this occurs in the field remains unknown. Adult *P. maculata* also readily eat conspecific egg masses and smaller individuals (Horn *et al*., 2008). However, given the number of eggs produced, egg or juvenile cannibalism probably does not significantly hamper reproductive potential of *P. maculata*, although it would be interesting to develop a model of survivorship. The near absence of predation on *P. canaliculata* eggs (Yusa, 2001) seems also to be the case for *P. maculata* eggs in both their native and non-native ranges (Burks, unpublished). A carotenoprotein, similar to ovorubin in the eggs of *P. canaliculata* (Dreon *et al*., 2010, 2013) occurs in *P. maculata* eggs (Pasquevich *et al*., 2014) and these proteins play a role in the defence of embryos (Giglio *et al*., 2016), probably limiting the overall impacts of predation.

Only a few studies have investigated predators of recently hatched, juvenile or adult *P. maculata* (Horn *et al*., 2008; Dorn & Hafsadi, 2016; Cattau *et al*., 2016). Given the chemical defences of the eggs (Pasquevich *et al*., 2014, Giglio *et al*., 2016) and the large size of the adults, hatchlings and juveniles probably experience the highest predation rates. Possible predators of *P. maculata* (Fig. 3) include a similar suite of species as prey on the better studied *P. canaliculata*: crustaceans (shrimp, crayfish, crabs), insects (dragonfly larvae and beetles, among others), fish (including catfish, carp, tilapia, perch, among other benthic predatory fishes), reptiles (turtles), leeches, birds (ducks, crows, storks and various wading birds) and mammals (rats and other mammals feeding in littoral areas) (see Carlsson *et al*., 2004b; Yusa, 2006; Yusa *et al*., 2006).

Among invertebrate predators, the red swamp crayfish (*Procambarus clarkii*) consumed individuals of *P. maculata* under 4 mm in size in feeding trials (Carter, unpublished). Furthermore, in experimental wetland mesocosms (Dorn & Hafsadi, 2016), predation by native crayfish (*Procambarus fallax*) on hatchlings was eight times stronger on non-native *P. maculata* than on native *P. paludosa*. Crayfish differentially selected the smaller *P. maculata* hatchlings, to the extent that none of them survived the 44 days of exposure to predators. Regarding vertebrate predators, *P. maculata* has been found in the gut contents of blue catfish (*Ictalurus furcatus*) in Texas and alligators in Florida (Bernatis, unpublished). Glossy ibis (*Plegadis falcinellus*) have been seen feeding on *P. maculata* stranded in a recently drained pond (Howells *et al*., 2006). Limpkins (*Aramus guarauna*) specialise on snails (though not exclusively) and in the southeastern USA primarily occur in Florida (Kale & Maehr, 1990). Limpkins eat *P. maculata* in South America and limpkins have been photographed eating *P. maculata* in Florida, although there have been no published studies.

However, in Florida, the best-known apple snail predator (Fig. 3) is the Florida snail kite, *Rostrhamus sociabilis plumbeus*, the natural food of which was the native Florida apple snail, *P. paludosa*. These birds initially appeared less efficient at consuming the larger *P. maculata* than the smaller *P. paludosa* (Cattau *et al*., 2010) and studies recently documented a preference for medium-sized snails regardless of species (Wilcox & Fletcher, 2016; Fig. 3). Thus, feeding patterns appear to depend more on size of thesnails. Management efforts have sought to help increase the kites' efficiency of extracting larger *P. maculata* from their shells by providing perches that offer a closer, more stable platform than nests, which may be further away (Pias *et al*., 2012). Although not clearly predicted early on in the invasion, the abundance of *P. maculata* has benefited kite populations (Cattau *et al*., 2016; Wilcox & Fletcher, 2016). Water fluctuations may also affect which apple snail the kites consume. For example, in 2011, a drought and agricultural water withdrawal caused water levels to drop in certain areas in Lake Okeechobee. This water level drop in turn caused localised populations of *P. padulosa* to collapse. However, *P. maculata* did not appear to suffer the same high level of mortality. Consequently, in 2012, *P. maculata* represented nearly 100 % of the snail kite's diet in certain locations (Gray, 2013). Even if management actions could help to increase the number of Florida snail kites, their populations are unlikely to increase enough to control invasions of *P. maculata* adequately. Furthermore, if the birds did increase significantly, they could have a negative effect on the declining populations of *P. paludosa*.

Pomacea maculata *as a parasite vector and toxin transmitter*

Along with many other snail species, *P. maculata* acts as an intermediate host of the parasitic nematode, *Angiostrongylus cantonensis* (rat lungworm) (Kim *et al*., 2014). Humans are infected by *A. cantonensis* when they ingest raw snails containing the parasite larvae. In the southern USA, infected *P. maculata* have been reported in Louisiana (Qvarnstrom *et al*., 2013; Teem *et al*., 2013). However, Teem *et al*. (2013) did not find any infected *P. maculata* in the sites they sampled in Texas, Mississippi and Florida, although the parasite is present in southern Florida in giant African snails (Iwanowicz *et al*., 2015; Stockdale-Walden *et al*., 2015). However, very few locations were sampled and the true extent to which *P. maculata* is infected across the southern USA remains unknown.

Angiostrongylus cantonensis is not the only health threat posed by *P. maculata*. In its native range (i.e. Brasil), *P. maculata* can act as a host for a parasitic trematode (*Stomylotrema gratiousis*) (Pinto *et al*., 2015). No doubt *P. maculata* can act as a host for a large number of parasites, just as can *P. canaliculata* and other ampullariid species (Damborenea et al., 2017). As parasites may modify the anti-predator responses and behaviour of their intermediate hosts to facilitate infection of their final hosts (Poulin, 2011),the distribution of *P. maculata* parasites, infection intensity and possible resulting changes in behaviour merit further study.

In addition, in a laboratory feeding study, Dodd *et al*. (2016) found that *P. maculata* transferred an undescribed cyanotoxin associated with avian vacuolar myelinopathy to domestic chickens. Other *Pomacea* species are also capable of toxin bioaccumulation (Berry & Lind, 2010) and this capacity also warrants further investigation as a threat to human and ecosystem health.

Management

To some degree, there exists a prevailing sense that *P. maculata* may not be as harmful as *P. canaliculata,* despite its rapid range expansion in the southern USA. However, long-term impacts may just be emerging and the history of invasion in the genus warrants application of the precautionary principle. Options that could have realistically resulted in early eradication did not receive enough attention. Almost in a self-defeating manner, United States Federal Aquatic Nuisance Species Task Force admits "there is no way to eradicate all invasive apple snails once established" (Pasko *et al.*, 2011). The United States Department of Agriculture through its Plant Protection and Quarantine Program prohibits interstate transport of *P. maculata* without a permit but such regulations are difficult to enforce. The rapid proliferation of snails in Florida and Texas allowed populations to grow swiftly and ultimately spread throughout the southeastern USA. Annual surveys of Florida's 460 public water bodies conducted by the Florida Fish and Wildlife Conservation Commission noted an increase in the number of lakes infested (Table 1; R. Kipker, pers. comm.). Management of apple snails also faces budgetary issues, especially in Florida where resources are spent battling a number of invasive species, which costs millions of dollars per year (Dodds *et al*., 2014). The efficacy of control measures depends on frequency, intensity and accuracy of application.

Table 1. Number of Florida lakes and total area infested with invasive apple snails, primarily *Pomacea maculata*. Data from annual lake surveys by the Florida Fish and Wildlife Conservation Commission.

Year	Number	Hectares
2006	4	1934
2007	46	275,423
2008	59	299,704
2009	81	308,822
2010	98	317,982
2011	110	337,188
2012	126	342,449
2013	147	365,546
2014	164	366,871

Control strategies

Prohibiting or restricting apple snail species

Prevention measures represent the best front-line strategy for combatting any invasive species. This premise holds especially true for species that exhibit extremely high fecundity (Keller *et al*., 2007), such as *P. maculata* (Barnes *et al*., 2008; Kyle *et al*., 2013). Along with *P. canaliculata*, *P. maculata* is listed as a prohibited species in some states (Texas, Florida, Louisiana, South Carolina) but not all. Other states where snails have invaded (i.e. Alabama, Mississippi, Georgia) should consider similar measures. Often, even if *P. maculata* appears on a prohibited list, little enforcement of the prohibition takes place. However, some stakeholders take the problem more seriously. For example, to prevent the further spread of *Pomacea* species, the State of Louisiana made buying, selling or possession of apple snails illegal in August 2012. When the State of Louisiana becomes aware of commercial activity, receives commercial permit requests, or learns of individuals threatening to spread the snails, they have taken enforcement action immediately (Carter, unpublished). Unfortunately, the trade still continues informally and established populations get dispersed through events such as flooding. Overall, detection, prevention and management of apple snails all require constant diligence.

Manually removing snails and eggs

Hand removal involves a substantial time commitment and is tedious work (Bernatis & Warren, 2014). Dense shoreline vegetation greatly complicates the task, as does steep bathymetry and deep organic sediments. However, this simple action often constitutes the first response by managers who simply want to do something quickly. The appearance of pink egg clutches signals the start of invasion and examination of the clutches can help species identification (Hayes *et al*., 2012). In addition, understanding the mechanisms and preferences behind oviposition behaviour may help identify particular areas where snails congregate (Burks *et al*., 2010; Kyle *et al*., 2011). Unless snails invade a very small area, handpicking may be unaffordable without the help of volunteers (Martin *et al*., 2012).

Reducing egg-laying habitat

Pomacea maculata prefers sturdy emergent vegetation for egg deposition (Burks *et al*., 2010; Kyle *et al*., 2011), although snails will also seek out any other hard substrate when emergent plants are not available. From observations in South Carolina, apple snails may also climb shoreline plants to escape water treated with copper sulphate or chelated copper (M. Hook, pers. comm.), so that removal of emergent vegetation not only reduces their ability to escape from the copper treatment but also reduces oviposition sites. Consequently, managed removal of emergent plants, especially ones that are also non-native, invasive species, may reduce the reproductive success of *P. maculata*. Consequently, management efforts should target multiple life history stages using an integrated pest management approach such as hand removal of egg clutches, application of deterrent substances to artificial substrates and removal of nearshore vegetation to effectively cut down oviposition sites.

Baiting and trapping

Pomacea maculata appears highly susceptible to baiting and trapping, but as with manual removal, trapping also requires intensive labour and some costs for materials. By tending to traps weekly in Wellman Pond, Florida (see following case study), this effort temporarily reduced the snail population to the point that the emergent littoral vegetation (which was being heavily consumed) stabilised (Van Dyke, unpublished). In Langan Lake, Alabama (see case study), trapping proved an effective way to monitor trends in the snail population (D. Armstrong, pers. comm.).

Stocking predators

The redear sunfish (*Lepomis microlophus*; Fig. 3) is commonly called the “shellcracker” for good reason. They use thick, pharyngeal teeth to crack open shells of small molluscs. Larger *P. maculata* are too big to be vulnerable, but these fish could readily consume hatchling and juvenile snails. The largest management effort to date, which included stocking redear sunfish in an Alabama lake and downstream portions of a tidal creek, achieved some success in combatting snail invasion (Martin *et al*., 2012). Biological control with crayfish may also be possible, as red swamp crayfish *(P. clarkii*), native to states bordering the Gulf of Mexico, do eat *P. maculata* (Carter, unpublished) and native *P. fallas* readily consume *P. maculata* (Dorn & Hafsadi, 2016). However, given the propensity for crayfish to be invasive themselves, this species may not be the best choice in non-native habitats as their preference for *P. maculata* over other prey items remains unknown.

Chemical control

Copper and other metals can be toxic to snails and other aquatic invertebrates because metals can disrupt metabolic processes by damaging cell membranes, denaturing enzymes and binding to proteins and lipids (Dummee *et al*., 2015). In 2007, Florida lake managers attempted to control an isolated population of *P. maculata* in Newnan's Lake with granular copper sulphate. In spite of three treatments, the snails spread throughout the system, and there was unacceptable mortality of non-target organisms, e.g. odonates, fish and shrimp (Bernatis & Warren, 2014).

At identical rates, chelated copper is just as acutely toxic to sensitive organisms as inorganic copper. In either case, copper does not remain in the water column, but accumulates in the sediments. Precipitation takes longer for chelated forms than for inorganic forms, thus requiring less copper to kill target organisms. Therefore, compared to copper sulphate, the relatively expensive, chelated copper products are effective at lower rates, thus reducing potential for chronic non-target impacts (Leslie, 1992). In 2009, four chelated copper products were tested on *P. maculata* in the laboratory. A double-chelated product, "Captain" by SePRO (28.2 % copper ethanolamine complex), proved to be the most effective. Effects were directly related to dose and water temperature. At 23 °C, the snails produced copious mucus at 0.2 ppm and, at 0.4 ppm, they lay on their sides with opercula open and died within 96 h. At 27 °C, 0.2 ppm was lethal after 48 h (Van Dyke, unpublished).

In the future, use of copper treatments may wane as alternative chemicals become available for snail control that pose fewer threats of non-target mortality than copper. For example, "Bayluscide" by Bayer (17.1 % niclosamide) is used extensively to control the snail hosts of schistosomiasis in other countries (Takougang *et al*., 2006). In laboratory studies, label rates of niclosamide effectively killed *P. maculata*, but did not appear to harm red swamp crayfish (*P. clarkii*) or redear sunfish (*L. microlophus*) (Carter, unpublished). Niclosamide is approved for use in commercial fishponds in the USA but has not been used to control *P. maculata* because of a lack of registration for that specific use by the United States Environmental Protection Agency (USEPA). Perhaps, the most environmentally safe option for aquatic snail control is iron phosphate. “Ferroxx AQ” by Neudorff (3 % iron phosphate) is a pelletised formulation that includes a snail feeding attractant. This product was tested on snails with 0.5, 1.0, 2.0 and 4.0 g of pellets per 5.7 L of water, plus a “control” of flour-based pellets (eight snails per treatment). Feeding was suppressed in all treatments and ceased completely at rates of 2.0 g and 4.0 g. At those higher rates, 75 % of the adult snails were dead 6 days after treatment (Van Dyke, unpublished). The registration of “Ferroxx AQ” for aquatic use is currently under review by the USEPA.

Adjusting water level

As suggested by experimental data, a rapid change in water level can have lethal consequences for the egg clutches of *P. maculata*. A rapid rise floods the eggs and drowns some proportion of the embryos. However, such manipulations require careful consideration because some proportion of the clutch may still produce viable offspring, depending on its age (see above). Also, flooding may unintentionally result in increased snail dispersal. On the other hand, a rapid decline in water level may reduce water availability for hatchlings or expose adults to extreme desiccation and predation, as occurs in some South Florida water treatment areas (Bernatis, unpublished). In Wellman Pond (see below), for instance, the invasive, non-native red imported fire ant (*Solenopsis invicta*) quickly invaded the exposed littoral vegetation and attacked adult snails and eggs (Van Dyke, unpublished).

Case studies

While the initial reactions to most *P. maculata* invasions in the southern USA have been minimal, we report on three noteworthy case studies from Florida, Alabama and South Carolina that provide insight into the control of new populations of *P. maculata.*

Florida

Prior to plant restoration, hand collection of eggs and adult snails began in Wellman Pond (6.1 ha, Leon County, Tallahassee, Florida) in the summer of 2007. In spring 2008, efforts were begun to control the snail population using various baiting and trapping methods. The snails were easily attracted to combinations of certain grains and sugars

(Van Dyke, unpublished). They also tended to hide in black plastic containers made of high-density polyethylene (HDPE). Based on these observations, a large (7 L) trap, bait and method of use were designed and patented (Van Dyke *et al.*, 2013). After being attracted to the non-toxic bait, the snails entered the trap from above where a floating grate blocked their exit. In 2008 and 2009, 30 traps were deployed, and baited and emptied weekly (Van Dyke, unpublished). The traps performed well in shallow wadeable habitat with a hard substrate. As workers went from trap to trap, they also collected any eggs and adults observed in the pond. By the end of 2008, the management project removed 170,000 snails (3800 kg) and 2135 egg masses. In 2009, 35,000 snails (800 kg) and 1106 egg masses were removed, reflecting a smaller population.

Extensive landscaping occurred along the entire shoreline of Wellman Pond and involved planting 250,000 native plants (*Juncus effusus*, *Sagittaria* spp., *Pontederia cordata*). However, after one growing season, *P. maculata* had consumed 75 % of these plants. Besides deploying traps, native, snail-eating redear sunfish (*L. microlophus*) were stocked into the pond to control juvenile snails. Managers also painted a large interlocking metal sheet-pile wall, a retaining structure constructed to retain water within the pond, with chelated copper to discourage clutch deposition by snails left in the pond (Van Dyke, unpublished).

This program temporarily stabilised the emergent plant community, but littoral macrophyte abundance declined in subsequent years. All 27,500 plantings of pickerelweed (*Pontederia cordata*) disappeared, although soft-stemmed rush (*Juncus effusus*), smartweed (*Polygonum* spp.), and maidencane (*Panicum hemitomon*) appeared somewhat resistant to the voracious herbivory of *P. maculata*. Thus, partial removal of snails slowed impacts on plant community structure.

Alabama

The Wildlife and Freshwater Fisheries Division of the Alabama Department of Conservation and Natural Resources and the United States Fish and Wildlife Service initiated an on-going ambitious effort involving cooperation among numerous experts, volunteer organisations and public agencies, to eradicate *P. maculata* in autumn 2009 in the Three Mile Creek watershed, Mobile County (Martin *et al.*, 2012). Langan Lake (16 ha) flows into Three Mile Creek and while both these habitats have their own ecological and social importance, the urgency of this effort comes from their connection to the Mobile-Tensaw delta, the second largest river delta in the USA. In summer 2008, *P. maculata* had established a population only 12 km upstream from the delta. As one of the most biologically diverse deltas in the USA, more than 780 km^2 of marshes, swamps and bottomlands within this National Natural Landmark are at high risk from invasion by *P. maculata*.

The control project involved collecting eggs, trapping adults, treating with copper sulphate and reducing emergent vegetation. In Langan Lake, managers initially relied on only copper sulphate because of its relatively low cost but copper concentrations declined rapidly and reached undetectable limits only 24 h after the applications (B. Ricks & A. Ford, personal communication). Volunteers have played a large role in egg collection (Martin *et al*., 2012). Traps served not only to capture snails but also to assess the efficacy of copper sulphate treatments. For instance, 60-70 % of the snails inside the traps were dead after the initial treatment. Granular copper sulphate was first applied to Langan Lake using a blower to achieve the target concentration of 2.54 ppm elemental copper. No fish died. The treatment resulted in the added bonus of killing non-native, invasive Asiatic clams (*Corbicula fluminea*). Routine applications of copper sulphate now occur in Langan Lake and tidal portions of Three Mile Creek. Any impacts on the fauna downstream of the application sites have not been reported.

As additional management efforts, volunteers continued to remove snails manually. Volunteers also applied herbicides to reduce the abundance of native, but rapidly spreading, giant cutgrass (*Zizaniopsis miliacea*), the plant the snails appeared to prefer for egg deposition. In addition, a copper-based paint was applied to various human-made structures to discourage egg deposition. Finally, native redear sunfish have been stocked annually at a rate of 2500 ha^{-1} to provide biological control of juvenile snails. *Pomacea maculata* also invaded two other ponds (< 8 ha) in Alabama, in Baldwin and Coffee counties. Quick agreements with landowners that facilitated copper sulphate treatments, trapping and hand picking of egg masses eliminated both populations. The goal of complete eradication in Alabama remains elusive (Martin *et al*., 2012), but the effort continues.

South Carolina

In May 2008, *P. maculata* was discovered in 35 water bodies (0.25-7.12 ha) and a portion of the Waccamaw River near Socastee (M. Hook & C. Page, pers. comm.). Immediately, surveys of 200 water bodies in the area were undertaken and 39 contained apple snails. Most of these water bodies consisted of interconnected drainage ditches and retention ponds within housing communities and golf courses. Less than a month after the discovery of *P. maculata*, all infested ponds were treated with granular copper sulphate at a rate of 11.2 kg ha^{-1}. The extent of control varied, so the ponds were retreated in September.

The ponds were surveyed again in May 2009 when egg production had begun. In June, ponds with snails were treated again with copper sulphate and eggs were removed by hand. Unfortunately, this control effort was not effective and snails persisted into July. Subsequently, the infested ponds were treated with double chelated copper at 0.3 ppm (an order of magnitude lower than applications in Alabama), with a follow-up treatment in August to ensure a long exposure time. Initial treatment resulted in high mortality. While doing informal surveys the week after the second treatment, managers stopped counting at 500 shells of dead snails when they examined a single 5 m section of shoreline in several of the ponds. By September 2009, there was little to no egg production in all ponds except two, which were retreated with the same low dose of chelated copper. This level of application did not eliminate other wildlife in the ponds including fish, ducksand turtles, although other invertebrates were not monitored (M. Hook, pers. comm.). By November, no reproduction was observed in any of the ponds, although seasonality probably contributed to reduced snail activity. Through the summer of 2014, routine use of multiple treatments of double chelated copper maintained low abundances when snails were active. Since 2014, chemical control of algae by homeowners and management associations in some of the ponds has helped to keep snail abundances low (M. Hook, pers. comm.). However, the interconnectedness of the systems allows apple snails to persist and the remnant populations that survive make continued monitoring essential.

Future research

Based on these three case studies, no one single approach works for all systems. Management efforts must weigh the ecological costs of ignoring *P. maculata* invasions against the time and economic costs of fighting an on-going battle with snails that exhibit high fecundity (Barnes *et al*., 2008). Continued spread of *P. maculata* in the southern USA warrants increased concern about the possibility that it may follow the devastating path of *P. canaliculata* in other parts of the world. *Pomacea maculata* has a broader geographic distribution in the continental USA, as *P. canaliculata* only occurs in a few localities in Arizona, Florida and California) (Rawlings *et al*., 2007). There is less published research on *P. maculata* than *P. canaliculata*. We reiterate the need for more taxonomic rigour and advocate for use of scientific names rather than common names to emphasise the diversity of apple snails present. Given recent taxonomic and phylogenetic clarity (Hayes *et al*., 2012), more studies are appearing regarding the threat of *P. maculata*, correctly identified, as an expanding, invasive species. In this contribution, we sought to synthesise what is known about *P. maculata* in the USA and conclude by identifying research questions that deserve further attention (Table 2). We hope that this chapter serves as a wake-up call to pay attention to *P. maculata* as a species that warrants concern.

Table 2. Future research areas to advance our understanding of *Pomacea maculata*.

Area	Open research topics	Related references
Identity and genetics	• Investigating invasion via genetic analysis • Possibility/likelihood of hybridisation • Microsatellite approach to determine paternity • Sequence of entire genome	Hayes *et al*., 2008 Hayes *et al*., 2012 Yoshida *et al*., 2014 Matsukura *et al*., 2016a
Fecundity, growth and reproduction	• Biochemistry of eggs and predation effects • Age and size at first reproduction • Hatchling growth and survival • Maximum reproductive potential per female • Longevity of different sexes	Dreon *et al*., 2010; 2013 Giglio *et al*., 2016 Dorn & Hafsadi, 2016 Kyle *et al*., 2011
Distribution	• Determining vectors of introduction • Refinement of ecological niche modelling • Dispersal ability of life history stages • Proportion of snails infected by *A. cantonensis*	Byers *et al*., 2013 Teem *et al*., 2013
Abiotic tolerances	• Hatching efficiency and hatchling survival in a range of environmental conditions • Ability to survive low water levels	Barnes *et al*., 2008 Martin & Valentine, 2014 Ramakrishnan, 2007

Trophic interactions	• Quantitative measures of consumption of macrophytes in natural field settings • Determination of trophic position • Role as parasite vector and infection rates	Connor *et al*., 2008 Cattau *et al*., 2010, 2016 Posch *et al*., 2013 Kim *et al*., 2014
Impact	• Potential for agricultural impact on rice • Threats to endangered species • Screening of snails for *A. cantonensis*	Burlakova *et al*., 2010 Teem *et al*., 2013
Control	• Effective, non-toxic ways to remove snails • Effects of water level fluctuations on dispersal	Martin *et al*., 2012
Education	• Aquarium and aquaculture industry outreach • Establish clearinghouse for new information	*Pomacea* Project, 2013
Big picture	• Ability to shift alternative ecological stable states • Contributions to invasional meltdown theory • Model organisms for amphibiousness	Carlsson *et al*., 2004a Meza-Lopez & Siemann, 2015 Hayes et al. 2009b

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References

Ackleh, A.S., Allen, L.J.S. & Carter, J. 2007. Establishing a beachhead: a stochastic population model with an Allee effect applied to species invasion. *Theoretical Population Biology* 71: 290-300.

Andree, K.B. & López, M.A. 2013. Species identification from archived snail shells via genetic analysis: a method for DNA extraction from empty shells. *Molluscan Research* 33: 1-5.

Arnold, T.E., Brenner, M., Curtis, J.H., Dutton, A., Baker, S.M., Escobar, J.H. & Ortega, C.A. 2014. Application of stable isotopes ($\delta^{18}O$) to determine growth patterns of the invasive gastropod *Pomacea maculata* in Florida Lakes. *The Florida Scientist* 77: 126-143.

Baker, P., Zimmanck, F. & Baker, S.M. 2010. Feeding rates of an introduced freshwater gastropod *Pomacea insularum* on native and nonindigenous aquatic plants in Florida. *Journal of Molluscan Studies* 76: 138-143.

Barnes, M.A., Fordham, R.K., Hand, J.J. & Burks, R.L. 2008. Fecundity of the exotic apple snail, *Pomacea insularum*. *Journal of the North American Benthological Society* 27: 738-745.

Batzer, D.P., Dietz-Brantley, S.E., Taylor, B.E. & DeBiase, A.E. 2005. Evaluating regional differences in macroinvertebrate communities from forested depressional wetlands across eastern and central North America. *Journal of the North American Benthological Society* 24: 403-414.

Bernatis, J.L. 2014. Morphology, ecophysiology, and impacts of nonindigenous *Pomacea* in Florida. Ph.D. dissertation. University of Florida, Gainesville.

Bernatis, J.L. & Warren, G.L. 2014. Effectiveness of a hand removal program for management of nonindigenous apple snails in an urban pond. *Southeastern Naturalist* 13: 607-618.

Bernatis, J.L., Mcgaw, I. & Cross, C.L. 2016. Abiotic tolerances in different life stages of apple snails *Pomacea canaliculata* and *Pomacea maculata* and the implications for distribution. *Journal of Shellfish Research* 35(4): 1013-1025.

Berry, J.P. & Lind, O. 2010. First evidence of “paralytic shellfish toxins” and cylindrospermopsin in a Mexican freshwater system, Lago Catemaco, and apparent bioaccumulation of the toxins in “tegogolo” snails (*Pomacea patula catemacensis*). *Toxicon* 55: 930-938.

Boland, B.B., Meerhoff, M., Fosalba, C., Mazzeo, N., Barnes, M.A. & Burks, R.L. 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of applesnails (*Pomacea*). *Journal of Molluscan Studies* 74: 47-54.

Burks, R.L., Kyle, C.H. & Trawick, M.K. 2010. Pink eggs and snails: field oviposition patterns of an invasive snail, *Pomacea insularum*, indicate a preference for an invasive macrophyte. *Hydrobiologia* 646: 243-251.

Burks, R.L., Hensley, S.A. & Kyle, C.H. 2011. Quite the appetite: juvenile island apple snails (*Pomacea insularum*) survive consuming only exotic invasive plants. *Journal of Molluscan Studies* 77: 423-428.

Burks, R.L., Miller, A.E. & Hill, A.L 2016. *Pomacea maculata*. CABI Invasive Species Compendium. http://www.cabi.org/isc/datasheet/116486. Accessed 2 November 2016.

Burlakova, L.E., Karatayev, A.Y., Padilla, D.K., Cartwright, L.D. & Hollas, D.N. 2009. Wetland restoration and invasive species: apple snail (*Pomacea insularum*) feeding on native and invasive aquatic plants. *Restoration Ecology* 17: 433-440.

Burlakova, L.E., Padilla, D.K., Karatayev, A.Y., Hollas D.N., Cartwright, L.D. & Nichol, K.D. 2010. Differences in population dynamics and potential impacts of a freshwater invader driven by temporal habitat stability. *Biological Invasions* 12: 927-941

Byers, J.E., McDowell, W.G., Dodd, S.R., Haynie, R.S., Pintor, L.M. & Wilde, S.B. 2013. Climate and pH predict potential range of the invasive apple snail (*Pomacea insularum*) in the southeastern United States. *PloS ONE* 8(2): e56812.

Carlsson, N.O.L., Brönmark, C. & Hanson. L.-A. 2004a. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575-1580.

Carlsson, N.O.L., Kestrup, A., Martensson, M. & Nystrom, P. 2004b. Lethal and non lethal effects of multiple indigenous predators on the invasive golden apple snail (*Pomacea canaliculata*). *Freshwater Biology* 49: 1269-1279.

Cattau, C.E., Martin, J. & Kitchens, W.M. 2010. Effects of an exotic prey species on a native specialist: example of the snail kite. *Biological Conservation* 143: 513-520.

Cattau, C.C., Fletcher, R.J., Jr., Reichert, B.E. & Kitchens, W.M. 2016. Counteracting effects of a non-native prey on the demography of a native predator culminate in positive population growth. *Ecological Applications* 26: 1952-1968, Conner, S.L., Pomory, C.M. & Darby, P.C. 2008. Density effects of native and exotic snails on growth in juvenile apple snails *Pomacea paludosa* (Gastropoda: Ampullariidae): a laboratory experiment. *Journal of Molluscan Studies* 74: 355-362.

Cowie, R.H. 2015. The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa* 3940(1): 1-92.

Cowie, R.H. & Héros, V. 2012. Annotated catalogue of the types of Ampullariidae (Mollusca: Gastropoda) in the Muséum national d'Histoire naturelle, Paris, with lectotype designations. *Zoosystema* 34: 793-824.

Cowie, R.H. & Thiengo, S.C. 2003. The apple snails of the Americas (Mollusca: Gastropoda: Ampullariidae: *Asolene*, *Felipponea*, *Marisa*, *Pomacea*, *Pomella*): a nomenclatural and type catalog. *Malacologia* 45: 41-100.

Cowie, R.H., Hayes, K.A. & Thiengo, S.C. 2006. What are apple snails? Confused taxono- my and some preliminary resolution. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 3-23. Philippine Rice Research Institute, Nueva Ecija.

Cowie, R.H., Hayes, K.A. & Strong, E.E. 2015. Types of Ampullariidae (Mollusca: Gastropoda) in the National Museum of Natural History, Smithsonian Institution, with lectotype designations. *Smithsonian Contributions to Zoology* 645: [i-iii], 1-13.

Cowie, R.H., Strong, E.E., Rosenberg, G. & Hayes, K.A. 2017. Types of Ampullariidae (Mollusca: Gastropoda) in the Academy of Natural Sciences of Philadelphia. *Proceedings of the Academy of Natural Sciences of Philadelphia* forthcoming.

Damborenea, C., Brusa, F. & Negrete, L. 2017. Symbionts and diseases associated with invasive apple snails. In: *Biology and Management of Invasive Apple Snails* (Joshi, R.C., Cowie, R.H. & Sebastian, L.S, ed.), p. 73-97. Philippine Rice Research Institute, Nueva Ecija.

Darby, P.C., Bennetts, R.E. & Percival, H.F. 2008. Dry down impacts on apple snail (*Pomacea paludosa*) demography: implications for wetland management. *Wetlands* 28: 204-214.

Deaton, L.E., Schmidt, W., Leblanc, B., Carter, J., Mueck, K. & Merino, S. 2016. Physiology of the invasive apple snail *Pomacea maculata:* tolerance to low temperatures. *Journal of Shellfish Research* 35: 207-210.

Dodd, S.R., Haynie, R.S., Williams, S.M. & Wilde, S.B. 2016. Alternate food-chain transfer of the toxin linked to avian vacuolar myelinopathy and implications for the endangered Florida snail kite (*Rostrhamus sociabilis*). *Journal of Wildlife Diseases* 52: 335-344.

Dodds, N.M.W., Miller, H.M. & Lamm, A.J. 2014. Floridians' perspectives on invasive species. University of Florida Institute of Food and Agricultural Sciences, Gainesville. http://edis.ifas.ufl.edu/wc186.

Dorn, N.J. & Hafsadi, M. 2016. Native crayfish consume more non-native than native apple snails. *Biological Invasions* 18(1): 159-167.

Dreon, M.S., Ituarte, S. & Heras, H. 2010. The role of the proteinase inhibitor ovorubin in apple snail eggs resembles plant embryo defense against predation. *PLoS ONE* 5(12): e15059.

Dreon, M.S., Frassa, M.V., Ceolin, M., Ituarte, S., Qiu, J.-W., Sun, J., Fernandez, P.E. & Heras, H. 2013. Novel animal defenses against predation: a snail neurotoxin combining lectin and pore-forming chains that resembles plant defense and bacteria attack toxins. *PloS ONE* 8(5): e63782.

Dummee, V., Tanhan, P., Kruatrachue, M., Damrongphol, P. & Pokethitiyook, P. 2015. Histopathological changes in snail, *Pomacea canaliculata*, exposed to sub-lethal copper sulfate concentrations. *Ecotoxicology and Environmental Safety* 122: 290-295.

Fontanilla. I.K.C., Sta. Maria, I.M.P., Garcia, J.R.M., Ghate, H., Naggs, F. & Wade, C.M. 2014. Restricted genetic variation in populations of *Achatina* (*Lissachatina*) *fulica* outside of East Africa and the Indian Ocean islands points to the Indian Ocean islands as the earliest known common source. *PLoS ONE* 9(9): e105151.

Gettys, L.A., Haller, W.T., Mudge, C.R. & Koshnick, T.J. 2008. Effect of temperature and feeding preference on submerged plants by the island apple snail, *Pomacea insularum* (d'Orbigny, 1839) (Ampullariidae). *The Veliger* 50: 248-254.

Giglio, M.L., Ituarte, S., Pasquevitch, M.Y. & Heras, H. 2016. The eggs of the apple snail *Pomacea maculata* are defended by indigestible polysaccharides and toxic proteins. *Canadian Journal of Zoology* 94(11): 777-785.

Gray, P. 2013. *Everglade Snail Kite Nesting Summary 2012*. Audubon Florida, Miami. http://fl.audubon.org/sites/g/files/amh666/f/audubon_evergladesnailkite_jan2013.pdf

Guisan, A., Tingley, R., Baumgartner, J.B., Naujokaitis-Lewis, I., Sutcliffe, P.R., Tulloch, A. I.T., Regan, T.J., Brotons, L., McDonald-Madden, E., Mantyka-Pringle, C., Martin, T.G., Rhodes, J.R., Maggini, R., Setterfield, S.A., Elith, J., Schwartz, M.W., Wintle, B.A., Broennimann, O., Austin, M., Ferrier, S., Kearney, M.R., Possingham, H.P. & Buckley, Y.M. 2013. Predicting species distributions for conservation decisions.

Ecology Letters 16: 1424-1435.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of the non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Cowie, R.H. & Thiengo, S.C. 2009a. A global phylogeny of apple snails: Gondwanan origin, generic relationships, and the influence of outgroup choice (Caenogastropoda: Ampullariidae). *Biological Journal of the Linnean Society* 98: 61-76.

Hayes, K.A., Cowie, R.H., Jørgenson, A., Schultheiß, R., Albrecht, C. & Thiengo, S.C. 2009b. Molluscan models in evolutionary biology: apple snails (Gastropoda: Ampullariidae) as a system for addressing fundamental questions. *American Malacological Bulletin* 27: 47-58.

Hayes, K.A., Cowie, R.H., Thiengo, S.C. & Strong, E.E. 2012. Comparing apples to apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society* 166: 723-753.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.-W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Horgan, F.G., Stuart, A.M. & Kudavidanage, E.P. 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica* 54: 90-100.

Horn, K.C., Johnson, S.D., Boles, K.M., Moore, A., Siemann, E. & Gable, C.A. 2008. Factors affecting hatching success of golden apple snail eggs: effects of water immersion and cannibalism. *Wetlands* 28: 544-549.

Howells, R.G., Burlakova, L.E., Karatayev, A.Y., Marfurt, R.K. & Burks, R.L. 2006. Native and introduced Ampullariidae in North America: history, status, and ecology. In *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 73-112. Philippine Rice Research Institute, Nueva Ecija.

Iwanowicz, D.D., Sanders, L.R., Schill, W.B., Xayavong, M.V., da Silva, A.J., Qvarnstrom, Y. & Smith, T. 2015. Spread of the rat lungworm (*Angiostrongylus* *cantonensis*) in Giant African land snails (*Lissachatina fulica*) in Florida, USA. *Journal of Wildlife Diseases* 51:749–753.

Jordan, P.J. & Deaton, L.E. 1999. Osmotic regulation and salinity tolerance in the freshwater snail *Pomacea bridgesi* and the freshwater clam *Lampsilis teres*. *Comparative Biochemistry and Physiology Part A* 122: 199-205.

Joshi, R.C. & Sebastian, L.S. 2006. *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija.

Kale, H.W., II. & Maehr, D.S. 1990. *Florida's Birds: a Handbook and Reference*. Pineapple Press, Sarasota.

Karatayev, A.Y., Burlakova, L.E., Karatayev, V.A. & Padilla, D.K. 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619: 181-194.

Karraker, N.E. & Dudgeon, D. 2014. Invasive apple snails (*Pomacea canaliculata*) are predators of amphibians in South China. *Biological Invasions* 16: 1785-1789.

Keller, R.P., Drake, J.M. & Lodge, D.M. 2007. Fecundity as a basis for risk assessment of nonindigenous freshwater molluscs. *Conservation Biology* 21: 191-200.

Kim, J.R., Hayes, K.A., Yeung, N.W. & Cowie, R.H. 2014. Diverse gastropod hosts of *Angiostrongylus cantonensis*, the rat lungworm, globally and with a focus on the Hawaiian Islands. *PLoS ONE* 9(5): e94969.

Kratzer, E.B. & Batzer, D.P. 2007. Spatial and temporal variation in aquatic macroinvertebrates in the Okefenokee Swamp, Georgia, USA. *Wetlands* 27: 127-140.

Kyle, C.H., Trawick, M.K., McDonough, J.P. & Burks, R.L. 2009. Population dynamics of an established reproducing population of the invasive apple snail (*Pomacea insularum*) in suburban southeast Houston, Texas. *Texas Journal of Science* 61: 1-5.

Kyle, C.H., Kropf, A.W. & Burks, R.L. 2011. Prime waterfront real estate: apple snails choose wild taro for oviposition sites. *Current Zoology* 57: 630-641.

Kyle, C.H., Plantz, A.L., Shelton, T. & Burks, R.L. 2013. Count your eggs before they invade: identifying and quantifying egg clutches of two invasive apple snail species (*Pomacea*). *PLoS ONE* 8(10): e77736.

Leslie A.J. 1992. Copper-herbicide use patterns in Florida water. Florida Department of Natural Resources, Tallahasse.

Lockwood, J.L., Hoopes, M.F. & Marchetti, M.P. 2013. *Invasion Ecology*, second edition. Blackwell Publishing, Malden, USA.

Lodge, D.M., Brown, K.M., Klosiewski, S.P., Stein, R.A., Covich, A.P., Leather, B.K. & Brönmark, C. 1987. Distribution of freshwater snails: spatial scale and the relative importance of physiochemical and biotic factors. *American Malacological Bulletin* 5: 73-84.

Lowe S., Browne, M., Boudjelas, S. & De Poorter, M. 2004. *100 of the world's worst invasive alien species*. Updated and reprinted version. Invasive Species Specialist Group, Auckland.

Martín, P.R., Estebenet, A.L. & Cazzaniga, N.J. 2001. Factors affecting the distribution of *Pomacea canaliculata* (Gastropoda: Ampullariidae) along its southernmost natural limit. *Malacologia* 43: 13-23.

Martin, C.W. & Valentine, J.F. 2014. Tolerance of embryos and hatchlings of the invasive apple snail *Pomacea maculata* to estuarine conditions. *Aquatic Ecology* 48: 321-326.

Martin, C.W., Bayha, K.M. & Valentine, J.F. 2012. Establishment of the invasive island apple snail *Pomacea insularum* (Gastropoda: Ampullaridae [*sic*]) and eradication efforts in Mobile, Alabama, USA. *Gulf of Mexico Science* 30: 30-38.

Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions* 15: 2039-2048.

Matsukura, K., Hayes, K.A. & Cowie, R.H. 2016a. Eleven microsatellite loci for two invasive freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* (Ampullariidae). *Biological Invasions* 18: 3397-3400.

Matsukura, K., Izumi, Y., Yoshida, K. & Wada, T. 2016b. Cold tolerance of invasive freshwater snails, *Pomacea canaliculata*, *P. maculata* and their hybrids helps explain their different distributions. *Freshwater Biology* 61: 80-87.McMahon, R.F. 1983. Physiological ecology of freshwater pulmonates. In: *The Mollusca*, *Volume 6, Ecology*. (Russell-Hunter, W.D., ed.), p 359-430. Academic Press, London.

Meza-Lopez, M.M. & Siemann, E. 2015. Experimental test of the invasional meltdown hypothesis: an exotic herbivore facilitates an exotic plant but the plant does not reciprocally facilitate the herbivore. *Freshwater Biology* 60: 1475-1482.

Monette, D., Ewe, S. & Markwith, S.H. 2016. Effects of the consumptive behavior of adult *Pomacea maculata* and *Pomacea paludosa* on *Vallisneria americana*. *The Southeastern Naturalist* 15(4): 689-696.

Morrison, W.E. & Hay, M.E. 2011a. Feeding and growth of native, invasive and non-invasive alien apple snails (Ampullariidae) in the United States: invasives eat more and grow more. *Biological Invasions* 13: 945-955.

Morrison, W.E. & Hay, M.E. 2011b. Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS ONE* 6(3): e17227.

Pasko, S., Eich, A.M., & Britton, D. 2011. Applesnail (*Pomacea canaliculata*, *Pomacea insularum*). United States Federal Aquatic Nuisance Species Task Force. http://www.anstaskforce.gov/spoc/applesnail.php.

Pasquevich, M.Y., Dreon, M.S. & Heras. H.H. 2014. The major egg reserve protein from the invasive apple snail *Pomacea maculata* is a complex carotenoprotein related to those of *Pomacea canaliculata* and *Pomacea scalaris*. *Comparative Biochemistry and Physiology Part B* 169: 63-71.

Perlman, H. 2016. Water hardness. USGS [United States Geological Survey] Water Science School. http://water.usgs.gov/edu/hardness.html. Accessed 27 November 2016.

Pias, K.E., Welch, Z.C. & Kitchens, W.M. 2012. An artificial perch to help snail kites handle an exotic apple snail. *Waterbirds* 35: 347-351.

Pinto, H.A., Cantanhede, S.P.D., Thiengo, S.C., de Melo, A. & Fernandez, M.A. 2015. The apple snail *Pomacea maculata* (Caenogastropoda: Ampullariidae) as the intermediate host of *Stomylotrema gratiosus* (Trematoda: Stomylotrematidae) in Brazil: the first report of a mollusc host of a stomylotrematid trematode. *Journal of Parasitology* 101: 134-139.

Pizani, N.V., Estebenet, A.L. & Martín, P.R. 2005. Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae). *American Malacological Bulletin* 20: 55-63.

Pomacea Project. 2013. Website on the Florida apple snail (*Pomacea paludosa*) and its predators. The *Pomacea* Project, Inc., Pensacola. http://www.pomaceaproject.org. Accessed 26 September 2016.

Posch, H., Garr, A.L. & Reynolds, E. 2013. The presence of an exotic snail, *Pomacea maculata*, inhibits the growth of juvenile Florida apple snails, *Pomacea paludosa*. *Journal of Molluscan Studies* 79: 383-385.

Poulin, R. 2011. *Evolutionary Ecology of Parasites*. Princeton University Press. Princeton.

Qiu, J.-W. & Kwong, K.L. 2009. Effects of macrophytes on feeding and life-history traits of the invasive apple snail *Pomacea canaliculata*. *Freshwater Biology* 54: 1720-1730.

Qvarnstrom, Y., Bishop, H.S. & da Silva, A.J. 2013. Detection of rat lungworm in intermediate, definitive, and paratenic hosts obtained from environmental sources. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 63-69.

Ramakrishnan V. 2007. Salinity, pH, temperature, desiccation and hypoxia tolerance in the invasive freshwater snail *Pomacea insularum*. Ph.D. dissertation. University of Texas at Arlington. https://uta-ir.tdl.org/uta-ir/handle/10106/131.

Rawlings, T.A., Hayes, K.A., Cowie, R.H. & Collins, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology* 7: 97.

Scott T.M., Means, G.H., Means, R.C. & Meegan, R.P. 2002. *First Magnitude Springs of Florida. Open File Report No. 85*. Florida Department of Environmental Protection, Florida Geological Survey, Tallahassee. http://www.dep.state.fl.us/geology/geologictopics/springs.htm. Accessed 20 November 2016.

Seuffert, M.E. & Martín, P.R. 2009a. Dependence on aerial respiration and its influence on microdistribution in the invasive freshwater snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Biological Invasions* 12: 1695-1708.

Seuffert, M.E. & Martín, P.R. 2009b. Influence of temperature, size and sex on aerial respiration of *Pomacea canaliculata* (Gastropoda: Ampullariidae) from southern Pampas, Argentina. *Malacologia* 51: 191-200.

Sharfstein, B. & Steinman, A.D. 2001. Growth and survival of the Florida apple snail (*Pomacea paludosa*) fed 3 naturally occurring macrophyte assemblages. *Journal of the North American Benthological Society* 20(1): 84-95.

Shuford, R.B.E., McCormick, P.V. & Magson, J. 2005. Habitat related growth of juvenile Florida applesnails (*Pomacea paludosa*). *Florida Scientist* 68:11-19.

Smith, C., Boughton, E.H. & Pierre, S. 2015. *Pomacea maculata* (island apple snail) invasion in seasonal wetlands on Florida ranchland: association with plant community structure and aquatic-predator abundance. *Southeastern Naturalist* 14: 561-576.

Stockdale-Walden, H.D., Slapcinsky, J., Qvarnstrom, Y., McIntosh, A., Bishop, H.S. & Rosseland, B. 2015. *Angiostrongylus cantonensis* in introduced gastropods in southern Florida. *Journal of Parasitology* 101: 156-159.

Takougang, I., Meli, J. & Angwafo, F. 2006. Field trials of low dose Bayluscide on snail hosts of schistosome and selected non-target organisms in Sahelian Cameroon. *Memórias do Instituto Oswaldo Cruz* 101: 355-358.

Tamburi, N.E. & Martín, P.R. 2009. Reaction norms of size and age at maturity of *Pomacea canaliculata* (Gastropoda: Ampullariidae) under a gradient of food deprivation. *Journal of Molluscan Studies* 75: 19-26.

Tamburi, N.E. & Martín, P.R. 2011. Effects of food availability on reproductive output, offspring quality and reproductive efficiency in the apple snail *Pomacea canaliculata*. *Biological Invasions* 13: 2351-2360.

Teem, J.L., Qvarnstrom, Y., Bishop, H.S., da Silva, A.J., Carter, J., White-McLean, J. & Smith, T. 2013. The occurrence of the rat lungworm, *Angiostrongylus cantonensis*, in nonindigenous snails in the Gulf of Mexico region of the United States. *Hawai'i Journal of Medicine and Public Health* 72(6) Supplement 2: 11-14.

Tran, C.T., Hayes, K.A. & Cowie, R.H. 2008. Lack of mitochondrial DNA diversity in invasive apple snails (Ampullariidae) in Hawaii. *Malacologia* 50: 351-357.

Van Dyke, J.M., McGlynn, S.E. & Ingram, L.E. 2013. Aquatic snail trap, bait, and method of use thereof. Patent number US8341875 B2

Verbrugge, L.N.H., Schipper, A.M., Huijbregts, M.A.J., Van der Velde, G. & Leuven, R.S.E.W. 2012. Sensitivity of native and non-native mollusc species to changing river water temperature and salinity. *Biological Invasions* 14: 1187-1199.

White, M., Chejlava, M., Fried, B. & Sherma, J. 2007. The concentration of calcium carbonate in shells of freshwater snails. *American Malacological Bulletin* 22: 139-142.

Wilcox, R.C. & Fletcher, R.J., Jr. 2016. Experimental test of preferences for an invasive prey by an endangered predator: implications for conservation. *PLoS ONE* 11(11): e0165427.

Yoshida, K., Matsukura, K., Cazzaniga, N.J. & Wada, T. 2014. Tolerance to low temperature and desiccation in two invasive apple snails, *Pomacea canaliculata* and *P. maculata* (Caenogastropoda: Ampullariidae) collected in their original distribution area (northern and central Argentina). *Journal of Molluscan Studies* 80: 62-66.

Youens, A.K. & Burks, R.L. 2008. Comparing applesnails with oranges: the need to standardize measuring techniques when studying *Pomacea*. *Aquatic Ecology* 42: 679-684.

Yusa, Y. 2001. Predation on eggs of the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) by the fire ant *Solenopsis geminata*. *Journal of Molluscan Studies* 67: 275-279.

Yusa, Y. 2006. Predators of the introduced apple snail, *Pomacea canaliculata* (Gastropoda Ampullariidae): their effectiveness and utilization in biological control.

In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S., ed.), p. 345-361. Philippine Rice Research Institute, Nueva Ecija.

Yusa, Y., Suguira, N. & Wada, T. 2006. Predatory potential of freshwater animals on an invasive agricultural pest, the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae), in southern Japan. *Biological Invasions* 8: 137-147.

MANAGEMENT AND USE

The Invasive Species Compendium: information for the management of invasive species

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Abstract

The Invasive Species Compendium (ISC) is an open access encyclopaedic knowledge base that draws together scientific information on invasive species with coverage extending to all taxa affecting managed and natural ecosystems and a focus on those with the greatest economic and environmental impact. Globally relevant species datasheets (e.g. for *Pomacea canaliculata* and *P. maculata*) are commissioned from expert authors and include text sections, database tables, maps, images and links to abstracts, full text articles and other sources of further information. This contribution describes the features of the ISC and its context within CABI's Compendium Programme.

Additional keywords: Ampullariidae, apple snail, CABI, database, ISC, Mollusca, *Pomacea*

Introduction

Accurate scientific information is needed to make decisions on the management of invasive species, whether they are introduced species that are already present in an area and causing damage, or whether they represent a risk to livelihoods and/or the environment by their introduction or spread. The Invasive Species Compendium (ISC) currently provides detailed datasheets on almost 2000 of the most invasive species and animal diseases worldwide and basic information on a further 7000 invasive species. In addition, the ISC includes a bibliographic database that is updated weekly and contains over 185,000 relevant abstracts, of which 3600 have full text documents. A glossary and links to further web resources for identification keys and other supporting materials are also provided.

There are a number of routes into the data, including a site search and powerful database searches by which the user can generate a list of species matching selected criteria. Navigation to related content is provided by hyperlinks, making the ISC easy to explore. The text found can be pasted into reports or extension and training materials, and geographic records can be exported to mapping software. The ISC is used in over 150 countries for a wide range of purposes, including pest management. It is one of a series of Compendia published by CABI on the internet. Compendia development is funded and guided by international groups of stakeholders made up of government departments, development assistance organizations and private companies. A full description can be found at: http://www.cabi.org/isc.

Invasive species datasheets

Information is presented in a standardized datasheet format, accompanied by distribution maps, illustrations and bibliographic sources. Text sections cover the following aspects:

- Names and taxonomy
- Summary of invasiveness
- Description
- Geographic distribution
- History of introduction and spread
- Risk of introduction
- Habitat
- Host range
- Symptoms
- Biology and ecology
- Natural enemies
- Means of movement and dispersal
- Impact (economic, environmental and social)
- Uses
- Detection and inspection
- Prevention and control

- References and further information
- Gaps in knowledge
- Contributors

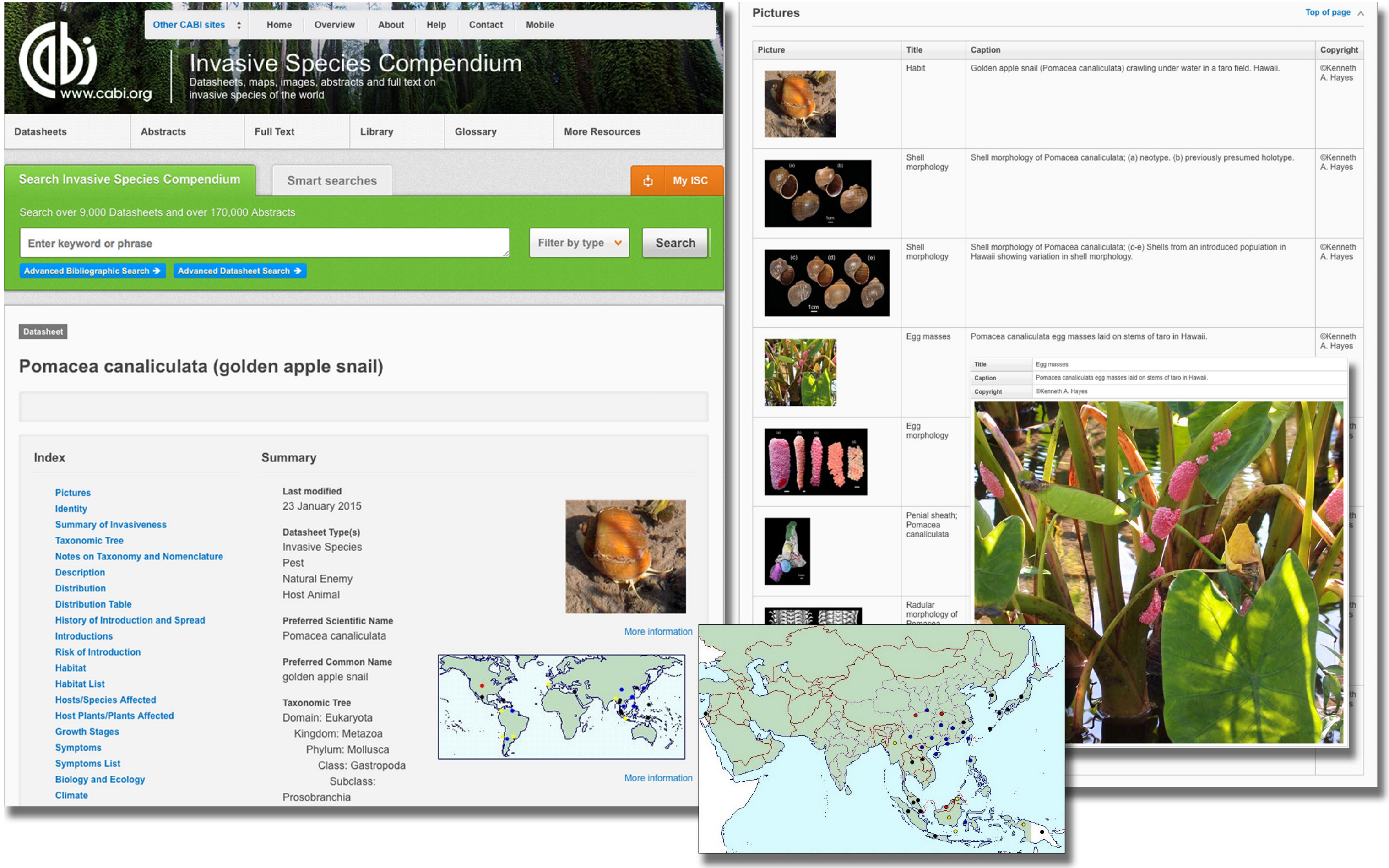
The fully-referenced, detailed datasheets have been compiled by over 1000 selected experts, edited by CABI, and peer reviewed by specialists. There is a programme for reviewing datasheets and updates are published to the website as soon as they have been finalized. Particular effort is allocated to updating species distributions and host records. New datasheets are added in response to recent research, alerts and funded projects.

A datasheet on *Pomacea canaliculata* (http://www.cabi.org/isc/datasheet/68490) was first commissioned for CABI's Compendium Programme in 1996 and included in the first edition of the Crop Protection Compendium (CPC). The original text was compiled by James Litsinger. The content has since been reviewed, in 2003 by Ravindra Joshi and in 2013 by Robert Cowie, and enhanced for inclusion in the ISC, with extensive new and revised material including sections describing its invasiveness (Fig. 1).

The datasheet on *Pomacea maculata* (http://www.cabi.org/isc/datasheet/116486) was added more recently. The original text was compiled by Romi Burks, Amy Miller and Alexandria Hill in 2013, and reviewed, revised and augmented with extensive additional information by Robert Cowie in 2015.

Decision support

The ISC presents well referenced scientific information that may be otherwise inaccessible or poorly compiled. There are several ways to access the species information. For example, you can search for a datasheet using the preferred scientific name, or any of its synonyms or common names, using the site search. You can also browse through a list of species recorded for a particular country by visiting the country datasheet. Linkage of datasheets allows you to navigate to related content, for example, to find information on the invasive species threatening an endangered species, or those transported by a particular pathway. The "Advanced Datasheet Search" enables you to search within the controlled vocabulary to generate more accurate species lists that can be filtered by country status, taxonomic group, host, habitat, pathway or risk factor. You can also search for pests that are present in one country and not in another.



Pomacea canaliculata (golden apple snail)

Summary

Last modified
23 January 2015

Datasheet Type(s)
Invasive Species
Pest
Natural Enemy
Host Animal

Preferred Scientific Name
Pomacea canaliculata

Preferred Common Name
golden apple snail

Taxonomic Tree
Domain: Eukaryota
Kingdom: Metazoa
Phylum: Mollusca
Class: Gastropoda
Subclass:
Prosobranchia

Picture	Title	Caption	Copyright
	Habit	Golden apple snail (Pomacea canaliculata) crawling under water in a taro field. Hawaii.	©Kenneth A. Hayes
	Shell morphology	Shell morphology of Pomacea canaliculata; (a) neotype. (b) previously presumed holotype.	©Kenneth A. Hayes
	Shell morphology	Shell morphology of Pomacea canaliculata; (c-e) Shells from an introduced population in Hawaii showing variation in shell morphology.	©Kenneth A. Hayes
	Egg masses	Pomacea canaliculata egg masses laid on stems of taro in Hawaii.	©Kenneth A. Hayes
	Egg morphology		
	Penial sheath; Pomacea canaliculata		
	Radular morphology of Pomacea		

Title	Egg masses
Caption	Pomacea canaliculata egg masses laid on stems of taro in Hawaii.
Copyright	©Kenneth A. Hayes

Fig. 1. Elements of the *Pomacea canaliculata* datasheet from the ISC.

The ISC is used worldwide for various purposes including checking the status of species in a country, pest risk analysis, prioritizing invasive species and pathways for management, selecting management options, research, policy development, preparing extension and public awareness materials, and training.

Compendia developments

The ISC is CABI's fifth Compendium and its first major open access publication. It complements the Crop Protection Compendium (CPC), the Forestry Compendium (FC), Animal Health and Production Compendium (AHPC) and Aquaculture Compendium (AC) (see http://www.cabi.org/publishing-products/compendia/). A further, sixth Compendium, covering the production, protection and marketing of horticultural food crops, both pre- and post-harvest, is in development and will be launched in 2017.

The CPC has CABI's most comprehensive coverage of crop pests with over 2700 full datasheets on pests, 800 on the crops they attack and their natural enemies, and basic data on a further 23,000 species of relevance. The bibliographic database contains over 364,000 abstracts and full text articles. This publication is available from CABI on subscription (see www.cabi.org/cpc).

Current projects to enhance the ISC are focusing on increasing the invasive species coverage for African, Caribbean and Pacific countries (following an outreach project funded by the European Union), documenting the invasive species threatening species listed as endangered in the United States (with the U.S. Department of Agriculture), extending coverage of invasive plants in the Caribbean (with the Smithsonian Institution), and improving harmonization and data sharing between other major invasive species databases.

CABI is working with partners to provide training and promote the use of Compendia in a variety of situations. Further details of both content development and dissemination activities can be found on the Compendium website at www.cabi.org/isc.

Neem, *Azadirachta indica*, as a potential biopesticide for controlling the apple snail, *Pomacea canaliculata*

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Abstract

Experiments were undertaken to assess the efficacy of methanol and water extractions of fresh neem (*Azadirachta indica*) seed against apple snails. Each assay included five neem treatments (10-50 % dilutions of concentrated extract), a control treatment and a chemical treatment using Niclosamide. Assays were replicated. Snail mortality was recorded at 24, 48, 72 and 96 h. There was little difference between the extracts obtained by methanol and water extractions. Mortality increased over time and with neem extract concentration. The highest concentration of neem extract (50 % dilution) resulted in similar mortality levels to the Niclosamide treatment. The study showed that fresh neem seed extract has potential as a botanical pesticide against apple snails.

Additional keywords: Ampullariidae, botanical pesticide, Malaysia, Mollusca, neem seed, pest, rice

Introduction

The South American apple snail *Pomacea canaliculata*, listed among 100 of the world's most invasive species (Lowe *et al.*, 2000), was intentionally introduced and cultivated as a protein source in Taiwan, but rapidly spread to other parts of Asia (Ng & Tan, 2011). It now occurs in most Southeast Asian countries (Hayes *et al*., 2008), where it is a serious invasive agricultural pest, especially in paddy cultivation fields (Cowie, 2002). Several characteristics may facilitate the snails' invasive potential including that it is a food generalist and has a very fast growth rate and reproduction capacity (Hayes *et al*., 2015).

Damage to rice plants occurs primarily as a result of the snails feeding on the young leaf tips within the first 14 days after transplanting or emergence of the seedlings (Ito, 2002). Older seedlings with hardened leaves are less vulnerable to attack. In cases of severe infestation, the snails can cause extensive crop losses and farmers may even lose their entire crop. The damage level in the field depends on the size and number of the snails (Morallo-Rejesus *et al*., 1989). Naylor (1996) reported that a density of eight snails/m^2 can decrease rice yields by 90 %.

Various strategies are being used by farmers to eradicate or control the snails, including cultural and mechanical, biological, chemical and botanical control. Hand picking and crushing of adult snails and eggs is widely practiced in most rice growing regions in Asia, including Malaysia. Significant control can be achieved by hand picking if sufficient labour is available. The efficacy of hand picking can be improved by using attractants such as leaves of various plants (Teo, 1999). Other common cultural and mechanical practices that are still implemented widely include installing metal screens on irrigation inlets to prevent entry of apple snails from irrigation canals, maintaining a shallow water depth after transplanting to suppress snail activities, transplanting seedlings that are more than 30 days old, dry rotavation and keeping the fields dry during the off planting season to inhibit breeding (Teo, 1999).

Biological control using fish such as the common carp, Nile tilapia, black carp and hybrid fish have been experimentally used to control apple snails in rice fields in the Philippines and Vietnam with encouraging results. However, field trials of the use of hybrid catfish in Malaysia have thus far not been so successful (Jambari & Suryanto, 2000). The use of fish may not be practical because fish culture requires maintaining relatively deeper water in the fields (Cowie, 2002).

Synthetic chemical molluscicides are used extensively in Asia to control these snails, but it may be costly to both the farmer and the environment. Niclosamide, metaldehyde, endosulfan, tea seed cake (residue) and copper sulphate have been used but they are lethal to non-target organisms and pollute water bodies (Joshi, 2005). For example, Niclosamide, which is the only compound recommended for control of aquatic snails by the World Health Organization (WHO), is effective against apple snails at 0.5-1.0 mg a.i./l, but the LC_{50} for carp is only 0.14 mg a.i./l (San Martin *et al*., 2008). The major environmental concern with these pesticides is their ability to leach down to the subsoil and contaminate the ground water, or they may persist on the top soil and become harmful to microorganisms, plants, animals and people (Tomašević & Gašić, 2012). Such concerns have led to a focus on isolation and characterization of natural products that are as effective as synthetic pesticides without posing the threats to the environment.

Botanical pesticides are of great interest because they occur naturally. Historically, plant materials have been in use longer than any other type of pesticide. The flowers, leaves, bark, seeds and roots are finely ground and used in this form, or the toxic ingredients are extracted and used alone or in combination with other toxicants. Neem (*Azadirachta indica*) has been recognised for its pest control properties and is regarded as the most reliable source of eco-friendly botanical pesticide. In Thailand, Benchawattananon & Boonkong (2006) compared the toxicity of crude extracts of neem leaf and garlic (*Allium sativum* L.); 1000 mg/l of neem killed 96 % of apple snails in 96 hours, while 1000 mg/l of garlic killed 92 % in 96 hours. In Malaysia, showed that neem leaf extracts killed 93 % of small snails (10-20 mm) and 84 % of large snails (20-40 mm) after 96 hours in the laboratory (Massaguni & Latip, 2012).

A major reason for the interest in neem is the widely held view that neem has numerous valuable attributes, including that it is safe to both the environment and non-target organisms, and it is degraded rapidly in the environment. The present study assessed the efficacy of water and methanol extractions of fresh neem seeds against apple snails.

Material and methods

Apple snails and neem seed were collected at the Federal Land Consolidation and Rehabilitation Authority (FELCRA), Seberang, which is located in the Kampung Gajah Sub-district, Perak, Malaysia. Ten apple snails in the 20-25 mm size range were selected for the laboratory study. Neem seed extract was obtained from fresh neem seeds by extraction with either methanol or water as the solvent. For the methanol extraction, the protocol of Parekh *et al.* (2005) was used, and for the water extraction that of Polaquini *et al*. (2006) was used, both with some modification. In both cases the neem extract was diluted to concentrations of 100,000 ppm, 200,000 ppm, 300,000 ppm, 400,000 ppm and 500,000 ppm by volume. 1 ml of Tween 20 was added as an emulsifier in the water dilutions.

Each assay included five neem treatments (100,000 ppm (T1), 200,000 ppm (T2), 300,000 (T3), 400,000 ppm (T4) and 500,000 ppm (T5), a control treatment and a chemical treatment using Niclosamide. Each assay was replicated eight times for each of the water and methanol extractions. Snail mortality was recorded at 24, 48, 72 and 96 h. Snails were considered dead when the body was contracted within the shell and no response to a needle probe could be elicited (Singh *et al*., 1996). Dead snails were removed as soon as they were found.

The data were analyzed using Minitab 16 and POLO PLUS software. Analysis of variance (ANOVA) followed by Tukey Simultaneous tests were performed to assess differences among treatments in overall mortality after 96 h within each of the assays for methanol and water extractions. Probit analysis also performed to determine LC_{50} values (the concentration at which 50 % mortality would occur after 96 h exposure).

Results and discussion

The total percentage mortality differed significantly among treatments for both extraction protocols (ANOVA: methanol, $F = 386.42$, $P < 0.0001$; water, $F = 205.66$, $P < 0.0001$), with every treatment differing significantly from every other ($P < 0.0012$ to $P < 0.00001$) with the exception of the comparison of the 50 % neem dilution (water extraction) and Niclosamide ($P = 0.32$) (Figs. 1 and 2). The lowest mortality was in the control treatments; mortality increased with neem concentration; and the highest mortality was in the Niclosamide treatments. Mortality increased with exposure time (Figs. 1 and 2).

These results augment previous work that has shown that neem extract may be useful in the control of insect and other pests. For example, Tanu *et al*. (2010) showed that higher concentrations of neem extract resulted in higher mortality of adults of the red cotton stainer bug, *Dysdercus cingulatus*. Kudom *et al*. (2011) showed that mortality of pupae of the mosquito *Culex quinquefasciatus* increased to 80 % over 144 h.

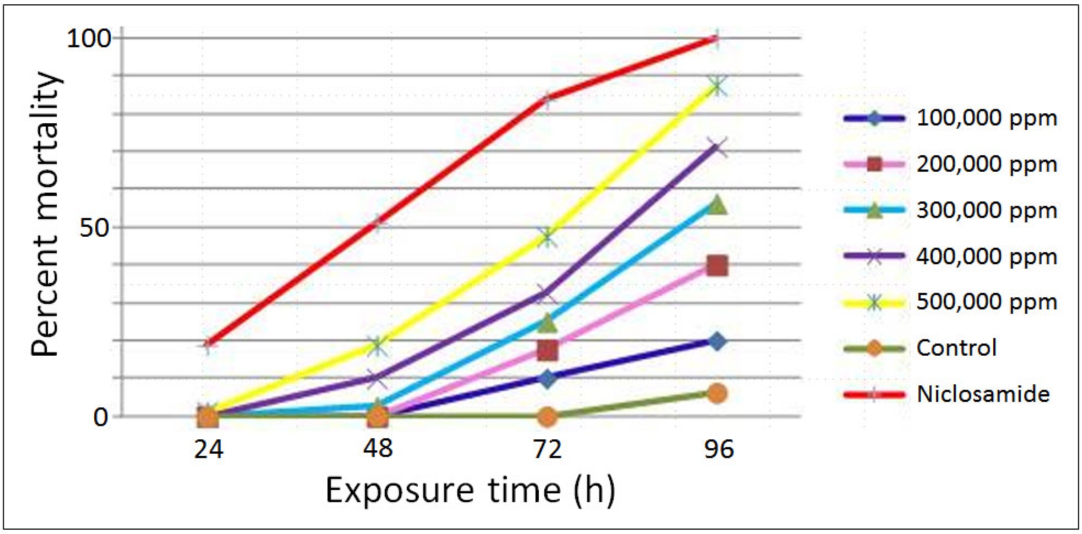

Fig.1. Percentage mortality of apple snails treated with different concentrations of neem extracted in methanol over 96 hours.

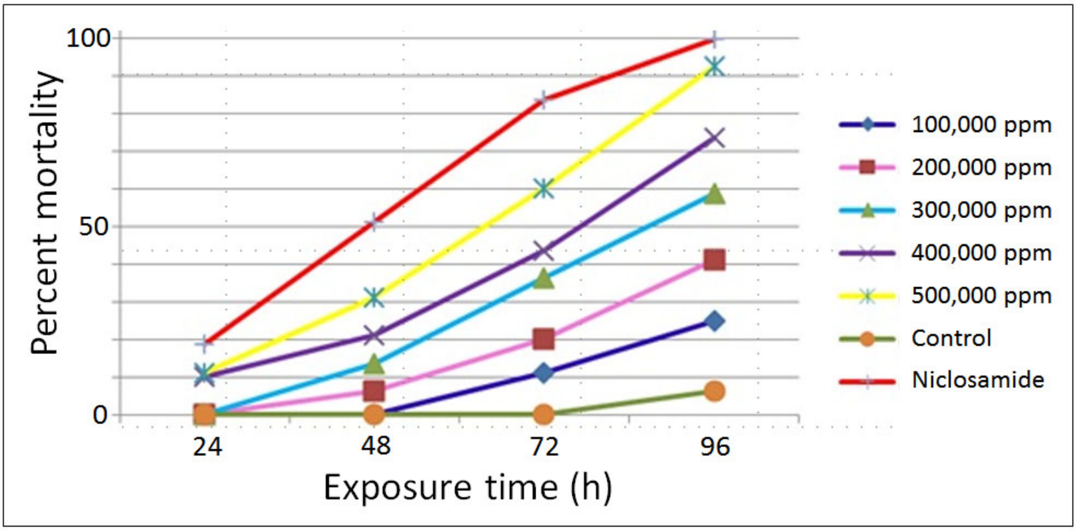

Fig.2. Percentage mortality of apple snails treated with different concentrations of neem extracted in waterover 96 hours.

Das *et al*. (2010) showed that the duration of each larval stage of the red slug caterpillar, *Eterusia magnifica*, lengthened with increase of neem kernel aqueous extract concentration. And Pinheiro *et al*. (2009) reported increased mortality of nymphs of the silverleaf or sweet potato whitefly, *Bemicia tabaci*, between three and five days after application of neem oil and in particular that neem oil caused mortality to third and fourth instar nymphs after two days application of treatment.

Probit analysis gave LC_{50} values for neem extract dilution at 96 h of 26.8 % (95 % confidence interval 22.5-31.8 %) for the methanol extraction and 24.9 % (95 % confidence interval 20.5-29.8 %) for the water extraction.

There was little difference in the results for the two extraction methods with the highest neem seed extract concentration (50 % dilution) resulting in the highest mortality due to neem, similar to the level of mortality due to Niclosamide. This study showed that fresh neem seed extract has potential as a botanical pesticide against apple snails.

References

Benchawattananon, R. & Boonkong, U. 2006. The toxicity of leave crude extract from neem tree (*Azadirachta indica* Juss.) and garlic (*Allium sativom* L.) on mortality rate of golden apple snail (*Pomacea* sp.), 32nd Congress on Science and Technology of Thailand. Queen Sirikit National Convention Center, Bangkok, 10-12 October.

Cowie, R.H. 2002. Apple snails as agricultural pests: their biology, impacts and management. In: *Molluscs as Crop Pests* (Barker, G.M., ed.), p. 145-192. CABI Publishing, Wallingford.

Das, R., Chutia, B.C., Sarmah, M. & Rahman, A. 2010. Effect of neem kernel aqueous extract (NKAE) on growth and development of red slug caterpillar, *Eterusia magnifica* Butl in Tea in North-East India, India. *Journal of Biopesticides* 3(2): 489-494.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.-W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Pilar Cadierno, M., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. & Cowie, R.H. 2013. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Ito, K. 2002. Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan. *Applied Entomology and Zoology* 37(4): 655-661.

Jambari, A. & Suryanto, E. 2000. Fish as biological control agent of golden apple snail: prospects and challenges. In: *Biological Control in the Tropics: Towards Efficient Biodiversity and Bioresource Management for Effective Biological Control. Proceedings of the Symposium on Biological Control in the Tropics held at MARDI Training Centre, Serdang, Malaysia from 18-19 March 1999* (Hong, L.W., Sastroutomo, S.S., Caunter, I.G., Ali, J., Yeang, L.K., Vijaysegaran, S. & Sen, Y.H., ed.), p. 92-95. CABI Publishing, Wallingford.

Joshi, R.C. 2005. Managing invasive alien mollusc species in rice. *International Rice Research Notes* 30: 5-13.

Kudom, A.A.., Mensah, B.A. & Botchey, M.A. 2011. Aqueous neem extract versus neem powder on *Culex quinquefasciatus*: implications for control in anthropogenic habitats. *Journal of Insect Science* 11: 142. doi: http://dx.doi.org/10.1673/031.011.14201.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. *100 of the World's Worst Invasive Alien Species. A Selection from the Global Invasive Species Database*. Invasive Species Specialist Group, Auckland.

Massaguni, R. & Latip, S.N.H.M. 2012. Neem crude extract as potential biopesticide for controlling golden apple snail, *Pomacea canaliculata*. In: *Pesticides. Advances in chemical and botanical pesticides* (Soundararajan, R.P., ed.), p. 233-254. InTech, Rijeka, Croatia.

Morallo-Rejesus, B., Sayaboc, A.S. & Joshi, R.C. 1989. The distribution and control of the introduced golden snail (*Pomacea* spp.) in the Philippines. In: *Introduction of Germplasm and Plant Quarantine Procedures. PLANTI Proceedings No. 4* (Jalil, A.W., Manalo, P.L., Sastroutomo, S.S., Ganapathi, A., Chan, K.C., Lim, L.G., Rahim, M.A.A., Durai, P.S.S. & Stephenson, M.C., ed.), p. 213-223. ASEAN PLANTI, Kuala Lumpur.

Naylor, R. 1996 Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25: 443-448.

Ng, T.H. & Tan, S.K. 2011. Observations of land snails feeding on the eggs of *Pomacea canaliculata* (Lamarck, 1822) (Mollusca: Gastropoda). *Nature in Singapore* 4: 79-83.

Parekh, J., Jadeja, D. & Chanda, S. 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology* 29: 203-210.

Pinheiro, P.V., Quintela, E.D., de Oliveira, J.P. & Seraphin, J.C. 2009. Toxicity of neem oil to *Bemisia tabaci* biotype B nymphs reared on dry bean. *Pesquisa Agropecuária Brasileira* 44(4): 354-360.

Polaquini, S.R.B., Svidzinski, T.I.E., Kemmeimeier, C. & Gasparetto, A. 2006. Effect of aqueous extract from neem (*Azadirachta indica* A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by *Candida albicans*. *Archives of Oral Biology* 51: 482-490.

San Martín, R., Ndjoko, K. & Hostettmann, K. 2008. Novel molluscicide against *Pomacea canaliculata* based on quinoa (*Chenopodium quinoa*) saponins. *Crop Protection* 27: 310-319.

Singh, K., Singh, A. & Singh, D.K. 1996. Moluscicidal activity of neem (*Azadirachta indica* A. Juss). *Journal of Ethnopharmacology* 52: 35-40.

Teo, S.S. 1999. Control of the golden apple snail *(Pomacea* spp.) by handpicking using herbage as attractants. In: *Sustainable Crop Protection Practices in the Next Millennium. Proceedings of MCB-MAPPS Plant Protection Conference '99. 2-3 November, 1999, Kota Kinabalu, Sabah, Malaysia* (Sidek, Z., Bong, C.L., Kanapathipillai, V.S., Ong, C.A. & Kadir, H.A., ed), p. 78-81. Malaysian Plant Protection Society, Kota Kinabalu.

Tomašević, A.V. & Gašić, S.M. 2012. Photoremediation of carbamate residues in water. In: *Insecticides - Basic and Other Applications* (Soloneski, S. & Larramendy, M., ed.), p. 39-60. InTech, Rijeka, Croatia.

Use of apple snail (*Pomacea* sp.) shell as a catalyst for biodiesel production: full factorial design optimisation

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Abstract

Use of apple snail (*Pomacea* sp.) shell as a catalyst for biodiesel production was studied using full factorial experimental design optimisation to determine the optimum conditions for production. The calcium oxide (CaO) catalyst was produced by calcination of apple snail (*Pomacea* sp.) shell at 900°C for 2 h in a tubular furnace. The catalyst and shell were characteriszed using nitrogen sorption analysis, Fourier transform infra-red (FTIR) analysis, X-ray fluorescence (XRF) spectrometry and X-ray diffraction (XRD). The optimum conditions as determined by the model were obtained by employing 5% catalyst and a methanol/oil ratio of 5:1, while the best combination based on real data was 4% catalyst and a methanol/oil ratio of 7:1, with a difference in fatty acid methyl ester (FAME) yield between the data and the model of 2.1%. Apple snail (*Pomacea* sp.) shell has potential as a catalyst for biodiesel production, provided population growth and expansion of the distribution of the snails is prevented so as not to increase the already serious impacts they have on rice production.

Additional keywords: Ampullariidae, calcium oxide, Mollusca, transesterification

Introduction

Development of more sustainable fuel is vital for human society, especially in the industrial sector, because of the severe environmental impacts of greenhouse gas emission of carbon dioxide, nitrogen and sulphur compounds, and the long term fuel availability issues associated with traditional fossil based fuels. The major challenge is to create sustainable alternative fuel that can address these issues.One of the alternative fuels that has been developed over the past decades is biodiesel, which has the advantages of being renewable, having lower emissions, a high flashpoint and high cetane number (CN), and providing good lubrication (Kouzu *et al.*, 2008; Margaretha *et al.*, 2012). Chemically, biodiesel is a homogeneous mixture of methyl esters with long chain fatty acids and is normally produced through transesterification of waste cooking oil, animal tallow and non-edible and edible vegetable oils (Gui *et al.*, 2008; Demirbas, 2009; Kusuma *et al.*, 2013).

The current technology used in second generation biodiesel production often employs a homogeneous catalyst. The use of this kind of catalyst has several drawbacks includingnon-recyclability after the reaction and production of toxic wastewater that requires special treatment, which significantly adds to production cost (Liu *et al.*, 2008). A new, alternative, third generation method of biodiesel production uses microalgae, which have a short harvesting cycle and can produce greater yield than traditional vegetable oils or animal fats. However, scaling-up production of biodiesel from microalgae can face unsustainable demands on energy, water (to produce 1litreof biodiesel requires 3726 litres of water) and nutrients (nitrogen, phosphorus and CO_2) required for cultivating this particular feedstock (Yang *et al.*, 2011). Thus, this option is not currently feasible for large-scale production.

The development of a heterogeneous catalyst for biodiesel production could be the best alternative to the use of homogeneous catalysts. The advantages of using a heterogeneous catalyst are re-usability, easy separation, low sensitivity to free fatty acids (FFA) and being more environmentally friendly (Suryaputra *et al.*, 2013). Different kinds of heterogeneous catalysts have been studied in terms of their performance in the preparation of biodiesel from various kinds of oils and lipids. Some of the catalysts are expensive (Garcia-Sancho *et al.*, 2011; Ghiaci *et al.*, 2011; Li *et al.*, 2011; Ramachandran *et al.*, 2011; Quintella *et al.*, 2012; Xie &Wang, 2013) and do not have any potential economic viability for industrial application. Cheaper minerals, notably calcium oxide

(CaO) from mining or natural resources, have also been used (Kouzu *et al*., 2008; Boro *et al*., 2011; Tang *et al*., 2011; Margaretha *et al*., 2012; Suryaputra *et al*., 2013).

Shells of molluscs are composite materials composed of polymorphs of calcium carbonate ($CaCO_3$),either in the form of calcite or aragonite, and organic molecules (mainly proteins and polysaccharides). Since the shells of molluscs contain significant amounts of $CaCO_3$, they have attracted the interest of many scientists for possible use as a renewable catalyst for biodiesel production (Agrawal *et al*., 2012; Birla *et al*., 2012; Boey *et al*., 2012; Jairam *et al*., 2012; Margaretha *et al*., 2012; Taufiq-Yap *et al*., 2012; Suryaputra *et al*., 2013; Zhang & Liu, 2013). In this study, we used apple snail (*Pomacea* sp.) shell as a catalyst for biodiesel production.

Apple snails in the genus *Pomacea* are not native to Indonesia, but have both ecological and economic impacts in some parts of the country. In Asia, they werefirst introduced toTaiwan from Argentina as a commodity for aquaculture business ventures in early 1980, and were subsequentlyspread, intentionally and unintentionally, to much of Southeast Asia, becoming a major pest of rice (Halwart, 1994). Now, this species (probably *Pomacea canaliculata* but possibly also *P. maculata*) is widely established in Indonesia and is having serious effects on thousands of hectares of rice fields. The meat of the snails has high protein content and can be used as animal feed or as an alternative food for human consumption (Margaretha *et al*., 2012). In some parts of the country, the meat has been used as raw materials for various kinds of fried food, while the shell is discarded as waste (Viriya-empikul *et al*., 2010). In our previous study (Margaretha *et al*., 2012) we successfully used this waste shell as a basic catalyst for transesterification of palm oil into biodiesel. In this contribution, a full factorial design was employed to determine the optimum conditions for biodiesel production using a CaO catalyst derived from apple snail shells.

Materials and methods

Materials

Waste apple snail (*Pomacea* sp.) shells were obtained from Pati, Central Java, Indonesia. The raw shell was washed repeatedly with tap water to remove dirt and other unwanted materials.The cleaned shell was then dried in an oven at 100°C for 24 hours. Then the shell was pulverized using a Janke and Kunkel hammer mill. The resulting shell powder was calcined in a tubular furnace at 900°C for 2 hours. During the calcination process, the $CaCO_3$ was converted to CaO according to the reaction:

$CaCO_3 \rightarrow CaO + CO_2$

In order to cool the system after the reaction was complete, nitrogen gas with a flow rate of 3 $lmin^{-1}$ was introduced to the tubular furnace. The solid product was crushed and sieved to pass through 140 mesh (0.105 mm) screens. The catalyst powder was stored in desiccators at room temperature for further use.

Methanol used in this study was purchased as analytical grade from MERCK, Germany. Refined palm oil (Bimoli^{TM}) was purchased from Giant Supermarket, Surabaya, Indonesia. The chemical composition of the oil was determined by gas chromatography (GC-2014, Shimadzu, Japan) (Table 1). The FAME (fatty acid methyl esters) standard was purchased from Sigma-Aldrich, Singapore.

Table 1. Chemical characteristics of refined palm oil.

Fatty acid	**%**
Lauric acid (C12:0)	0.83
Myristic acid (C14:0)	1.18
Palmitic acid (C16:0)	42.22
Palmitoleic acid (C16:1)	0.23
Stearic acid (C18:0)	4.72
Oleic acid (C18:1)	41.02
Linoleic acid (C18:2)	9.36
Linolenaic acid (C18:3)	0.14
Arachinoeic acid (C20:0)	0.30
Water content, %	0.03
Acid number, mg KOH/g oil	0.74

Transesterification procedure

The transesterification of palm oil using the CaO catalyst derived from the apple snail shell was carried out in in a three-neck round bottom flask equipped with a reflux condenser, heating mantle controller and mechanical stirrer. A brief description of the procedure for transesterification is as follows.Methanol and palm oil were mixed at molar ratios of either 5:1, 7:1, 9:1 or 11:1. The CaO catalyst was then added at a ratio of 1, 2, 3, 4 or 5%, by weight. All experiments were conducted at constant temperature (60°C) and over a standard reaction time (4 h). During the process, the mixture was stirred at constant speed (700 rpm). After the process was complete, the catalyst was separated from the liquid product by vacuum filtration. The filtrate was kept in a funnel separator for 24 hours until it separatedby gravity into two layers. The top layer consisted of biodiesel, non-reacted palm oil, and a small amount of excess methanol, while the bottom layer was composed of glycerol, excess methanol and other products from secondary reactions. After this gravitational separation, the excess methanol was removed by evaporation under vacuum.

Characterization of biodiesel

The composition of biodiesel (fatty acid methyl esters) was analysed using a GC-2014 Shimadzu gas chromatograph (Japan). The column used for FAME determination was the Agilent J&W DB-Wax capillary column (Agilent Technologies, USA). A flame ionization detector (FID) was used as the detector. The following operational conditions were used for determination of FAME content in the biodiesel. Helium was used as the carrier gas at 40 $cm.s^{-1}$. The injector temperature was 250°C and a splitless technique was used. The FID was set at 300°C. The initial oven temperature was 50°C with an equilibration time of 3 min. After an isothermal period, the oven temperature was increased to 250°C at a heating rate of 10°C/min and held for 8 min. Peaks of methyl esters were identified by comparing them with the FAME reference standard.The biodiesel yield percentage was calculated by the expression Yield % = (weight of biodiesel x % FAME) /[weight of oil x 100] % where % FAME is the concentration of FAME obtained by GC analysis.

Some physical and chemical properties of biodiesel such as density, viscosity, cetane index, flash point, water and sediment content and acid number were determined according to the ASTM International standardand the results were compared with the SNI (Indonesian National Standard) standard for biodiesel (SNI-04-7182-2006). The density was determined by ASTM D1298, kinematic viscosity by ASTM D445-10, cetane number by ASTM D613 (standard method for diesel fuel oil), flash point by ASTM D93 (standard method using Pensky-Martens closed cup tester), water and sediment content in biodiesel by ASTM D2709 (standard method for water and sediment in middle distillate fuels using centrifuge) and acid number by ASTM D664-11a (potentiometric titration).

Characterization of apple snail shell and catalyst

Characterizations of apple snail shell and the CaO catalyst were conducted using several methods including nitrogen sorption analysis, Fourier transform infrared spectroscopy (FTIR), X-ray fluorescense (XRF) spectrometry and X-ray diffraction

(XRD). The nitrogen sorption analysis was conducted to characterize the pore structure of the shell and CaO catalyst using a Quadrasorb SI analyzer. The measurement was conducted at the boiling point of nitrogen gas (-196°**C**). Prior to the analysis the samples were degassed under vacuum for 24 hours at 150°**C**.The standard Brunauer–Emmett–Teller (BET) equation was used to calculate the BET surface area of the catalyst (Brunauer *et al.*, 1938). The BET calculation was performed in the relative pressure (p/p_o) range of 0.06 to 0.3.

The FTIR analysis was conducted using the KBr method in a FTIR spectrophotometer (Shimadzu 8400,Japan). The FTIR spectra were obtained over a wave number range of 4000-700 cm^{-1}. The XRD analysis was conducted to investigate the crystalline structure of both snail shell and the CaO catalyst. A Philips X'Pert diffractometer (USA) was used to obtain the diffraction spectra of both materials, employing CuKα radiation.The measurement was conducted in 2θ angle between 8 and 72°. The bulk composition of the CaO catalyst was measured by XRF spectrometry using a Rigaku ZSX100e spectrometer (Japan).

Statistical analysis

Table 2 is the complete factorial design constructed by considering the weight ratio of the catalyst (X_1) and the oil to methanol molar ratio (X_2) as the factors that determine the FAME yield percentage (Y). The experiments were performed in random sequence constituting a single block, assuming that all materials used had a long shelf time and no consistent error arose unintentionally.

Regression analysis was conducted to build the appropriate model to describe the effect of the variable factors on the FAME yield. Linear, quadratic and cubic models incorporating the interaction of the two factors were tested to find the best fitting model, as indicated by improvement of the sums of squares error, fitting R^2, adjusted R^2, predictive R^2 and *p*-value, which, respectively provide the variance of the model to the data mean, the correlation of the factors with the response value, the adjusted correlation of the factors including the number of the variables in relation to the response value, the predictive capability of the model compared to the real data and the significance of the regression model, respectively. The best model, in terms of the harmonic distribution and correspondence to the real data, was then further upgraded to an effective equation by the backward elimination of non-significant terms. Analysis of variance (ANOVA) was used with a significance level (α) of 5% to assess the significance of each model and its terms. All statistical analyses were performed in Minitab 16.

Table 2. Full factorial design of biodiesel production using *Pomacea* sp. shell as a catalyst. The order (top row to bottom row) reflects the sequence in which the experiments were carried out.

% catalyst (X_1)	MeOH/oil (X_2)	Observed % FAME yield (Y)	Predicted % FAME yield
3	11	89.22	89.22
4	7	95.61	90.69
3	5	83.90	87.63
2	5	81.91	81.90
4	5	91.91	91.51
2	9	83.51	85.65
4	11	88.73	89.05
2	11	84.07	87.53
5	7	90.13	91.54
1	5	70.86	74.31
5	9	87.45	89.20
1	9	82.12	80.76
3	9	91.26	88.69
1	11	83.50	83.99
2	7	86.33	83.77
1	7	81.91	77.54
4	9	90.16	89.87
3	7	87.60	88.16
5	5	93.48	93.54
5	11	87.72	87.04

Results and discussion

Full factorial design optimisation

Fig. 1A shows mean FAME yield values for the five CaO percentages (averaged across all methanol/oil ratios), and Fig. 1B shows mean yield values for the four methanol/oil ratios (averaged across all CaO percentages). The steeper slope of the catalyst percent effect (Fig.1A) than of the methanol/oil ratio (Fig. 1B) indicates that the catalyst percent has a greater effect on the yield than the methanol/oil ratio. Nevertheless, at some point, increased catalyst or methanol have a negative impact on FAME yield.

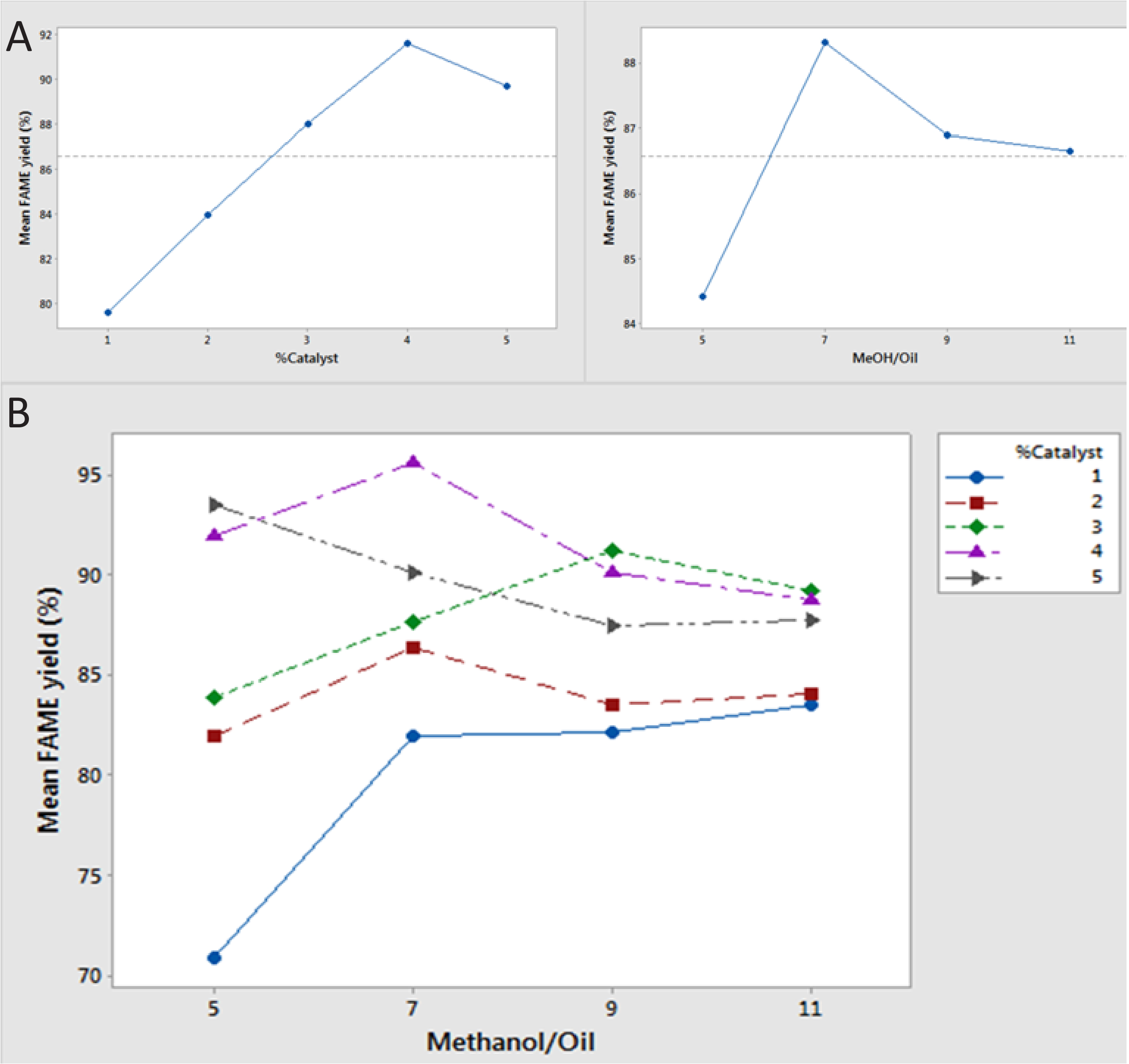

Fig. 1. Mean FAME yield. A: effect of percent CaO catalyst averaged over all values of the methanol/oil ratio (left panel); effect of the methanol/oil ratio averaged over all values of percent CaO catalyst (right panel). B: interaction effect of CaO catalyst and methanol/oil ratio.

This decrease in FAME yield may reflect the formation of soap caused by the highly basic conditions and the active site blockade of the catalyst by the formation of glycerol. The interaction between the catalyst percentage and the methanol/oil ratio (Fig.1B) shows a rapid increase in FAME yield only with 1% catalyst, a gradual increase followed by a decrease with 2%, 3 % and 4% catalyst and a decreasing trend with 5% catalyst. These trends can be interpreted as a combination of each main effect, which exhibit drops in yield above 4% catalyst and 7:1 methanol/oil ratio.

Table 3. ANOVA analysis of linear, quadratic and cubic models.

Model	**Sums of squares error**	**Fitting R^2**	**Adjusted R^2**	**Predictive R^2**	***p*-value**
Linear	407.979	0.7251	0.6736	0.5270	0.000095
Quadratic	477.577	0.8488	0.7948	0.6839	0.000026
Cubic	498.893	0.8867	0.8206	0.6506	0.000082

Table 4. Backward elimination of non-significant terms in the quadratic model.

Terms[1]	**Original**		**Modified**	
	Coefficient	***p*-value (α = 0.05)**	**Coefficient**	***p*-value (α = 0.05)**
X_1^2	-0.9259	0.0014	-0.9259	0.0200
X_2^2	-0.2597	0.0800	eliminated	eliminated
X_1	13.7328	0.0000	13.7328	<0.0001
X_2	6.4430	0.0013	2.2870	0.0020
X_1*X_2	-0.6741	0.0020	-0.6741	0.0030
Constant	38.1134	0.0020	53.4386	0.0000

[1] X_1 is CaO mass percentage to the oil, X_2 is methanol/oil molar ratio.

Because the interaction of these two factors may influence the response, the regression models developed from the data involve both factors and their interaction.The results of regression analysis for the models are presented in Table 3. Although all three regressions were highly significant, the higher the order of the regression model did not correspond to a better fit of the model. Although the values of fitting and adjusted R^2 rise, greater deviation from the experimental data mean (as indicated by the higher sums of squares error), lower predictive R^2 and *p*-value (indicating significance of the regression model) are observed in the cubic model. This phenomenon may have arisen because some other influential but unknown experimental factor was not included. Additional experiments should be conducted involving varying the reaction temperature and/or reaction time in order to investigate this further.

By considering all statistical indicators, the best fitting model is the quadratic, which has the greatest significance, highest predictive capability (predictive R^2) and proportional correlation (fitting and adjusted R^2) of the data fitting. By applying the backward elimination strategy to the quadratic model (Table 4), the sums of squares error was considerably reduced to 455.986 and the values of fitting R^2, adjusted R^2 and predictive R^2, and the *p*-value decreased slightly to 0.8104, 0.7599, 0.6606 and

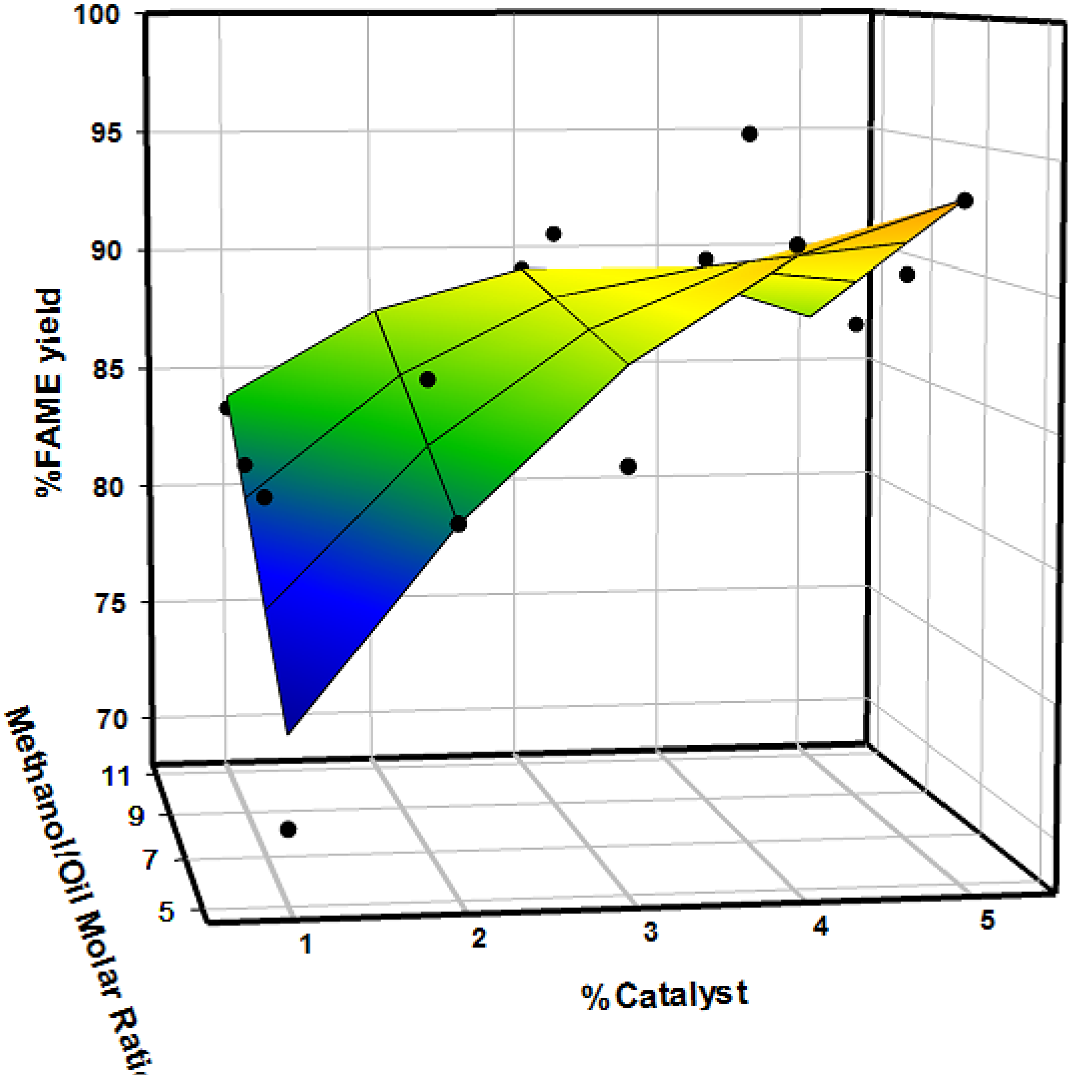

Fig. 2. The model (represented by the mesh plot) and the experimental data (represented by the scatter plot) of biodiesel production using *Pomacea* sp. shell as a catalyst.

0.0000271, respectively. This indicates that the removal of some variance, mainly from the methanol/oil molar ratio effect, reduces deviation from the data but provides only negligible improvement of the model fit.

The final model and the real experimental data are plotted together in Fig. 2. The model is:

% FAME yield = -0.9259 x % Catalyst^2 +13.7328 x % Catalyst + 2.2870 x Methanol / oil molar ratio – 0.06741 x % Catalysts x Methanol / oil molar ratio + 53.4386

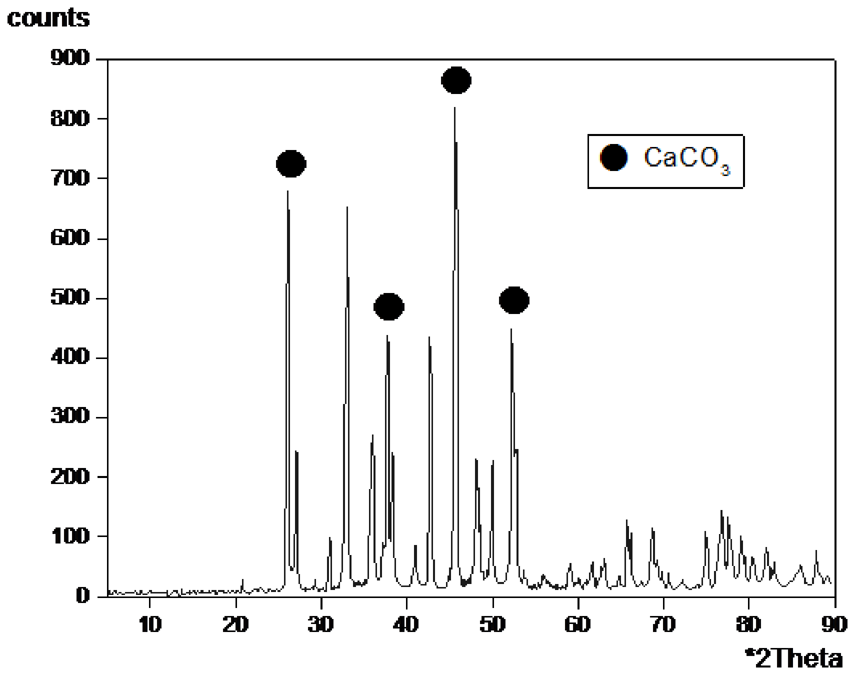

Fig. 3. X-Ray structure of apple snail shell (adapted from Margaretha *et al*., 2012).

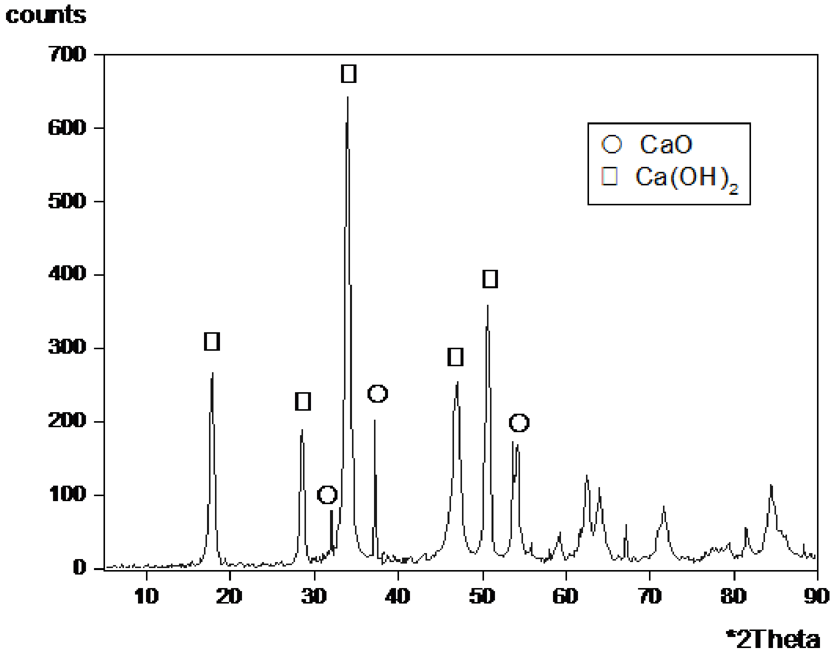

Fig. 4. X-Ray structure of CaO catalyst (adapted from Margaretha *et al*., 2012).

It is clear that the model can represent almost all the experimental data although the optimum point of the model data has shifted relative to the experimental data. The optimum conditions indicated by the model are 5% catalyst and a methanol/oil ratio of 5:1, whereas those from the real data are 4% catalyst and a methanol/oil ratio of 7:1, the difference in FAME yield percentage between the model and the data is 2.07%.

Characterization of catalyst and apple snailshell

The presence of $CaCO_3$ and CaO in the apple snail shell and the catalyst are indicated by the XRD patterns of both materials (Figs. 3 and 4). The presence of $CaCO_3$ is indicated by 2θ at 26.2, 33.1, 37.8, 45.8 and 52.4°, while CaO is indicated at 32.2, 37.3 and 53.8°. The presence of $Ca(OH)_2$ is also observed in Fig. 4, as indicated by diffraction peaks at 18.0, 28.6, 34.1, 47.0 and 50.8°. The presence of oxygen anions on the surface of the CaO results in highly basic conditions (Iizuka *et al*., 1971; Kouzu & Hidaka, 2012; Margaretha *et al*., 2012) and the exposure of this compound to ambient air results in the formation of $Ca(OH)_2$ because of the reaction of H_2O in the air with its highly basic surface (Margaretha *et al*., 2012).

Fig. 5 depicts the XRF analysis of the bulk composition of the CaO catalyst. It consists mainly of calcium oxide (96.8 %), with some other metal impurities. The FTIR spectra of both snailshell and CaO are depicted in Fig. 6. The characteristic peaks of the C=O bond stretching and bending modes of $CaCO_3$ are observed at 3117 cm^{-1}, 2513 cm^{-1}, 1420 cm^{-1} and 867 cm^{-1} for the combined shell catalyst and CO_2

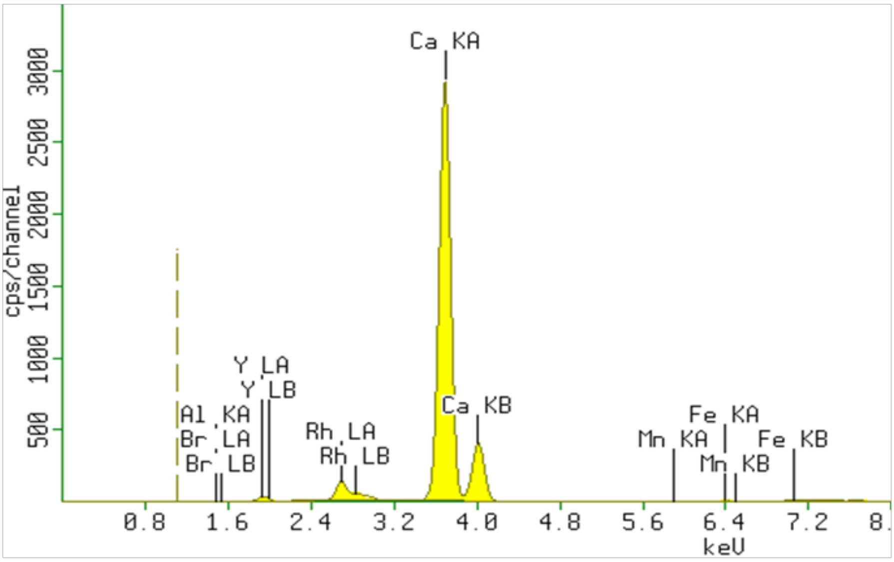

Fig. 5. XRF spectra of CaO catalyst (adapted from Margaretha *et al*., 2012).

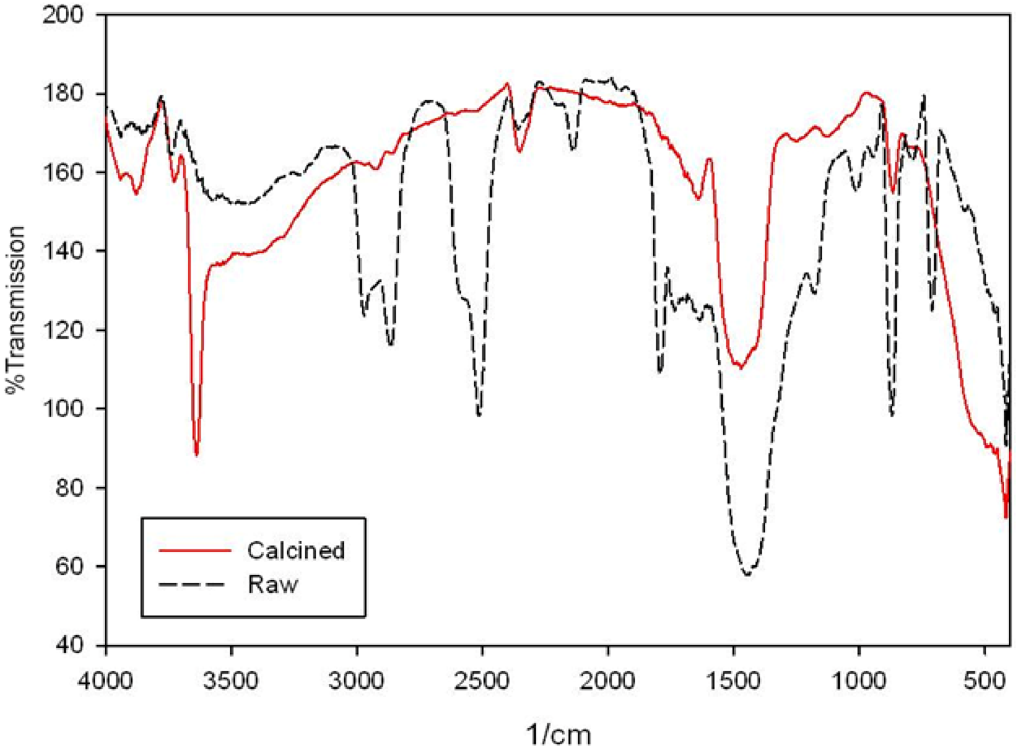

Fig. 6. FT-IR spectra of raw material and CaO catalyst (adapted from Margaretha *et al*., 2012).

adsorbed on it. During the calcination process, $CaCO_3$ is decomposed into CaO and CO_2. This is reflected by the FTIR spectra of the catalyst, which indicates the decreasing intensity of the characteristic peaks representing $CaCO_3$. After the calcination process, a new peak appears at 3620 cm^{-1}, indicating the formation of basic -OH groups attached to the calcium atoms (Margaretha *et al.*, 2012). The physical and chemical characteristics of the CaO catalyst are summarized in Table 5.

Characterization of biodiesel

Several selected physical and chemical characteristics of the biodiesel obtained in this study are summarized in Table 6 and are inaccordance with the values of the Indonesia National Standard (SNI-04-7182-2006) and the ASTM standard ((B100)-ASTM D6751-07b) for biodiesel.

Table 5. Physical and chemical characteristics of CaO catalyst (adapted from Margaretha *et al*., 2012).

Properties	**Value**
BET surface area, m^2/g	17
Pore volume, cm^3/g	0.04
Mean pore size, nm	3.2
Particle size, mesh	80/100
CaO content, %	96.83
SiO_2, %	0.60
FeO, %	0.42
Other chemical components, %	2.15

Table 6. Comparison of the properties of biodiesel produced from palm oil with catalyst from *Pomacea* sp.shell in this study with the Indonesia National Standard (SNI-04-7182-2006) and ASTM standard (B100)-ASTM D6751-07b) for biodiesel.

Properties	**Biodiesel produced in this study**	**SNI**	**ASTM**
Density at 15°C, g/mL	0.88 ± 0.07	0.85-0.89	-
Kinematic viscosity at 40°C, cSt	3.8 ± 0.2	2.3-6.0	1.9-6.0
Flash point, °C	164 ± 2.2	100 Min	93 Min
Cetane number	58 ± 1.5	51 Min	47 Min
Acid number mg KOH/g	0.42 ± 0.04	0.8 Max	0.5 Max
Water & sediment, % vol	0.02 ± 0.005	0.05 Max	0.05 Max

Conclusion

Apple snail (*Pomacea* sp.) shell has potential as a catalyst for biodiesel production, provided population growth and expansion of the distribution of the snails is prevented so as not to increase the already serious impacts the snails have on rice production.

Acknowledgments

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References

Agrawal, S., Singh, B.& Sharma, Y.C. 2012. Exoskeleton of a mollusk (*Pila globosa*) as a heterogeneous catalyst for synthesis of biodiesel using used frying oil. *Industrial and Engineering Chemistry Research* 51: 11875-11880.

Birla, A., Singh, B., Upadhyay, S.N. & Sharma, Y.C. 2012. Kinetic studies of synthesis of biodiesel from waste frying oil using a heterogeneous catalyst derived from snail shell. *Bioresource Technology* 106: 95-100.

Boey, P.L., Ganesan, S., Maniam, G.P. & Khairuddean, M. 2012. Catalysts derived from waste sources in the production of biodiesel from waste cooking oil. *Catalysis Today* 190: 117-121.

Boro, J., Thakur, A.J.& Deka, D. 2011. Solid oxide derived from waste shells of *Turbonilla striatula* as a renewable catalyst for biodiesel production. *Fuel Processing Technology* 92: 2061-2067.

Brunauer, S., Emmett, P.H.& Teller, E. 1938. Adsorption of gases in multimolecular layers. *Journal of the American Chemical Society* 60: 309–319.

Demirbas, A. 2009. Progress and recent trends in biodiesel fuels. *Energy Conversion and Management* 50: 14-34.

Garcia-Sancho, C., Moreno-Tost, R., Merida-Robles, J.M., Santamaria-Gonzalez, J., Jimenez-Lopez, A. & Maireles-Torres, P. 2011. Niobium-containing MCM-41 silica catalysts for biodiesel production. *Applied Catalysis B: Environmental* 108-109: 161-167.

Ghiaci, M., Aghabarari, B.& Gil, A. 2011. Production of biodiesel by esterification of natural fatty acids over modified organoclay catalysts. *Fuel* 90: 3382-3389.

Gui, M.M., Lee, K.T. & Bhatia, S. 2008. Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. *Energy*: 1646-1653.

Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming system: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Iizuka, T., Hattori, H., Ohno, Y., Sohma, J.& Tanabe, K. 1971. Basic sites and reducing sites of calcium oxide and their catalytic activities. *Journal of Catalysis* 22:130-139.

Jairam, S., Kolar, P., Sharma-Shivappa, R., Osborne, J.A.& Davis, J.P. 2012. KI impregnated oyster shell a solid catalyst for soybean oil transesterification. *Bioresource Technology* 104: 329-335.

Kouzu, M.& Hidaka, J.2012. Transesterification of vegetable oil into biodiesel catalyzed by CaO: a review. *Fuel* 93 : 1-12.

Kouzu, M., Kasuno, T., Tajika, M., Sugimoto, Y., Yamanaka, S. & Hidaka, J.2008. Calcium oxide as a solid base catalyst for transesterification of soybean oil and its application to biodiesel production. *Fuel* 87: 2798-2806.

Kusuma, R.I., Hadinoto, J.P., Ayucitra, A., Soetaredjo, F.E. & Ismadji, S. 2013. Natural zeolite from Pacitan Indonesia, as catalyst support for transesterification of palm oil. *Applied Clay Science* 74: 121-126.

Li, Y., Lian, S., Tong, D., Song, R., Yang, W., Fan, Y., Qing, R. & Hu, C. 2011. One-step production of biodiesel from *Nannochloropsis* sp. on solid base Mg-Zr catalyst. *Applied Energy* 88: 3313-3317.

Liu, X., He, H., Wang, Y., Zhu, S.& Piao, X. 2008. Transesterification of soybean oil to biodiesel using CaO as a solid base catalyst. *Fuel* 88: 216-221.

Margaretha, Y.Y., Prastyo, H.S., Ayucitra, A. & Ismadji, S. 2012. Calcium oxide from *Pomacea* sp. shell as a catalyst for biodiesel production. *International Journal of Energy and Environmental Engineering* 3: 33 (9 p.).

Quintella, S.A., Saboya, R.M.A., Salmin, D.C., Novaes, D.S., Araujo, A.S., Albuquerque, M.C.G. & Cavalcante, C.L., Jr. 2012. Transesterification of soybean oil using ethanol and mesoporous silica catalyst. *Renewable Energy* 38: 136-140.

Ramachandran, K., Sivakumar, P., Suganya, T. & Renganathan, S. 2011. Production of biodiesel from mixed waste vegetable oil using an aluminium hydrogen sulphate as a heterogeneous catalyst. *Bioresource Technology* 102: 7289-7293.

Suryaputra, W., Winata, I., Indraswati, N. & Ismadji, S. 2013. Waste capiz (*Amusium cristatum*) shell as a new heterogeneous catalyst for biodiesel production. *Renewable Energy* 50:795-799.

Tang, Y., Meng, M., Zhang, J. & Lu, Y. 2011. Efficient preparation of biodiesel from rapeseed oil over modified CaO. *Applied Energy* 88: 2735-2739.

Taufiq-Yap, Y.H., Lee, H.V. & Lau, P.L.2012. Transesterification of *Jatropha curcas* oil to biodiesel by using short necked clam (*Orbicularia orbiculata*) shell derived catalyst. *Energy Exploration and Exploitation* 30: 853-866.

Viriya-empikul, N., Krasae, P., Puttasawat, B., Yoosuk, B., Chollacoop N. & Faungnawakij, K. 2010. Waste shells of mollusk and egg as biodiesel production catalysts. *Bioresource Technology* 101: 3765-3767.

Xie, W.& Wang, T. 2013. Biodiesel production from soybean oil transesterification using tin-oxide-supported WO_3 catalysts. *Fuel Processing Technology* 109: 150-155.

Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M. & Chen, Y. 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresource Technology*102: 159-165.

Zhang, Y. & Liu, S. 2013. Solid alkali catalyst of mussel shell in producing biodiesel. *Advanced Materials Research* 608-609: 206-209.

Apple snails as animal feed

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Abstract

Apple snails potentially constitute a valuable source of food in Southeast Asia for poultry, ducks, pigs, fish, prawns and frogs for human consumption. Ducks will eat live snails, including their shells. However, for other livestock species, snail meal without the shells, created via silage, must be used. In general, only a portion of the normal diet should be replaced by snail meal because of the possible lower protein content and poorer amino acid profile compared to the normal high quality fish meal diet. The potential for snail meal to contain contaminants and pathogens may mean that sanitary procedures should be implemented. Evaluations of the economics of using snail meal as well as the performance of livestock fed on snail meal are needed.

Additional keywords: Ampullariidae, crustaceans, ducks, fish, pigs, *Pomacea*, poultry

Introduction

The apple snail *Pomacea canaliculata* was introduced to Taiwan in 1979-1980 as a new human food resource and spread rapidly through much of Southeast Asia in the 1980s (Mochida, 1991). It is now known that an additional species, *Pomacea maculata*, was also introduced and has become widespread, though for many years the two were rarely distinguished (Hayes *et al*., 2008, 2015). However, efforts to add these snails to the local gastronomy of Asian countries eventually failed. Nonetheless, apple snails constitute valuable biomass; they can be collected and processed into a good source of protein that can be used to replace other protein sources, notably fish meal, in the diets of various farmed species, including poultry, ducks, pigs, fish and crustaceans.

Distribution and processing

Apple snails can be fed live, freshly dead or processed. Live snails are eaten directly by fish and ducks in ponds and rice fields. Collected snails can be fed whole or without shells (snail meat). Whole snails or snail meat is fed fresh or ensiled, cooked and/or dried. Snail shells can be a source of minerals similar to oyster shells. A kilogramme of whole snails, when washed and unshelled, yields about 250 g of fresh snail meat and 100 g of dried snail meat (Ulep & Buenafe, 1991). Because fresh snail meat spoils easily, cooking, drying or ensiling should be considered whenever the snails cannot be eaten immediately.

Live snails and fresh snail meat

Live apple snails are a good feed for ducks, which are used in rice fields for natural apple snail population control (Pantua *et al*., 1992). Fish such as carp and tilapia have been tested as potential control agents (Caguan & Joshi, 2002; Halwart, 2006).

Fresh snail meat can be prepared as follows: the snails are cleaned, washed and crushed and the shells are separated from the meat (Salazar *et al*., 2003). A preliminary step may involve purging the snails for two days (Ulep & Santos, 1995).

Snail meal

Various methods for producing snail meal have been described in the literature (Table 1). Some authors recommend boiling the snails first in order to kill them, to

Table 1. Methods for preparing snail meal.

Method	Country	Reference
Whole snails boiled for 2 min, meat extracted and chopped into 1cm cubes	Philippines	Bombeo-Tuburan *et al*., 1995
Whole snails boiled for 5 min, meat extracted, sun-dried for 2-3 days, ground	Philippines	Ulep & Buenafe, 1991
Whole snails boiled for 5-15 min, meat extracted, oven-dried at 70°C for 3 days, ground	Philippines	Barcelo & Barcelo, 1991
Whole snails boiled at 100°C for 15 min, meat extracted, sun-dried until the meat contains less than 10% water, ground	Indonesia	Usman *et al*., 2007
Snail meat cooked for 30 min at 60°C	Indonesia	Firdus & Muchlisin, 2005
No boiling, meat extracted, cleaned with freshwater, sun-dried for 3 days, not ground	Vietnam	Chau Thi Da *et al*., 2012

remove pathogens and to facilitate the separation of the meat from the shell (Ulep & Buenafe, 1991).The resulting snail meat is sun-dried or oven-dried and then ground.

It is important to note that while apple snails are numerous, purchasing snail meat can be expensive because the price includes the labour costsof removing the shells and chopping. In Laos, this cost was estimated at 27% of the total cost of pig diets. Farmers who collect and process the snails themselves may get a more direct economic benefit than those who buy snail meat (Kaensombath & Ogle, 2005a).

In the papers cited in the rest of this contribution, apple snail meal refers to snail meat and does not include the shells unless otherwise stated.

Ensiled snails

Making silage from apple snail meat has been described as a low cost and simple method for small-scale production (Kaensombath, 2005). Because snail meat contains mostly protein and minerals, the addition of a source of carbohydrates such as molasses or rice bran is required for making good silage. Table 2 summarizes different methods for ensiling snail meat.

Chemical composition

Table 3 summarizes the composition and gross energy values of apple snail products reported in the literature. Whole snails are relatively poor in protein, about 14-18% dry matter basis (DM), particularly rich in calcium (28-31% DM) and poor in phosphorus

Table 2. Methods for preparing snail meat silage. All experiments took place in Thailand.

Snail preparation	Silage additive and ensiling method	Reference
Meat extracted, washed with clean water and drained, chopped into small pieces of 0.5-1.0 cm.	• Molasses and rice bran (1:9 fresh basis) • 1 kg molasses/bran for 1, 2 or 3 kg snail meat • Stored for 24 weeks • 1:2 additive:snail ratio preferable	Kaensombath & Ogle, 2005b
Whole snails boiled in water for 2 min, meat extracted, minced and blanched for 5 min	• Molasses with inoculum of lactic acid bacteria • 0.15 l molasses for 1 kg snail meat • Stored for 15 days • pH dropped from 8 to 4-5	Rattanaporn et al., 2006
Snail meat cleaned, chopped and ground	• 5% citric acid or 20% molasses (DM basis) • Stored 28 days in sealed plastic containers • Only the addition of 15-20% molasses resulted in acceptable silage with a brownish colour and a pleasant smell. Snails ensiled without additive or with citric acid deteriorated rapidly.	Phonekhampheng et al., 2009

(<0.5% DM). At the time of writing, no data were available on apple snail minerals other than calcium and phosphorus.

Snail meat (without shells) contains 52-63% protein (DM). It is similar to a fish meal of moderate quality. Ash content varies between 11 and 27% DM, and depends on the amount of residual shell material included. Snail meat contains about 3-4% calcium and 0.4-1.2% phosphorus. Fat content is generally less than 5%, much less than that of a typical fish meal (about 7-14%). Shells are mostly mineral matter and contain about 35% calcium with minimum amounts of residual protein.

There are few published amino acid profiles of apple snailproteins (Table 4). Unfortunately, theseprofiles are not very consistent with each other. The reported lysine content of the protein, for instance, ranges from 2.9 to 9.7% of crude protein, a range that is abnormally large for animal products. This could be explained by problems in analysing this unconventional material, or by differences in the tissue composition of the snail meat, which may include different proportions of muscle and viscera. The protein

Table 3. Composition of apple snail products.[1]

Product	Country	DM	CP	CF	Fat	Ash	Ca	P	GE	Reference
Whole snails, uncooked, dried	Philippines	89.9	17.2	3.5	0.6		28.6	0.26	2.8	Catalma *et al*., 1991a
Whole snails, cooked, dried	Philippines	90.3	14.6	3.4	0.9		30.9	0.30	2.5	Catalma *et al*., 1991a
Snail meat, fresh	Thailand	18.1	62.1			14.9				Kaensombath & Ogle, 2005b
Snail meat, fresh	Philippines		53.3	2.5	1.1	26.6	7.2	0.59		Salazar *et al*., 2003
Snail meat, uncooked, dried and ground	Philippines		53.2				6.0	0.49		Barcelo & Barcelo, 1991
Snail meat, boiled, dried and ground	Philippines		52.3				6.5	0.41		Barcelo& Barcelo., 1991
Snail meat, boiled, dried and ground	Philippines		54.3	2.0	3.7	21.9	6.2	1.20		Bombeo-Tuburan *et al*., 1995
Snail meat, boiled, dried and ground	Indonesia		56.9	2.8	5.2	11.2				Usman *et al*., 2007
Snail meat meal	Philippines	86.1	62.5	4.7	3.5		3.4	1.22	14.0	Catalma *et al*., 1991a
Snail meat, sun-dried	Vietnam		56.4	1.0	1.6	11.8			12.3	Da *et al*., 2012
Snail meat, ensiled, 1:1 molasses/rice bran:snail, 24 weeks	Thailand	49.6	17.9			6.6				Kaensombath & Ogle, 2005b
Snail meat, ensiled, 1:2 molasses/rice bran:snail, 24 weeks	Thailand	44.1	35.4			5.8				Kaensombath & Ogle, 2005b
Snail meat, ensiled, 1:3 molasses/rice bran:snail, 24 weeks	Thailand	35.8	43.5			7.8				Kaensombath & Ogle, 2005b
Snail shell meal	Philippines	98.6	4.3	3.0	0.5		35.1	0.01		Catalma *et al*., 1991a

[1] DM, dry matter (%); CP, crude protein (% DM); CF, crude fiber (% DM); Ca, calcium (% DM); P, phosphorus (% DM); GE, gross energy (MJ/kg DM). Note that fat was called "ether extract" by Catalma *et al*. (1991a).

content could also be influenced by the presence of undigested matter in the gut. Until more data are available, it is therefore difficult to draw conclusions about the suitability of apple snail protein for animal feeding.

Apple snails for poultry

Snail meal is a suitable substitute for more traditional protein sources in poultry diets. It can usually be added at 10-15% (diet DM).In chicks, feeding 10% of uncooked snail

Table 4. Amino acid composition of apple snail meat as % of crude protein.

Amino acid	**Whole snails**[1]	**Snail meat meal**[2]	**Snail meat meal**[3]	**Snail meat meal**[4]	**Fish meal**[5]	**Soybean meal**[6]
Alanine		6.1	5.5		6.1	4.4
Arginine	6.9	6.6	5.7	6.4	5.8	7.4
Aspartic acid		9.3	6.2		8.7	11.3
Cystine	1.4	trace			0.8	1.5
Glutamic acid		13.6	12.2		12.6	17.7
Glycine		5.5	4.3		5.9	4.2
Histidine	2.4	1.6	2.1	1.8	2.2	2.6
Isoleucine	9.1	3.2	3.3	4.6	4.3	4.6
Leucine	7.2	7.0	6.4	7.6	7.0	7.5
Lysine	3.5	9.7	2.9	5.5	7.5	6.1
Methionine	2.4	2.1	1.9		2.8	1.4
Methionine+cystine	3.8	2.1		0.6		
Phenylalanine	5.1	1.4	2.9	3.8	3.8	5
Proline		3.7	2.9		3.8	4.9
Serine		4.3	3.2		4.0	5
Threonine	4.8	4.0	2.0	4.1	4.1	3.9
Tryptophan		4.0			1.1	1.3
Tyrosine	4.4	1.9	3.2		2.9	3.5
Valine	7.0	3.8	3.7	4.6	4.9	4.8

[1]Cruz, 1997; [2]Bombeo-Tuburan *et al.*, 1995; [3]Da *et al.*, 2012; [4]Usman *et al.*, 2007; [5]Heuzé *et al.*, 2011; [6]Heuzé *et al.*, 2012

meal resulted in a 31% increase in total gain in weight and 35% improvement in feed efficiency compared to the control diet (Catalma *et al.*, 1991b).

In the Philippines, in broilers fed 12% cooked or raw snail meal, cooking improved the feed conversion ratio and the palatability of snail meal. Cooked snail meal (Table 5) led to better performance than raw snail meal and to slightly lower performance than the fish mealbased control diet (Barcelo & Barcelo, 1991). Snail meal fed at 4, 8, 12% levels in broiler diets replaced fish and meat and bone meals with good results, also in the Philippines (Ulep & Buenafe, 1991).

In the Philippines, studies with laying hens have produced contradictory results. Crushed snails given to White Leghorn layers as a supplement (20 g/bird/day) to a commercial mash resulted in 88% mean hen-day egg production rate compared to 84% without the supplement (Ancheta, 1990). Also in the Philippines, ground snail meal included at 11% or 25% in layer diets (i.e. diets for laying hens) resulted in lower hen-day egg production than for the control diet rate (72% and 84% respectively). However,

Table 5. Example of broiler diet based on snail meat meal (from Barcelo & Barcelo, 1991).

Ingredient	% as fed
Snail meat, cooked and dried	12.12
Yellow maize	70.00
Rice bran	9.10
Premix	0.23
Sodium chloride	0.25

feed intake, feed conversion, shell thickness and albumen weight were not affected and feeding snail meal to layers resulted in a higher value of eggs (Catalma *et al*., 1991b).

Apple snails for ducks

Ducks are commonly used for biological control of apple snails in paddies and taro patches. Both Mallards (*Anas platyrhynchos*) and Muscovy ducks (*Cairina moschata*) are used although the former is preferred by farmers (Serra, 1997). Ducks can be introduced to the fields after harvest and be removed from it before rice transplanting, or they can be introduced to the paddies three weeks after transplanting. Duck stocking rates suggested for snail control are highly variable, ranging from 5-10 ducks/ha in Malaysia (Teo, 2001) to 200, 400 and even 900 ducks/ha in the Philippines (Pantua *et al*., 1992; Vega, 1991;Cagauan, 1999; Cagauan & Joshi, 2002a). In China, young ducks readily eat young snails (hatchlings and juveniles) weighing less than 1.5 g, while 60 day old ducks are the main predators of older and adult snails (1.5 g to over 6.5 g) (Liang *et al*., 2013).

In experiments in the Philippines, Pekin ducks were fed fresh apple snail meat and fresh banana peels (1:1) replacing 50%, 70% or 90% of a commercial mash.The diet consisting of 45% banana peels, 45% snail meat and 10% commercial mash gave the best performance and yielded the highest profit (Ulep & Santos, 1995).

In the Philippines, laying Mallards fed fresh and crushed snails mixed with rice bran and broken maize grains at a ratio of 1.1:1 exhibited a 60-70% egg production rate in the Philippines (Tacio, 1987), while feeding *ad libitum* fresh snails and small amounts of rough rice resulted in a 68% egg production rate (Aquino, 1990). Use of a 2:1 ratio of fresh snails and rice bran has also been reported (Serrano, 1988). Mallards can be fed economically on a 50:50 mixture of apple snails and rice bran, and although the ducks fed the snail and bran diet had a lower final body weight and feed efficiency than ducks fed on commercial diets, economic returns were higher (PCARRD, 2006). The combination of snails and commercial duck layer feeds at a ratio of 1:1.3 resulted in optimum egg production rate and low production cost (Datuin *et al*., 1990).

Apple snails for pigs

Whole and uncooked apple snail meal (with shells) introduced at 15%in the diet of growing pigs in the Philippines resulted in performance (average daily gain, feed conversion ratio) similar to that obtained with a commercial mash (Catalma *et al*., 1991a), but in another trial, whole snail meal at 50% had no effect on performance (Garcia, 2010).

Fresh apple snails could replace 37.5% and 60% of the soybean meal in the commercial grower and finisher diets, respectively (Salazar *et al*., 2003; Table 6).

Fresh or ensiled golden apple snail meat has been used to replace fish meal in growing (30-70 kg) pig diets (Table 7). In a trial in Laos, the apparent digestibilities of crude protein and dry matter were about 81-83% and 55-59% respectively (Kaensombath & Ogle, 2005c). In a feeding trial, total replacement of fish meal with fresh snail meat (9% and 5.5% of diet DM for 30-50 kg and 50-70 kg pigs, respectively) or ensiled snail meat (15.5% and 9.5% for 30-50 kg and 50-70 kg pigs, respectively) reduced diet DM intake, perhaps because of the high moisture content of the snails, but did not alter daily weight gain and feed conversion ratio. It was concluded that replacing fish meal with

Table 6. Example of growing and finishing pig diets based on fresh snail meat (from Salazar *et al*., 2003).

Ingredient	**% as fed**	
	Grower	**Finisher**
Snail meat	6.00	6.00
Soybean meal	10.00	4.00
Yellow corn	40.00	40.00
Rice bran	40.88	47.63
Fish meal	1.00	-
Oyster shell	1.75	2.00
Salt	0.25	0.25
Premix	0.10	0.10
Amino acids	0.02	0.02

Table 7. Example of growing (30-50 kg and 50-70 kg pigs) pig diets based on fresh or ensiled snail meat (from Kaensombath & Ogle, 2005c).

Ingredient	% DM			
	Fresh snail meat		Ensiled snail meat	
	30-50 kg	50-70 kg	30-50 kg	50-70 kg
Snail meat	9.0	5.5	15.5	9.5
Broken rice	30.6	33.5	21.5	25.0
Rice bran	27.4	27.0	33.0	31.5
Maize	32.0	33.0	29.0	33.0
Salt	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5

fresh or ensiled snail meat could be economically effective for pig production and could increase rice yields in the fields (Kaensombath & Ogle, 2005a).

Apple snails for fish

Fish may help control apple snails (Cagauan &Joshi, 2002b). Common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) have been assessed as potential biocontrol agents. Common carp were more efficient predators, as they could prey on snails up to 12 mm in shell height while Nile tilapia did not prey on snails with shell height exceeding 4 mm (Halwart *et al*., 1998).

Good results have been obtained in several fish species raised for human consumption by replacing fish meal with snail meal. In Nile tilapia, comparison of diets containing various proportions of snail meal, rice bran and fish meal showed that higher growth rates were obtained in diets containing 100% snail meal or 75% snail meal and 25% rice bran. However, snail meal resulted in lower growth performance than fish meal when it was included at the level of 25% (Cagauan & Doria, 1989). In sex-reversed red tilapia (*O. niloticus* x *O. mossambicus*), minced snail meal could replace 50% of fish meal protein, whereas fermented snail meal (as proposed by Rattanaporn *et al.*, 2006, see Table 2) could replace up to 100% fish meal protein, though 75% has been recommended (Chimsung & Tantikitti, 2014).

In striped catfish (*Pangasianodon hypophthalmus*) fingerlings, apple snail meal could entirely replace fish meal (Table 8) without negative effects on feed intake, feed and protein utilisation and survival rate. Daily weight gain and specific growth rate of fingerlings in Vietnam did not differ from the control diet (Da *et al*., 2012). In African catfish (*Clarias gariepinus*), snail meal ensiled with molasses could replace 100% of the fish meal in the diet (20-27% of the total diet DM; Table 9) without affecting growth performance and feed utilisation (Phonekhampheng *et al*., 2009).

In tiger grouper (*Epinephelus fuscoguttatus*), apple snail meal could be used up to 20% (DM basis) without affecting performance. Higher inclusion rates resulted in reduced growth and survival (Usman *et al*., 2007). Feeding cultured grouper (*Epinephelus tauvina*) with 100% apple snails (fresh, cooked or a 50:50 mixture) resulted in lower survival than with a fish meal diet, though the highest growth among the snail-only diets was obtained with a 50:50 mixture of fresh and cooked snails (Firdus & Muchlisin, 2005).

Table 8. Example of striped catfish diet based on snail meat meal (from Da *et al*., 2012).

Ingredient	% as fed
Snail meat meal	24
Commercial catfish diet	48
Wheat flour	22
Squid liver oil	2
Premix	2
Carboxylmethyl cellulose	2

Table 9. Example of African catfish diet based on ensiled snail meat from (Phonekhampheng *et al*., 2009).

Ingredient	% DM
Snail meat ensiled with 20% molasses	27.1
Rice bran	71.4
Premix	1.0
Carboxymethyl cellulose	0.5

In seabass (*Lates calcarifer*), the replacement of fish meal by up to 25% of apple snail meal was found acceptable. Higher replacement rates decreased digestibility and performance (Hanafi, 2003).

Apple snails for prawns

Annually, some 20 to 25 tonnes of snails are collected and used as feed in giant freshwater prawn (*Macrobrachium rosenbergi*) farming in the Mekong Delta in Vietnam (Hasan& Halwart, 2009). In Thailand and with the same prawn species, apple snail meal could successfully replace 25% of the fish meal, with a maximum substitution rate of

50%; 100% replacement reduced performance (Jintasataporn *et al*., 2004, Table 10). In the Philippines, feeding giant tiger prawns (*Penaeus monodon*) with cooked snail meat and cooked cassava chips or maize grain (60:40 based on fresh weight) yielded the highest net income compared with maize or snails alone (Bombeo-Tuburan *et al*., 1995).

Apple snails for frogs

Apple snail meal could replace 50% of fish meal protein in the diets of young Chinese edible frogs (*Hoplobatrachus rugulosus*) and up to 100% of protein in grower frog diets in Thailand (Vongvichitch, 2006).

Table 10. Example of giant freshwater prawn diet based on snail meat meal(from Jintasataporn *et al*., 2004).

Ingredient	% as fed
Snail meat meal	8.8
Fish meal	22.7
Soybeal meal	35.0
Shrimp meal	4.0
Tuna oil	3.0
Cassava flour	19.0
Soybean oil	4.0
Cholesterol and lecithin	1.0
Premix	1.6
Dicalcium phosphate	1.1

Potential constraints

Concentration of contaminants

Apple snails may concentrate dangerous pollutants from freshwater bodies, such as mercury, arsenic and uranium, in their midgut, kidney and foot. They are thus considered good bio-indicators for water contamination but unrestricted feeding by humans and animals might be considered with caution (Vega *et al*., 2012).

Disease reservoirs

Apple snails are potential reservoirs of diseases (Hayes *et al*., 2015) and it has been recommended that snails intended for human food be thoroughly cooked. Apple snails are intermediate hosts of the rat lungworm (*Angiostrongylus cantonensis*), a parasite that can cause eosinophilic meningitis and meningoencephalitis in humans (Chao *et al*., 1987; Lv *et al*., 2011), in severe cases leading to paralysis and death (Murphy & Johnson, 2013). The snails may also host trematodes that cause skin irritations (e.g. Keawjam *et al*., 1993) and others that cause intestinal tract problems (Hayes *et al*., 2015).

Conclusion

Biomass derived from apple snails is a valuable substitute for more traditional protein sources for poultry, pigs, fish and prawns raised for human consumption. Snail meat is rich in protein and similar to fish meal of moderate quality. Feeding ducks with live snails or fresh snail meat seems particularly efficient from both a nutritional and an economic perspective. In other livestock species, snail meat can usually replace a portion of the fish meal. However, full substitution is not generally advisable, possibly because of the lower protein content and (possibly) poorer amino acid profile of snail meat compared to good quality fish meal. The potential presence of contaminants and pathogens may be a concern and require sanitary control procedures. Due to the variability of snail products, analysis of locally available snail meat is recommended. An economic evaluation of feeding snails is also necessary, since the benefits of using snails depend on their price, which may include the labour cost (collection, cooking, shell removal) when snails are purchased, on the price of competing protein sources (fish meal or meat and bone meal) and on the expected performance of animals fed on snail-based diets.

References

Ancheta, D.A. 1990. *Preliminary Study on Crushed Fresh Golden Snail (*Pomacea *sp.) as a Feed Supplement for Laying Hens*. Unpublished undergraduate thesis. Central Luzon State University, Muñoz, Nueva Ecija, Philippines.

Aquino, R.R. 1990. Golden snail, a major rice pest in the Philippines, is a potential feed for laying ducks. In: *Proceedings of the 5th Asian-Australian Association of Animal Production Society (AAAP) Animal Science Congress, May 27-June 1, 1990, Taipei, Taiwan, Republic of China, Vol. 3*, p. 25.

Barcelo, P.M.& Barcelo, J.R. 1991. The potential of snail (*Pila leopoldvillensis*) meal as protein supplement in broiler diet. *Tropicultura* 9: 11-13.

Bombeo-Tuburan, I., Fukumoto, S.& Rodriguez, E.M. 1995. Use of the golden apple snail, cassava, and maize as feeds for the tiger shrimp, *Penaeus monodon*, in ponds. *Aquaculture* 131: 91-100.

Cagauan, A.G.& Doria, L.S. 1989. Golden snail *Pomacea insularis* (?) *canalicuta*(?) meal as feed for Nile tilapia *Oreochromis niloticus* fingerlings in aquaria. *CLSU Scientific Journal* 9: 24-29.

Cagauan, A.G.& Joshi, R.C. 2002a. Golden apple snail *Pomacea* spp. in the Philippines. In: *Proceedings of the Special Working Group on the Golden Apple Snail (Pomacea spp.) at the Seventh International Congress on Medical and Applied Malacology (7th ICMAM) Los Banos, Laguna, SEAMEO Regional Center for Graduate Study and Research in Agriculture (SEARCA), Philippines* (Wada, T., Yusa, Y. & Joshi, R.C., ed.), p. 1-38. SEAMEO Regional Center for Graduate Study and Research in Agriculture. Los Banos

Cagauan, A.G.& Joshi, R.C. 2002b. Predation of freshwater fish on golden apple snail, *Pomacea canaliculata* Lam., under screenhouse conditions. *International Rice Research Notes* 27(2): 24-26.

Cagauan, A.G. 1999. *Production, Economics and Ecological Effects of Nile Tilapia (*Oreochromis niloticus *L.), a Hybrid Aquatic Fern Azolla (*Azolla microphylla *Kaulf. x* A. filiculoides *Lam.) and Mallard Duck (*Anas platyrhynchos *L.) in Integrated Lowland Irrigated Rice-Based Farming Systems in the Philippines*. Ph.D. dissertation. Institut des Sciences Naturelles Appliquées, Université Catholique de Louvain, Louvain-la-Neuve, Belgium.

Catalma, M.T.E., Capil, D.T., Antalan, R.A., Serra, A.B., Barroga, A.J.& Orden, E.A. 1991a. Golden snail (*Pomacea* sp.) use in animal feeds. *International Rice Research Newsletter* 16(6): 26-27.

Catalma, M.T., Serra, A.B., Orden, E.A., de Guia, A.S., Martin, E.A., Laureta, M.S., Rafael, E.J., Antalan, R.& Barroga, X.L. 1991b. Feeding value of golden snail (*Pomacea* sp.) meal for poultry and swine. In: *Proceedings of the 28th Philippine Society of Animal Science Annual Meeting, 21-22 November 1991*, p. 33-34. Philippine Society of Animal Science, Manila.

Chao, D., Lin, C.C.& Chen, Y.A. 1987. Studies on growth and distribution of *Angiostrongylus cantonensis* larvae in *Ampullarium canaliculatus* [sic]. *Southeast Asian Journal of Tropical Medicine and Public Health* 18: 248-252.

Chimsung, N.&Tantikitti, C. 2014. Fermented golden apple snails as an alternative protein source in sex-reversed red tilapia (*Oreochromis niloticus * O. mossambicus*) diets. *Walailak Journal of Science and Technology* 11: 41-49.

Cruz, P.S. 1997. *Aquaculture Feed and Fertilizer Resource Atlas of the Philippines. FAO Fisheries Technical Paper 366*. FAO, Rome.

Da, C.T., Lundh, T. & Lindberg, J.E. 2012. Evaluation of local feed resources as alternatives to fish meal in terms of growth performance, feed utilisation and biological indices of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. *Aquaculture* 364-365: 150-156.

Datuin, L.M., Aquino, L.T., Tucay, R.A.& Cayabyab, R.C. 1990. Utilization of golden kuhol on the egg production performance of Mallard duck. *Philippine Journal of Veterinary and Animal Sciences* 17: 65.

Firdus Muchlisin, Z.A. 2005. The possibility of golden snail (*Pomacea canaliculata*) as an alternative feed for cultured grouper (*Epinephelus tauvina*). *Enviro* 5: 64-66.

Garcia, J.P.S. 2010. *The Effect of Dried Pulverized Shell and Meat of Golden Apple Snail (Pomacea canaliculata) as a Supplementary Feed on the Growth of Swine (*Sus scrofadomesticus*)*. Unpublished report. Department of Science and Technology, Region III, Central Luzon, Philippines.

Halwart, M., Viray, M.C.& Kaule, G. 1998. *Cyprinus carpio* and *Oreochromis niloticus* as biological control agents of the golden apple snail *Pomacea canaliculata* – effects of predator size, prey size and prey density. *Asian Fisheries Science* 11:31-42.

Halwart, M., 2006. Fish as biological control agents of golden apple snails in Philippine rice fields. In: *Global advances in ecology and management of golden apple snails* (Joshi, R.C.& Sebastian, L.S.,ed.), p. 363-374. Philippine Rice Research Institute, Nueva Ecija, Philippines.

Hanafi, M. 2003. *Replacement of Fish Meal with Golden Apple Snail Meal in Artificial Feed for Seabass (*Lates calcarifer *Bloch)*. M.Sc. thesis, Kasetsart University, Bangkok.

Hasan, M.R.& Halwart, M. 2009. *Fish as Feed Inputs for Aquaculture: Practices, Sustainability and Implications*. *FAO Fisheries and Aquaculture Technical Paper No. 518*. FAO, Rome.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14(4): 701-712.

Hayes, K.A., Burks, R.L., Castro-Vazquez, A., Darby, P.C., Heras, H., Martín, P.R., Qiu, J.W., Thiengo, S.C., Vega, I.A., Wada, T., Yusa, Y., Burela, S., Cadierno, M.P., Cueto, J.A., Dellagnola, F.A., Dreon, M.S., Frassa, M.V., Giraud-Billoud, M., Godoy, M.S., Ituarte, S., Koch, E., Matsukura, K., Pasquevich, M.Y., Rodriguez, C., Saveanu, L., Seuffert, M.E., Strong, E.E., Sun, J., Tamburi, N.E., Tiecher, M.J., Turner, R.L., Valentine-Darby, P.L. and Cowie, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58: 245-302.

Heuzé, V., Tran, G. & Kaushik, S. 2015. *Fish Meal*. Feedipedia,a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/208. Accessed 16 June 2015.

Heuzé, V., Tran, G. & Kaushik, S. 2015.*Soybean Meal*. Feedipedia,a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/674. Accessed 16 June 2015.

Jintasataporn, O., Tabthipwon, P. & Yenmark, S. 2004. Substitution of golden apple snail meal for fishmeal in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) diets. *Kasetsart Journal: Natural Science* 38: 1-7.

Kaensombath, L. 2005. *Evaluation of the Nutritive Value of Ensiled and Fresh Golden Apple Snails (*Pomacea *spp) for Growing Pigs*. M.Sc. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Kaensombath, L. & Ogle, B. 2005a. Effect of ensiled or fresh golden apple snails (*Pomacea* spp) on pig growth performance and production economics. In: *Evaluation of the Nutritive Value of Ensiled and Fresh Golden Apple Snails (Pomacea* spp*) for Growing Pigs* (Kaensombath, L.), 10 p. M.Sc. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Kaensombath, L. & Ogle, B. 2005b. Laboratory-scale ensiling of golden apple snails (GAS) (Pomacea spp). In: *Evaluation of the Nutritive Value of Ensiled and Fresh Golden Apple Snails (*Pomacea spp*) for Growing Pigs* (Kaensombath, L.), 10 p. M.Sc. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Kaensombath, L.& Ogle, B. 2005c. Digestibility of ensiled and fresh golden apple snails (*Pomacea* spp) by growing pigs. In: *Evaluation of the Nutritive Value of Ensiled and Fresh Golden Apple Snails (Pomacea* spp*) for Growing Pigs* (Kaensombath, L.), 6 p. M.Sc. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Keawjam, R.S., Poonswad, P., Upatham, E.S. & Banpavichit, S. 1993. Natural parasitic infection of the golden apple snail, *Pomacea canaliculata*. *Southeast Asian Journal of Tropical Medicine and Public Health* 24: 170-177.

Liang, K., Zhang, J., Fang, L., Zhao, B., Luo, M., Parajuli, P. & Ouyang, Y. 2013. The biological control of *Pomacea canaliculata* population by rice-duck mutualism in paddy fields. *Biocontrol Science and Technology* 23: 674-690.

Lv, S., Zhang, Y., Steinmann, P., Yang, G.J., Yang, K., Zhou, X.N. & Utzinger, J. 2011. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biology* 56: 717-734.

Mochida, O. 1991. Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. *Micronesica* Supplement 3: 51-62.

Murphy, G.S. & Johnson, S. 2013. Clinical aspects of eosinophilic meningitis and meningoencephalitis caused by *Angiostrongylus cantonensis*, the rat lungworm. *Hawaii Journal of Medicine and Public Health* 72(6), Supplement 2: 35-40.

Pantua, P.C., Mercado, S.V., Lanting, F.O. & Nuevo, E.B. 1992. Use of ducks to control golden apple snail *Ampullarius* (*Pomacea canaliculata*) in irrigated rice. *International Rice Research Newsletter* 17(1): 27.

PCARRD. 2006. Mixed golden apple snail and rice bran as feed for Mallard ducks. *Philippine Council for Agriculture, Forestry and Natural Resources Research and Development, PCARRD Highlights* 2006: 97-98.

Phonekhampheng, O., Hung, L.T.& Lindberg, J.E. 2009. Ensiling of golden apple snails (*Pomacea canaliculata*) and growth performance of African catfish (*Clarias gariepinus*) fingerlings fed diets with raw and ensiled golden apple snails as protein source. *Livestock Research for Rural Development* 21(2): article 18.http://www.lrrd.org/lrrd21/2/phon21018.htm Accessed 30 September 2015.

Rattanaporn, K., Iuanloy, W., Ieng-ubol, S., Songsi-oon, W. & Chimsung, N. 2006. Effect of molasses on golden apple snail silage production. *Kasetsart Journal (Natural Science)* 40(5): 135-140.

Salazar, M.S., Sair, R.R. & Abalos, A.G. 2003. Golden Snail Meat as Feeds for Swine. Unpublished report. Mariano Marcos State University, Philippines

Serra, A.B. 1997. The use of golden snail (*Pomacea* sp.) as animal feed in the Philippines. *Tropicultura* 15: 40-43.

Serrano, R.C., 1988. Besting the golden apple snail. *Philippine Council for Agriculture and Resources Research and Development Monitor* 16(5): 6, 11.

Teo, S.S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* 20: 599-604.

Tacio, H.D. 1987. Raise snails for your ducks. *Agribusiness Weekly* (Philippines) 6-12 November 1987: 16-17.

Ulep, L.J.L. & Buenafe, M.M. 1991. Performance of broilers fed with snail (*Pomacea canaliculata*) meal as substitute to fish meal or meat and bone meal. *Tropicultura* 9: 58-60.

Ulep, L.J.L.& Santos, A.C. 1995. Growth performance of Pekin ducks fed with golden snail and fresh banana peelings. *Tropicultura* 13: 135-138.

Usman, Rachmansyah, Palinggi, N.N. & Ahmad, T. 2007. Utilisation of local feed ingredients in tiger grouper grow out moist diets. *Aquaculture Asia Magazine* 12(2): 33-36.

Vega, I.A., Arribére, M.A., Almonacid, A.V., Guevara, S.R. & Castro-Vazquez, A. 2012. Apple snails and their endosymbionts bioconcentrate heavy metals and uranium from contaminated drinking water. *Environmental Science and Pollution Research* 19: 3307-3316.

Vega, R.S.A. 1991. *Agro-economic Evaluation of Non-chemical Methods Against Golden Snail* (*Pomacea canaliculata* Lam.) *in Irrigated Lowland Rice*. M.Sc. thesis, University of the Philippines, Los Baños.

Vongvichitch, B. 2006. *Using Golden Apple Snail* (*Pomacea canaliculata*) *Meal and Betaine in Lowland Frog (*Rana rugulosa*) Feed*. M.Sc. thesis, Kasetsart University, Bangkok.

Apple snail use in small-scale aquaculture in the Philippines

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Abstract

The apple snail, *Pomacea canaliculata*, an alien invasive species that causes considerable damage to rice culture, is now being used as an alternative protein source in small-scale aquaculture in the Philippines. Interviews with selected fish farmers rearing Japanese koi and *Macrobrachium rosenbergii* (freshwater prawn) revealed that apple snail meal is the main source of protein given to the cultured species. The fish farms have observed satisfactory breeder performance and a reasonable culture period in attaining marketable size at reduced production costs as a result of substitution of apple snail meal for commercial feeds. Similarly, better growth, production and size-frequency distribution at harvest of *Penaeus monodon* (tiger shrimp) was observed when apple snail meal was combined with cassava. As use of apple snail meal becomes widespread in small-scale aquaculture, both the management and control of this invasive species may be addressed.

Additional keywords: Japanese koi, *Macrobrachium rosenbergii*, *Penaeus monodon*, *Pomacea canaliculata*, tiger shrimp

Introduction

The apple snail *Pomacea caniculata*, family Ampullariidae (sometimes known as the golden apple snail or GAS, although this name may also have been applied to *Pomacea maculata*, especially in areas where both occur together) is an alien invasive species that causes considerable economic losses due to agricultural damage, specifically to rice, which is one of the most important crops in Asia. *Pomacea canaliculata* was introduced to Southeast Asia inthe 1980s and has spread throughout the region, where it has come to be considered a significant agricultural pest. It has been reported as established in Japan, Laos, Malaysia, Philippines, Taiwan, Thailand, Vietnam and China (Hayes *et al*., 2008). In the Philippines alone, estimates of economic losses associated with apple snails ranged from US$425 million to US$1.2 billion in 1990 (Naylor, 1996). There is an urgent need to control the proliferation and spread of this pest, which has become cosmopolitan in distribution.

The introduction of *P. canaliculata* as a cheap protein source for humans has allowed its entry into and establishment in several new environments. This introduction has, however, backfired because of the Filipinos' preference for the endemic snail *Pila conica* (often referred to as its junior synonym *Pila luzonica*). The biological characteristics of *P. canaliculata*, which were its salient features and justification for its introduction, also became a nightmare. Its adaptability, voraciousness and rapid growth and reproduction provided more than what was needed and it inundated the rice ecosystems that it invaded to the point of almost replacing the endemic snail. This seemingly inexhaustible supply of *P. canaliculata* needs to be tapped to manage and control its populations.

Recognition of *P. canaliculata* as a good protein source for animal feeds and its abundance in rice fields have prompted the Camarines Sur State Agricultural College (CSSAC) through Marife Leonardo-Pesino to develop the golden *kuhol* crusher-grinder, designed and fabricated under a project implemented by CSSAC in partnership with a private manufacturer of agricultural machines. This machine is hoped to maximize the utility of the seemingly limitless food resource by processing the snails into a cheap source of protein for fish, poultry and livestock (Dela Cruz, 2006).

Rice-fish farming has been promoted to maximize farmers' income by using the same piece of land for both agriculture and aquaculture. It has been traditionally practiced in Asia, since irrigated, rainfed and deepwater rice systems all provide suitable environments for fish and other aquatic organisms. Small-scale aquaculture of food and ornamental fish can augment the incomes of farmers and local communities, using idle land and excess human capital (labour). Growing fish however, necessitates the addition of feed, which lessens the income from the activity. Using cheaper alternative sources of protein for feeding the fish is therefore essential to lower production costs and increase income. *Pomacea canaliculata*, which has become abundant throughout Southeast Asia, has been used as an alternative source of protein in aquaculture. By using *P. canaliculata* as an alternative to protein-rich feed for aquaculture, two important problems of small-scale farmers and fishers are mitigated: first, control of a very important pest of agriculture by directly using it as feed; and second, decrease of other inputs to aquaculture.

Control of apple snails

Natural predators of apple snails include a wide array of biological organisms: insects, fishes, amphibians, reptiles, crustaceans, mammals and birds (Yusa, 2006). In their native range in South America, snails in this family (Ampullariidae) are regarded as key prey organisms (Donnay and Beissinger, 1993) and are thus kept at relatively low densities (Halwart, 1994).

The adaptability and survival of these alien snails in their new environments are expected, given the similarity of the environmental conditions between their native range and those of the new environments. This has happened in most if not all of the countries where the species has been introduced. The abundance of this species in its introduced environments in Asia was thus initially surprising as such densities were almost never encountered in their natural environments. However, the initial absence of natural predators in the new introduced environments has to some extent allowed for the population explosion of the species, which has now become a major widespread problem.

Various control measures have been tried for *P. canaliculata*, including novel agricultural practices, mechanical and chemical control, crop rotation and biological control, with varying success in different countries (Wada, 2004) leading to numerous scientific publications. These control measures have specific problems and benefits, affecting control and management decisions of the farmers.

Biological control measures include the use of fish, particularly the common carp *Cyprinus carpio*, which are effective predators of apple snails in field experiments (Halwart, 1994; Ichinose *et al*., 2002). In rice paddies, fish feed on neonate snails, thereby potentially reducing snail populations, although this has not been formally demonstrated (Carlsson et al., 2004).

Apple snails and aquaculture systems

The use of apple snails in aquaculture systems is therefore a logical step towards both control of the pest and a cheap alternative to traditional processed fishmeal. The abundance and presence of an alternative high protein source for the species being cultured on site is enough incentive for use of apple snails. Furthermore, since it has been reported in several publications that snails are included in the diets of some fish species, their possible utility as an alternative protein source for aquaculture is evident. In Cambodia, where rice-fish culture has been traditionally practiced, several indigenous fish species from 10 genera have been reported in rice fields. These species subsist on available food within the ecosystem (Gregory, 1997).

Rice-prawn farming, which is quite common in China, has been reported to use crushed snails as a protein supplement (IIRR *et al*., 2001).

In Indonesia, three species of apple snails have been reported: *P. canaliculata*, *P. maculata* (as its junior synonym *P. insularum*) and *P. paludosa* (almost certainly a misidentification of one of the other two species). Farmers in some areas have started using apple snails, which they consider major pests, as duck and catfish feed. Apple snails have also been sold to small duck and catfish growers, who prefer purchasing over manufacturing or preparing feed. Nevertheless, apple snail supply has always surpassed demand (Suharto, undated).

Two indigenous predators (a fish and a wetland crab) of the invasive apple snails in Laos have been assessed for their efficacy as control agents. The climbing perch, *Anabas testudineus*, indigenous to wetlands, reduced the abundance of neonate snails in field experiments but their potential as control agents at a population level is as yet unclear (Carlsson *et al*., 2004).

A Food and Agriculture Organization (FAO) project in Vietnam entitled Integrated Golden Apple Snail Management in Rice used biological controls to combat apple snails, finding that rice-fish farming - where fish are raised in the rice fields – is one of the best ways to control the snails. In a training course on modern hatchery techniques for technicians from ten provinces in Vietnam, participants learned improved techniques for breeding and propagation of black carp, common carp and catfish. Research results point to the common carp as the most efficient control agent for apple snails, having a better survival rate than black carp and eating more snails. Field experiments showed that the common carp reduced populations of the snail, particularly young snails of less than 1 cm shell height, by 90% in rice fields over a period of 3 months (FAO, 1998).

The Philippine scenario

In the 1990s, rice field trials in the Philippines showed that fish can keep snail populations in check. Rice-fish farming serves a dual purpose: controlling the snail population and enhancing the food security of rice farmers by supplementing their diets and generating income (FAO, 1998). In Cavite, where rice-fish farming was being practiced, fish fingerlings of tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*) were stocked and grown. Fishwere fed with feed available on site, consisting of plankton growing in the trenches and fields, rice bran whenever available, given ad libitum twice a day (morning and afternoon), apple snails, azolla, *ipil-ipil* (*Leucaena leucocephala*) and *kangkong* (*Ipomoea aquatica*). These were all mixed with rice bran (if available) before being given to the fish (Velarde, undated). At the Central Luzon State University a study showed that red bellied pacu (*Piaractus brachyposum*) can reduce apple snail density by 63 % in rice plots (R.C. Joshi, pers. comm.).

Apple snails as food for humans and animals

Apple snails were intentionally introduced to the Philippines as an an alternative protein source for the growing Filipino population. But from being a potential food, the unabated proliferation of apple snail populations in rice fields is now widely viewed as an agricultural nuisance.

Although apple snails have become a pest of monumental magnitude, they still have potential in another agricultural system – in aquaculture, where feed accounts for a large percentage of production costs. Fish meal, an important feed component, is costly, especially because it is imported. The availability of apple snails in the natural environment provides fish farmers with a prospective feed additive, if not an outright substitute.

Tiger shrimp culture

Bombeo-Tuburan et al. (1995) reported their success in using apple snails as a feed component in the culture of tiger shrimp, *Penaeus monodon*. They tried to find a beneficial use for the snails, which are readily available and as of then had not been used in aquaculture. By studying the potential of substituting apple snails for trash fish, which is the common protein source in shrimp feeds, they showed that a pest in one agro-ecosystem can be an input into another.

In their study, shrimp fed with maize combined with apple snails showed significantly better growth, production and size frequency distribution at harvest. However, when only apple snails or maize was given, significantly less growth and production were evident. By combining these animal and plant components, the tiger shrimp were provided with better nutrition. The protein required for growth came from the snails, whereas the carbohydrate for energy came from either maize (or cassava). Apple snails have very high crude protein content at 54 %, which is similar to trash fish (58 %) (Bombeo-Tuburan et al., 1995). Although both maize (87 %) and cassava (92 %) contain high proportions of digestible carbohydrate, the apple snail cassava combination proved to be better.

In the same paper, analysis of the nutritional value (amino and fatty acid profiles) of apple snails was presented. Lipid content was 3.65 %, with a fatty acid profile that shows it can provide the polyunsaturated fatty acid requirement of *P. monodon*.

A comparison of the amino acid profiles of apple snails and tiger shrimp showed that the snails have higher arginine, leucine, lysine, threonine and tryptophan content than shrimps. On the other hand, histidine, isoleucine, methionine, phenylalanine and valine were higher in shrimps than in snails. The essential amino acid index of the snails was estimated as 0.84.

Freshwater prawn culture

Use of apple snails as feed for freshwater prawns is currently being practiced at the lsabela Green Valley Orchid and Giant Freshwater Prawn Hatchery in Cauayan City, Isabela. According to the farm owner (Floresma Dacuycuy, pers. comm.), the snails are the main diet of both the breeders and juveniles. Production cost is kept to a minimum, since the snails are simply collected from rice fields by school children who want to earn extra money.

Snail meal is prepared in bulk at the farm. First, the snails are boiled and then the meat is picked out of the shell. The meat is divided into serving sizes and placed inside plastic bags, which are kept frozen until needed. Prior to feeding time, the frozen apple snail meal is chopped into small pieces depending on the size of the prawns to be fed (Fig. 1). Although it may seem labour intensive, apple snail meal preparation is done by farm helpers during slack periods. Hence, collection cost is the only cash outlay required.

Apple snail meal is given twice a day, every morning and afternoon; vegetables and other trash meat provide variety. The use of apple snails as the breeder's main diet, 2 kg per feeding for 500 breeders, results in a shorter period between spawning. That is, breeders are ready to spawn after 18-25 days compared with 1 month when using commercial feeds. Juveniles maintained on apple snail meal grow to the marketable size of 50-60 g after about 6 months. Partial harvest after 4 months is possible because of the fast growth of some prawns, called shooters, which is common in any aquaculture system. Growth performance of juveniles is satisfactory relative to production costs. Fig. 2 shows freshwater prawns feeding on the snail/vegetable meal mixture.

Fig. 1. Preparation of apple snails and assorted trash vegetables as feed for freshwater prawns. (Photos: L.V. Castillo)

Fig. 2. Freshwater prawns in culture feeding on apple snail/vegetable meal. (Photo: L.V. Castillo)

The Bureau of Fisheries and Aquatic Resources (BFAR) of Region 2 provides technical support to the Isabela Green Valley Orchid and Giant Freshwater Prawn Hatchery. On the other hand, the farm provides the postlarvae needs of BFAR for their technology demonstrations in several provinces in northern Luzon, including Isabela, Cagayan, Nueva Vizcaya, Kalinga, Apayao, Benguet and Quirino. After these demonstrations, the grow-out farms in these provinces procure the postlarvae (PL25-30) from the hatchery. Although the use of apple snail meat has not been widely adopted, the owner is positive that in time, apple snail meal will become common in the region.

Common carp culture

FOR HUMAN CONSUMPTION Common carp (*Cyprinus carpio*) is one of the oldest and most widely cultured carp species. At the National Inland Fisheries Technology Center of the Bureau of Fisheries and Aquatic Resources, three strains of common carp (Sukabumi, Majalaya and Tanay) have been used in a genetic upgrading programme. The production performance of boiled apple snails (100 %) in comparison with the traditional feed formulation of fish meal (25 %) and rice bran (75 %) was used as feed for the three upgraded strains.

The study showed no significant effect of the type of feed formulation on the growth of the three strains although the Majalaya and Sukami strains performed better with snails as feed (Aida Palma, unpublished).

FOR ORNAMENTAL JAPANESE KOI Japanese koi (*Cyprinus carpio*) is a very popular ornamental freshwater fish for both aquaria and outdoor ponds in the Philippines. Although the beauty of koi is formally based on certain accepted patterns, colour and body shape, the ordinary Filipino hobbyist is satisfied with koi having deep and bright colours.

The colour of Japanese koi is melanins and carotenoids on the skin. The red, orange and yellow colours are due to the carotenoids, which the fish has to get from its diet. Koi breeders and growers must provide the koi with a diet that will enhance pigmentation. Plants, algae and crustaceans are the primary sources, although other organisms feeding on carotenoid-rich materials are also possible sources.

To have the desired color and intensity, a diet rich in carotenoids is essential. Koi are capable of metabolizing dietary sources of carotenoids as reported by several researchers, as cited by Stewart (1993). To koi growers and enthusiasts, these studies are significant because they show that the intensity of pigmentation is proportional to the amount of carotenoids in the diet.

These concepts were applied unknowingly at the Crismar Fishing Resort in Pila, Laguna, when they used apple snail meal for their Japanese koi breeders as well as during grow-outperiods. The Japanese koi are maintained largely on an apple snail meal diet. Although there is still no published report on the carotenoid content of *P. canaliculata*, it is probably carotenoid rich, being a herbivore that feeds on plants and algae. Since most collection is done in shallow ponds and rice fields, the possibility of the snails feeding on filamentous algae, perhaps the blue-green alga *Spirulina*, is not remote. *Spirulina* is a rich source of ß-carotene, echinenone, cryptoxanthin and zeaxanthin (Stewart, 1993). Since dietary carotenoids are metabolized by koi, a diet high in carotenoids is expected to give the desired colors and intensity. For example, dietary zeaxanthin is absorbed and transferred to the integument, where it is ultimately converted to astaxanthin (Stewart, 1993). By consuming snail meal every day, the Japanese koi at the Crismar Farm develop deep and brilliant colors that amaze even other seasoned ornamental fish growers in Pila.

Preparation of snails for feeding Japanese koi at the Crismar farm starts with collection of at least one pail (10 L capacity) of snails from nearby ponds and rice fields every morning. The snails are washed and crushed using improvised equipment designed by the farm owner. The crusher has a metal funnel with rotating spokes at the bottom. The spokes trap the snails, bringing them against the wall of the funnel. The pressure cracks the shells, exposing the meat. Crushed shells drop into a pail placed underneath the funnel. Finally, the shells are removed and the snail meal preparation is finished. About 30 koi breeders in an earthen pond are given this raw snail meal every feeding time. Feeding is twice a day, every morning and afternoon. The koi are very seldom given other supplemental feeds.

River catfish culture

The river catfish or cream dory, *Pangasius hypopthalmus*, is a recent favourite in most homes and restaurants in the country. A study by the Bureau of Fisheries and Aquatic Resources (D. Abalos & F. Mangabat, unpublished) revealed that *P. hypopthalmus* fed with a mixture of 60 % commercial feed to 40 % apple snails had similar weight gain to those fed with purely commercial feeds. Apple snail meal was prepared by extracting and boiling the meat of collected snails. Afterwards, the boiled snail meat was chopped and properly preserved. The amount of feed given daily was 5 % of the total fish biomass during the fingerling stage gradually reducing to 2.5 % of the total biomass one month before harvest. The results from this study open up the possibility of replacing commercial feeds with apple snail meal.

Giant gourami culture

Giant gourami (*Osphronemus goramy*) is a desirable alternative species for freshwater aquaculture because of its ability to thrive on a wide variety of feeds including *P. canaliculata*. For culture of giant gourami, feed consisting of water hyacinth (30 %), banana pseudostem (30 %) apple snails (30 %) and cassava (10 %) was given in the form of moist feed (Aida Palma, unpublished). The stocks attained an average final weight of 200 g after five months of culture with average survival rate of 95 %. However, experiments comparing growth on diets with and without apple snails have been performed.

Conclusion

The preceding examples in the Philippines point to the utility of *Pomacea canaliculata* as a substitute for fishmeal even in small-sale commercial production. This may lead to solving the problem of the management/control of an invasive species that has spread across the region as well as provision of a cheap and accessible alternate protein source for aquaculture. As more and more farmers use apple snails to feed their aquaculture animals, the snail populations may be reduced and impacts on agriculture lessened.

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References

Bombeo-Tuburan I., Fukumoto, S. & Rodriguez, E.M. 1995. Use of the golden apple snail, cassava, and maize as feeds for the tiger shrimp, *Penaeus monodon*, in ponds. *Aquaculture* 131: 91-100.

Carlsson, N., Kestrup, Å., Mårtensson, M. & Nyström, P. 2004. Lethal and non-lethal effects of multiple indigenous predators on the invasive golden apple snail (*Pomacea canaliculata*). *Freshwater Biology* 49: 1269-1279.

Dela Cruz, R.D. 2005. *Golden Kuhol Crusher-Grinder: a cheap, Gender-Friendly Machine*. https://www.bar.gov.ph/index.php/digest-home/digest-archives/73-2006-2nd-quarter/4409-aprjun06-goldenkuhol (Accessed 14 July 2016).

Donnay, T.J. & Beissinger, S.R. 1993. Apple snail (*Pomacea doliodes* [*sic*]) and freshwater crab (*Dilocarcinus dentatos*) population fluctuations in the Llanos of Venezuela. *Biotropica* 25: 206-214.

FAO [Food and Agriculture Organization of the United Nations]. 1998. Fish-farming in Vietnamese rice field fights golden apple snail pest. News and Highlights, FAO. http://www.fao.org/News/1998/980410-e.htm (Accessed 19 December 2005).

Gregory, R. 1997. *Ricefield Fisheries Handbook*. Cambodia-IRRI-Australia Project, Phnom Penh. [iv] + 37 p.

Halwart, M. 1994. The golden apple snail *Pomacea caniculata* in Asian rice farming systems: present impact and future threat. *International Journal of Pest Management* 40: 199-206.

Hayes, K.A., Joshi, R.C., Thiengo, S.C. & Cowie, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions* 14: 701-712.

Ichinose, K., Tochihara, M., Wada, T., Suguiura, N. & Yusa, Y. 2002. Influence of common carp on apple snail in a rice field evaluated by a predator-prey logistic model. *International Journal of Pest Management* 48: 133-138.

IIRR, IDRC, FAO, NACA & ICLARM. 2001. *Utilizing Different Aquatic Resources for Livelihoods in Asia: a Resource Book*. International Institute of Rural Reconstruction, International Development Research Centre, Food and Agriculture Organization of the United Nations, Network of Aquaculture Centers in Asia-Pacific and International Center for Living Aquatic Resources Management. 416 p.

Naylor, R. 1996. Invasions in agriculture: the cost of the golden apple snail in Asia. *Ambio*. 25: 443-448.

Stewart, L.J. 1993. Nutrition of koi, carp, and goldfish. In: *Fish Medicine* (Stoskopf, M.K., ed.), p. 461-469. W.B. Saunders, Philadephia.

Suharto, H. [undated]. Golden apple snail, *Pomacea canaliculata* (Lamarck) in Indonesia. http://applesnail.net/pestalert/conferences/icam07/indonesia_country_reports.pdf (Accessed 14 July 2016).

Velarde, A.V. 1996. Mang Dionisio Herrera switched over to rice-fish. *ILEIA Newsletter* 12(2): 12-13.

Wada, T. 2004. Strategies for controlling the apple snail *Pomacea caniculata* (Lamarck) (Gastropoda: Ampullariidae) in Japanese direct-sown paddy fields. *Japan Agricultural Research Quarterly* 38: 75-80.

Yusa, Y. 2006. Predators of the introduced apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae): their effectivenss and utilization in biological control. In: *Global Advances in Ecology and Management of Golden Apple Snails* (Joshi, R.C. & Sebastian, L.S. ed.), p. 345-361. Philippine Rice Research Institute, Nueva Ecija, Philippines.

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