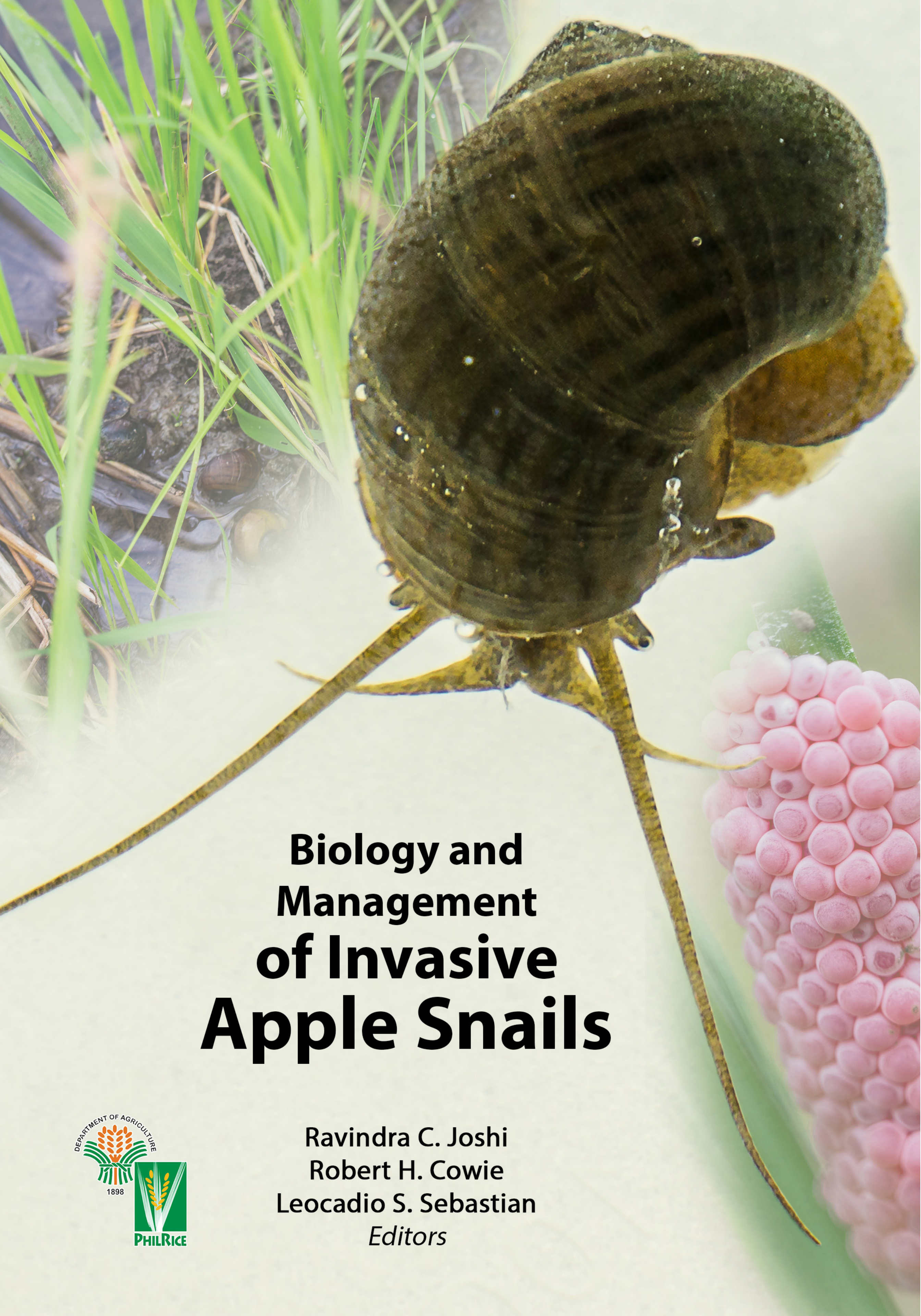

Biology and Management of Invasive Apple Snails

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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

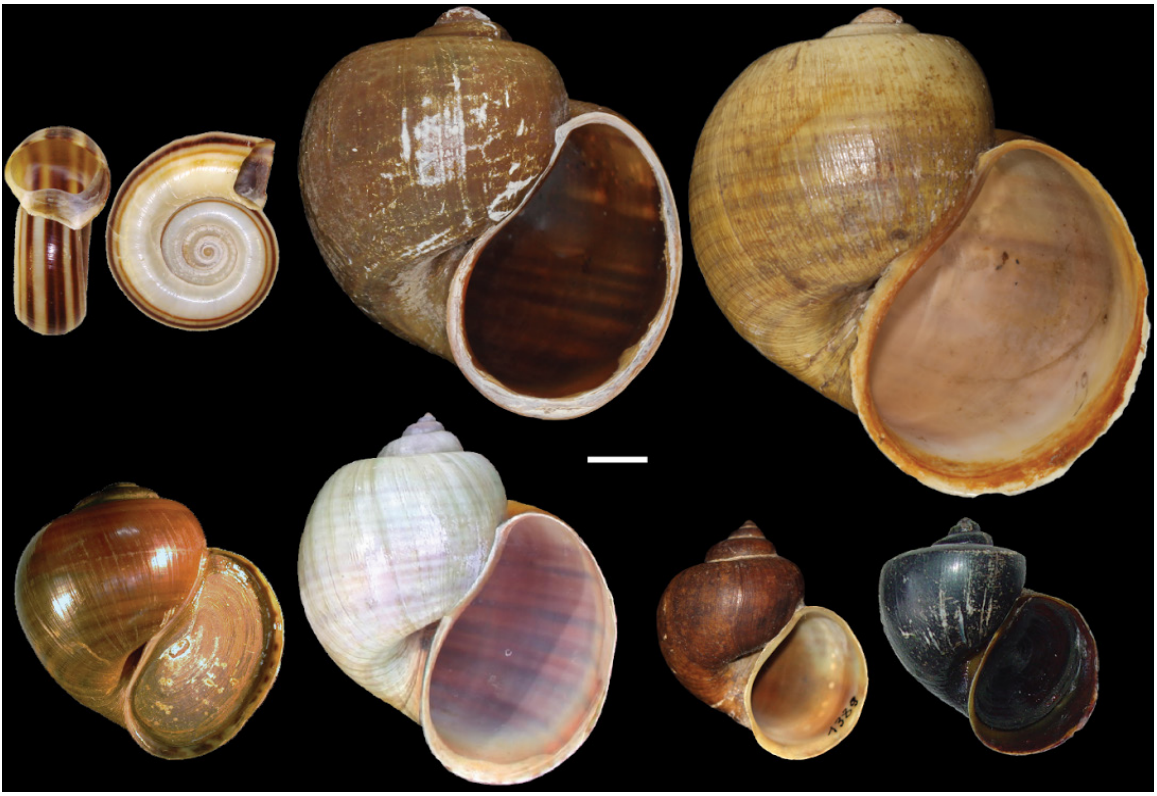

Fig. 1. Shells of introduced ampullariids. Top row, left to right: *Marisa cornuarietis*, *Pomacea canaliculata*, *P. maculata*; bottom row, left to right: *Pila scutata*, *Pomacea* sp. (incorrectly identified as *P. haustrum* by Rawlings *et al*., 2007), *P. diffusa*, *P. scalaris*. Scale bar: 1 cm. 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.

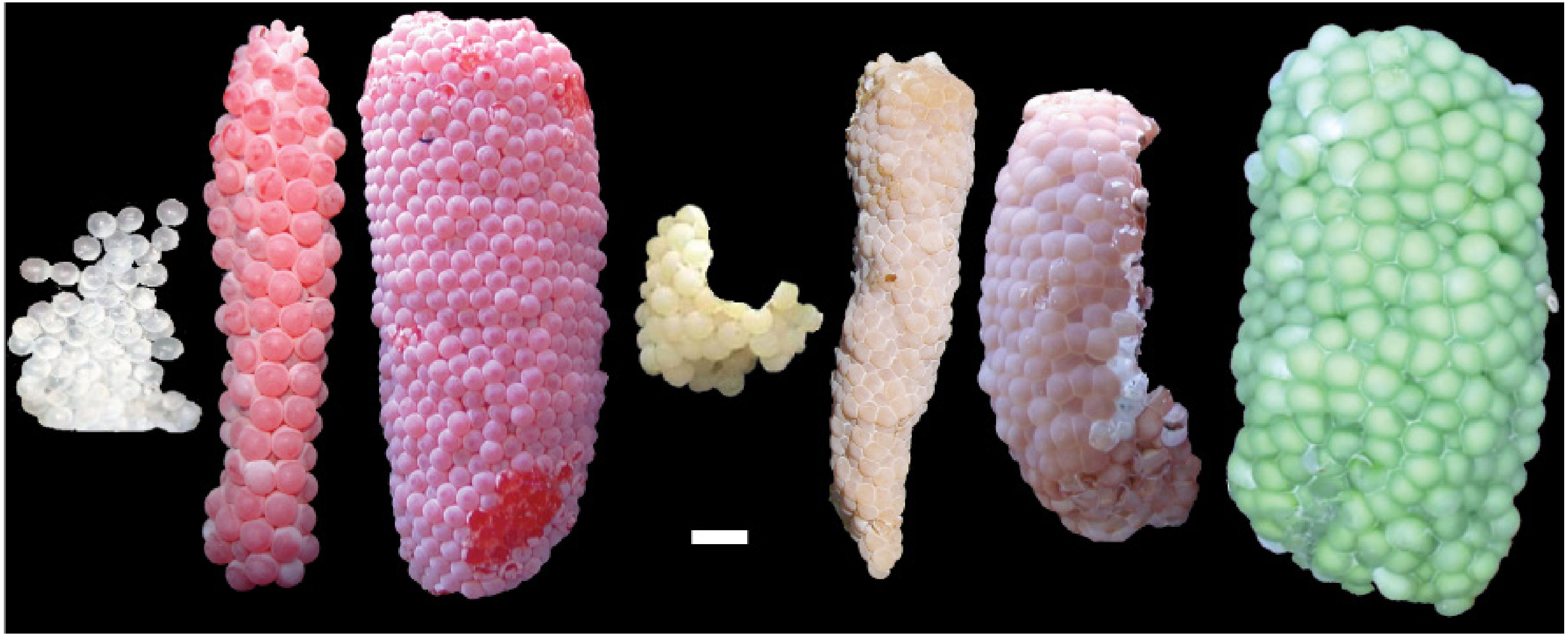

Fig. 2. Egg masses of introduced ampullariids. From left: *Marisa cornuarietis*, *Pomacea canaliculata*, *Pomacea maculata*, *Pila scutata*, *Pomacea. diffusa*, *Pomacea scalaris*, *Pomacea* sp. (incorrectly identified as *Pomacea haustrum* by Rawlings *et al*., 2007). Scale bar: 5 mm. Eggs of *M. cornuarietis* 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.

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.

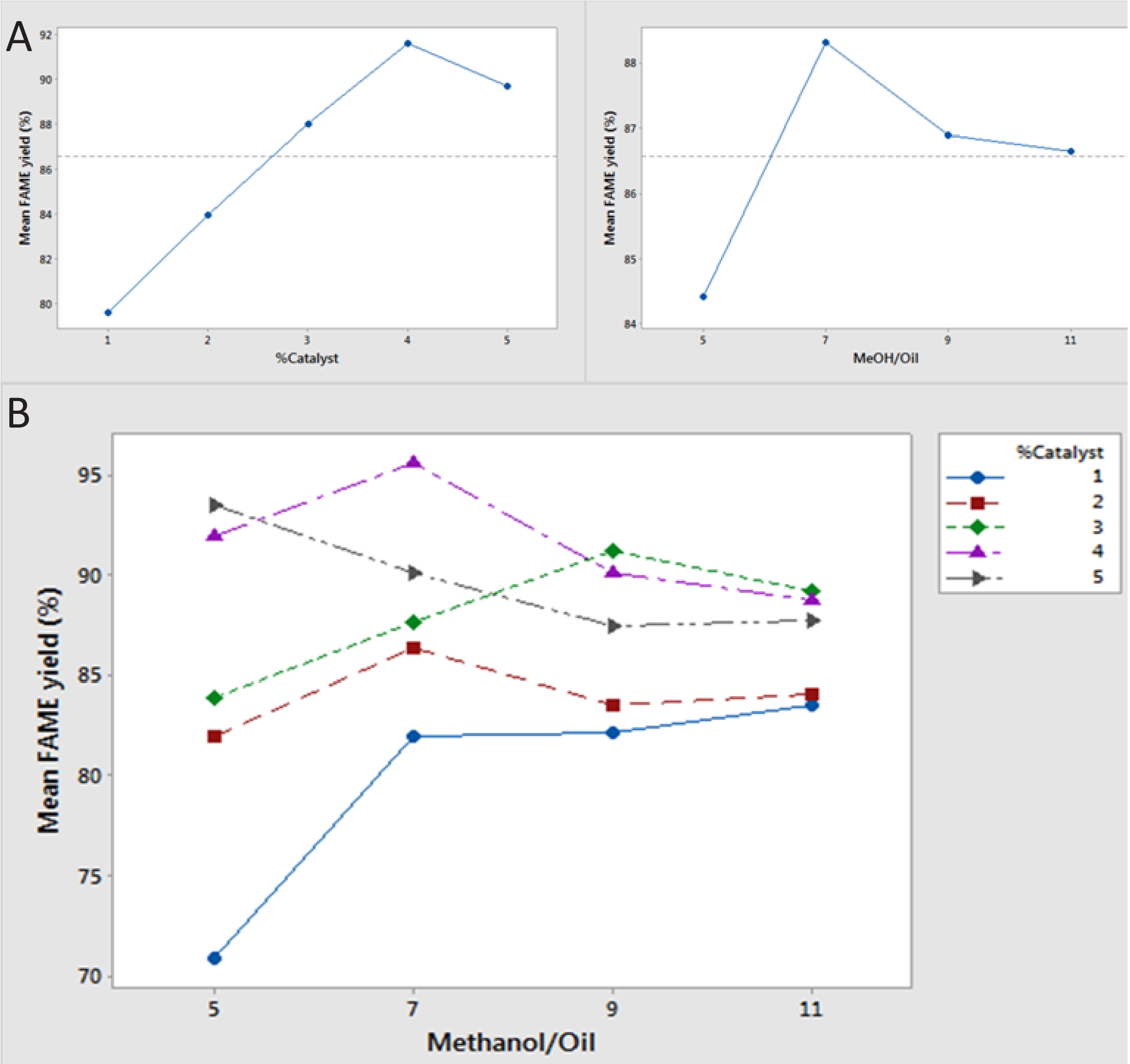

Fig. 1. Mean FAME yield. A: effect of percent CaO catalyst averaged over all values of the methanol/oil ratio (left panel); effect of the methanol/oil ratio averaged over all values of percent CaO catalyst (right panel). B: interaction effect of CaO catalyst and methanol/oil ratio.

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

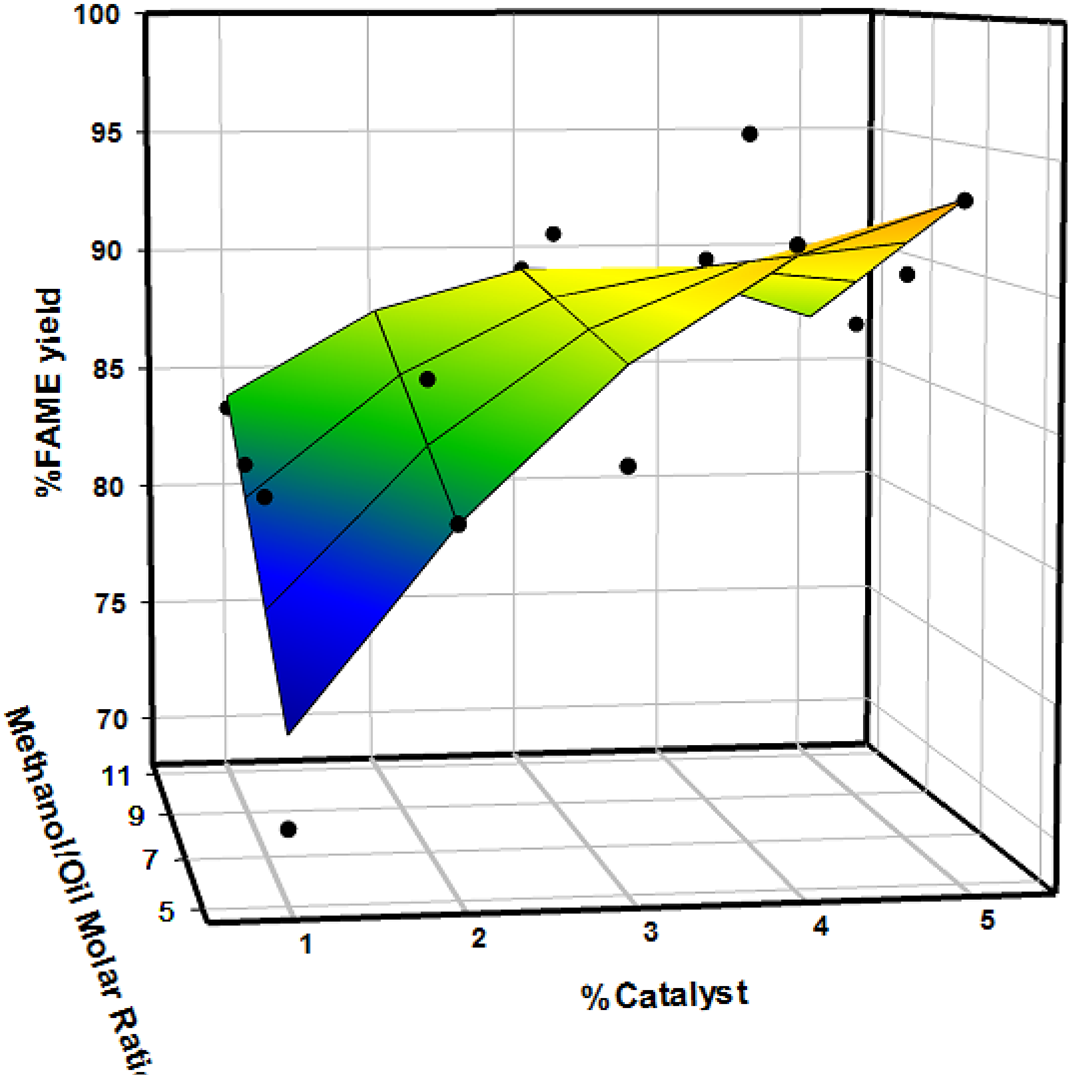

Fig. 2. The model (represented by the mesh plot) and the experimental data (represented by the scatter plot) of biodiesel production using *Pomacea* sp. shell as a catalyst.

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

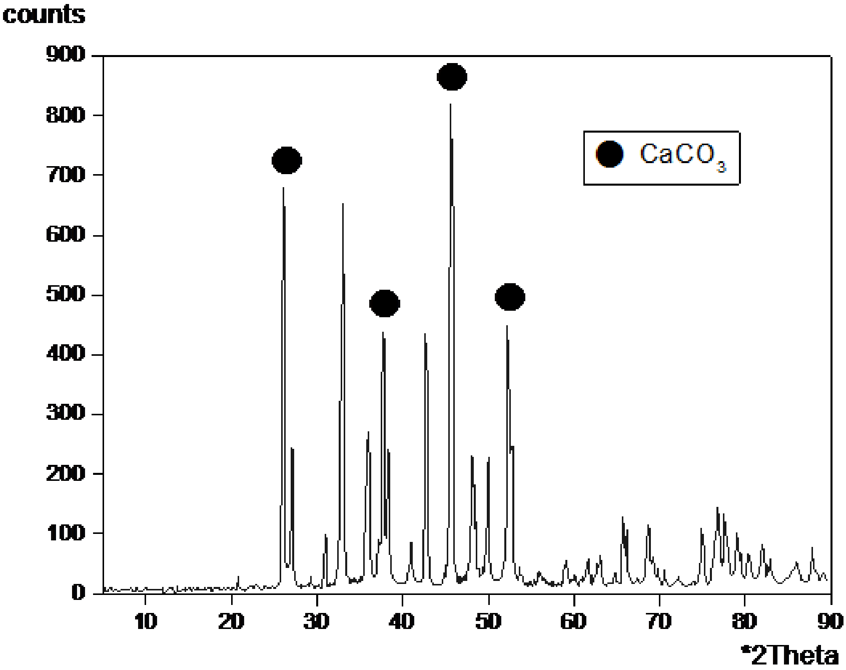

Fig. 3. X-Ray structure of apple snail shell (adapted from Margaretha *et al*., 2012).

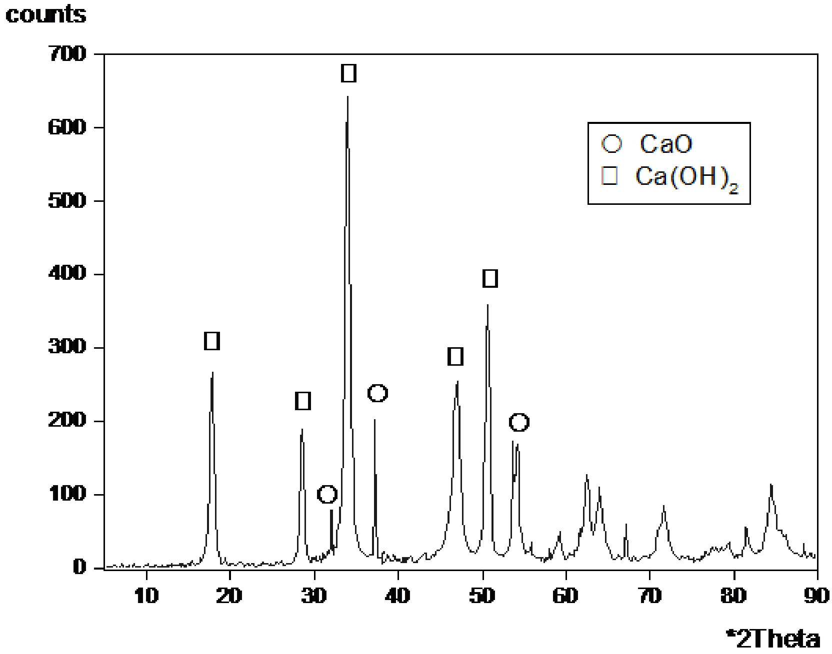

Fig. 4. X-Ray structure of CaO catalyst (adapted from Margaretha *et al*., 2012).

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

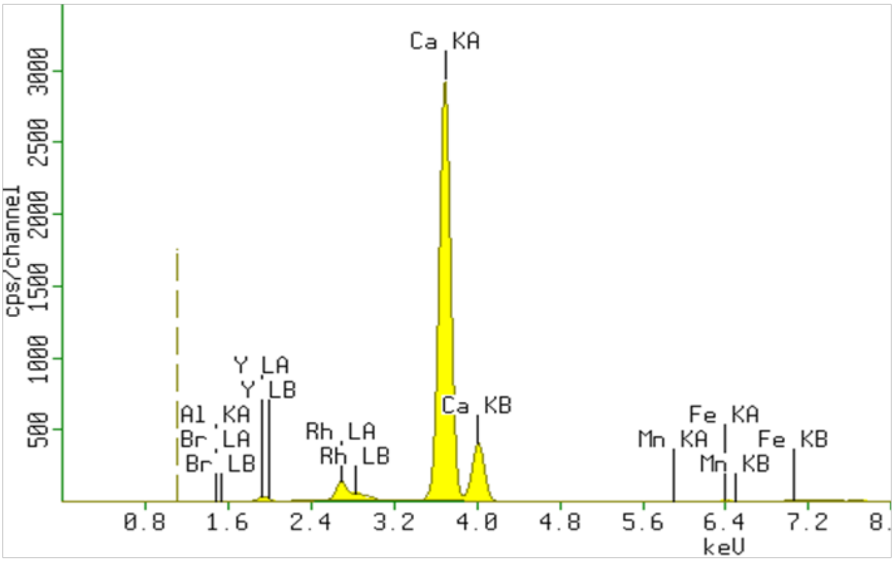

Fig. 5. XRF spectra of CaO catalyst (adapted from Margaretha *et al*., 2012).

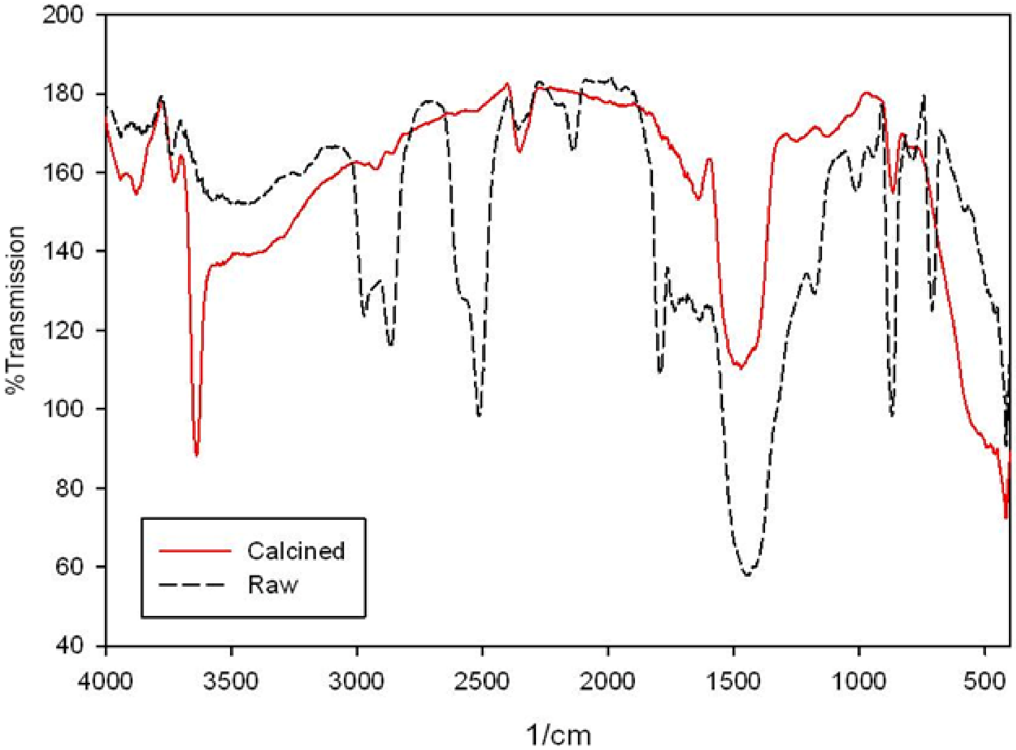

Fig. 6. FT-IR spectra of raw material and CaO catalyst (adapted from Margaretha *et al*., 2012).

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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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.

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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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