

# Biology and management of invasive apple snails

*by* Aning Ayucitra

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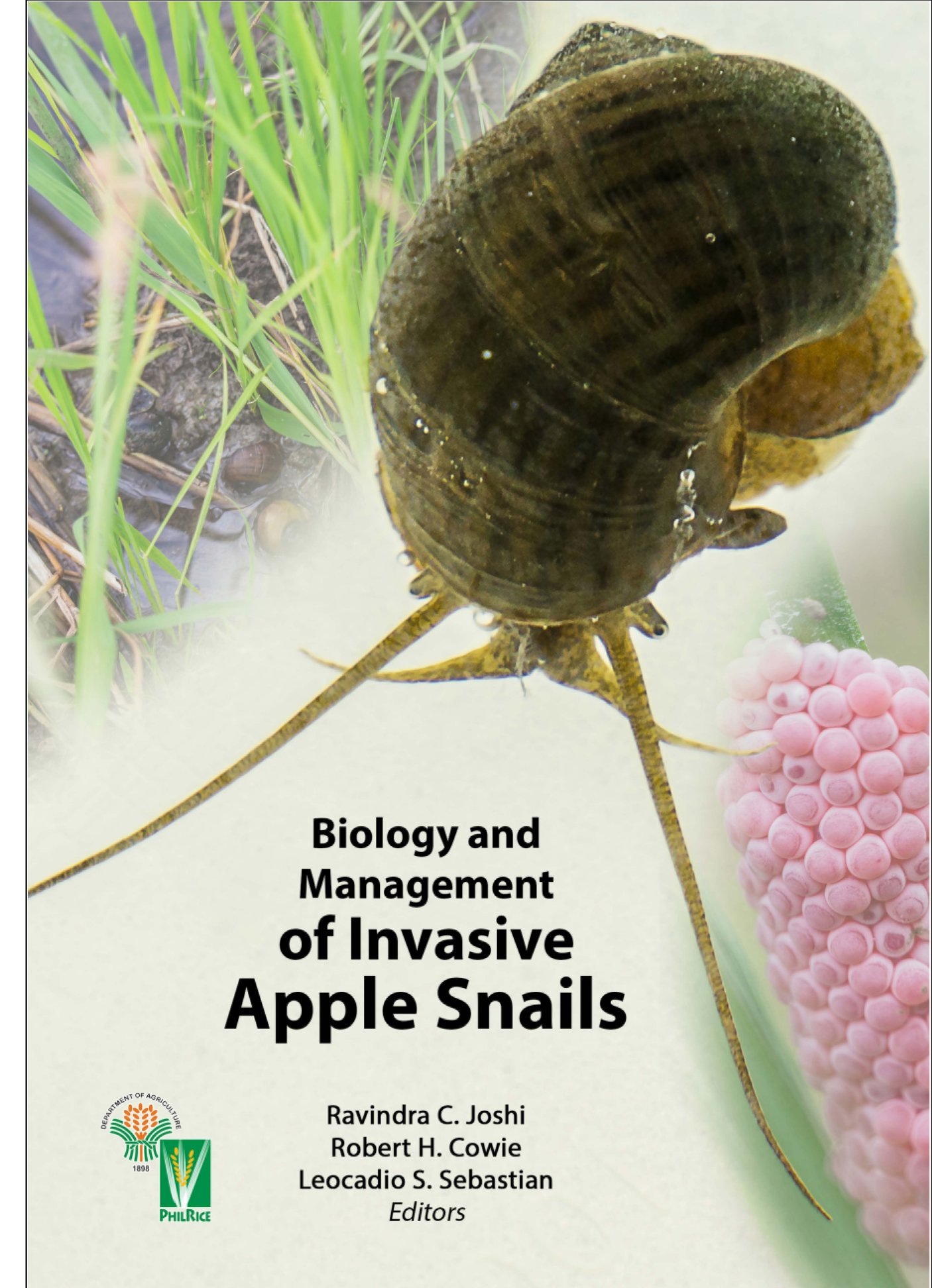
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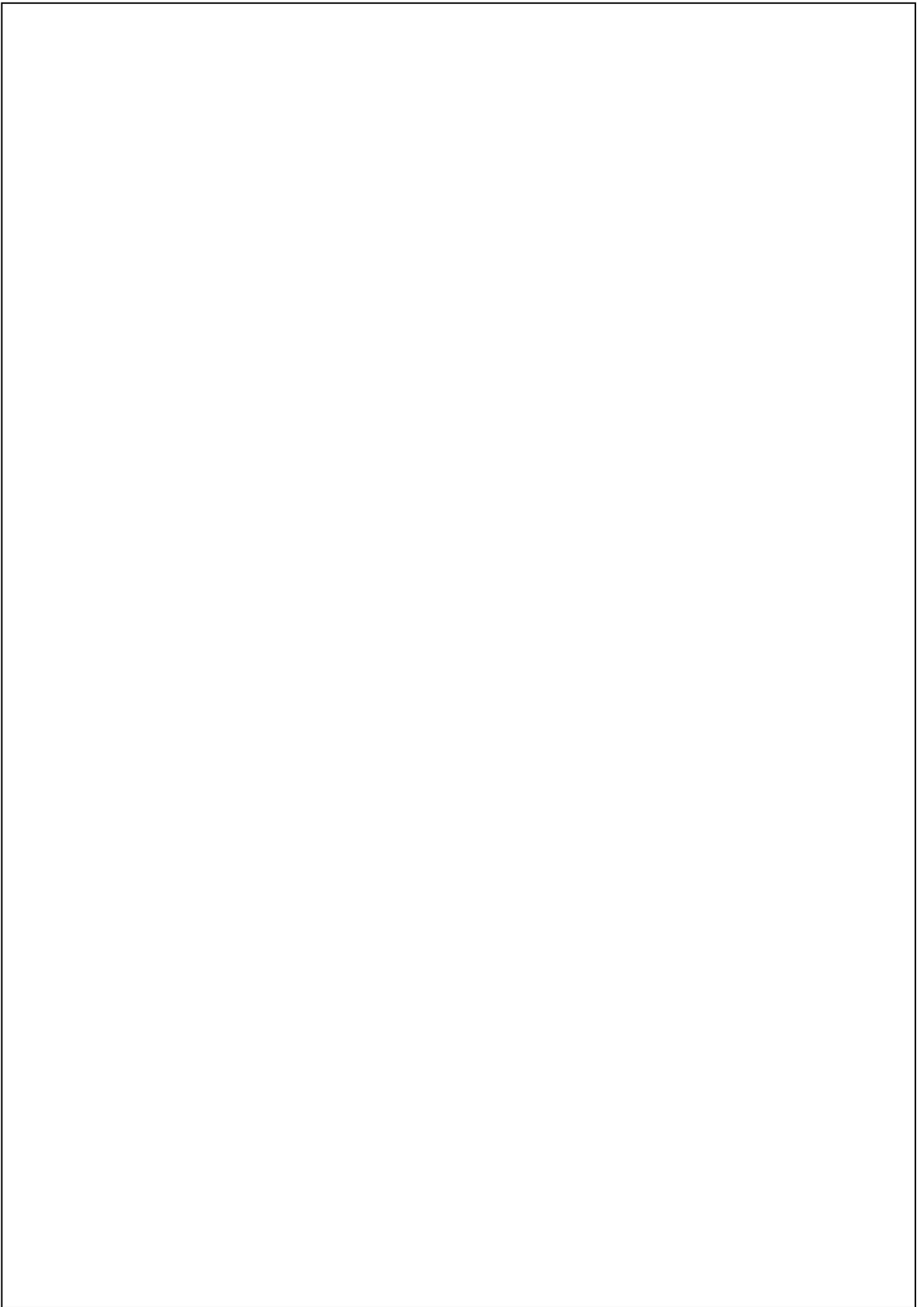
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# Biology and Management of Invasive Apple Snails

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





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BIOLOGY AND MANAGEMENT OF  
**INVASIVE APPLE SNAILS**

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



Philippine Rice Research Institute  
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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

<sup>6</sup> 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.

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# Use of apple snail (*Pomacea* sp.) shell as a catalyst for biodiesel production: full factorial design optimisation

Ong Lu Ki<sup>1</sup>, Suryadi Ismadji<sup>2\*</sup>, Aning Ayucitra<sup>2</sup>, Felycia Edi Soetaredjo<sup>2</sup>, Yosephine Yulia Margaretha<sup>3</sup> and Henry Sanaga Prasetya<sup>2</sup>

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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 characterized 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 including non-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 1 litre of biodiesel requires 3726 litres of water) and nutrients (nitrogen, phosphorus and CO<sub>2</sub>) 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 <sup>3</sup> *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<sub>3</sub>), 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<sub>3</sub>, 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.

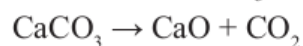
Apple snails in the genus *Pomacea* are not native to Indonesia, but have both ecological and economic impacts in some parts of the country. In Asia, they were first introduced to Taiwan from Argentina as a commodity for aquaculture business ventures in early 1980, and were subsequently spread, intentionally 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<sub>3</sub> was converted to CaO according to the reaction:



In order to cool the system after the reaction was complete, nitrogen gas with a flow rate of 3 lmin<sup>-1</sup> 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™) 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
Linolenic acid (C18:3)	0.14
Arachidonic 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 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 separated by 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<sup>-1</sup>. 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  $\text{Yield \%} = (\text{weight of biodiesel} \times \% \text{ FAME}) / [\text{weight of oil} \times 100] \%$  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 standard and 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 fluorescence (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 ( $-196^{\circ}\text{C}$ ). Prior to the analysis the samples were degassed under vacuum for 24 hours at  $150^{\circ}\text{C}$ . 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 ( $p/p_0$ ) 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  $4000\text{--}700\text{ cm}^{-1}$ . 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  $\text{CuK}\alpha$  radiation. The measurement was conducted in  $2\theta$  angle between  $8$  and  $72^{\circ}$ . 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 ( $\alpha$ ) of 5% to assess the significance of each model and its terms. All statistical analyses were performed in Minitab 16.

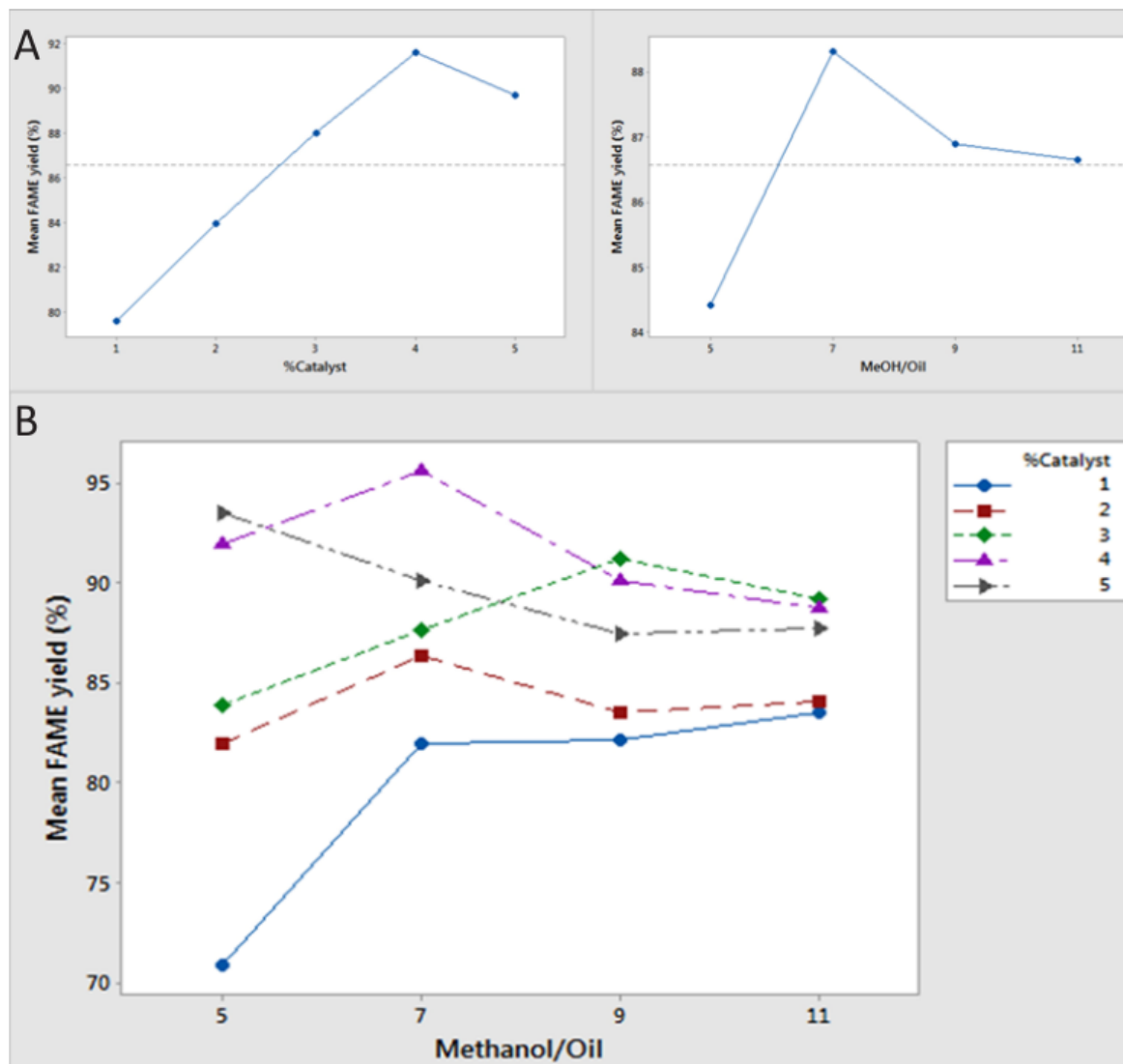
**Table 2.** Full factorial design of biodiesel production using *Pomacea* sp. shell as a catalyst. The order (top row to bottom row) reflects the sequence in which the experiments were carried out.

% catalyst (X <sub>1</sub> )	MeOH/oil (X <sub>2</sub> )	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

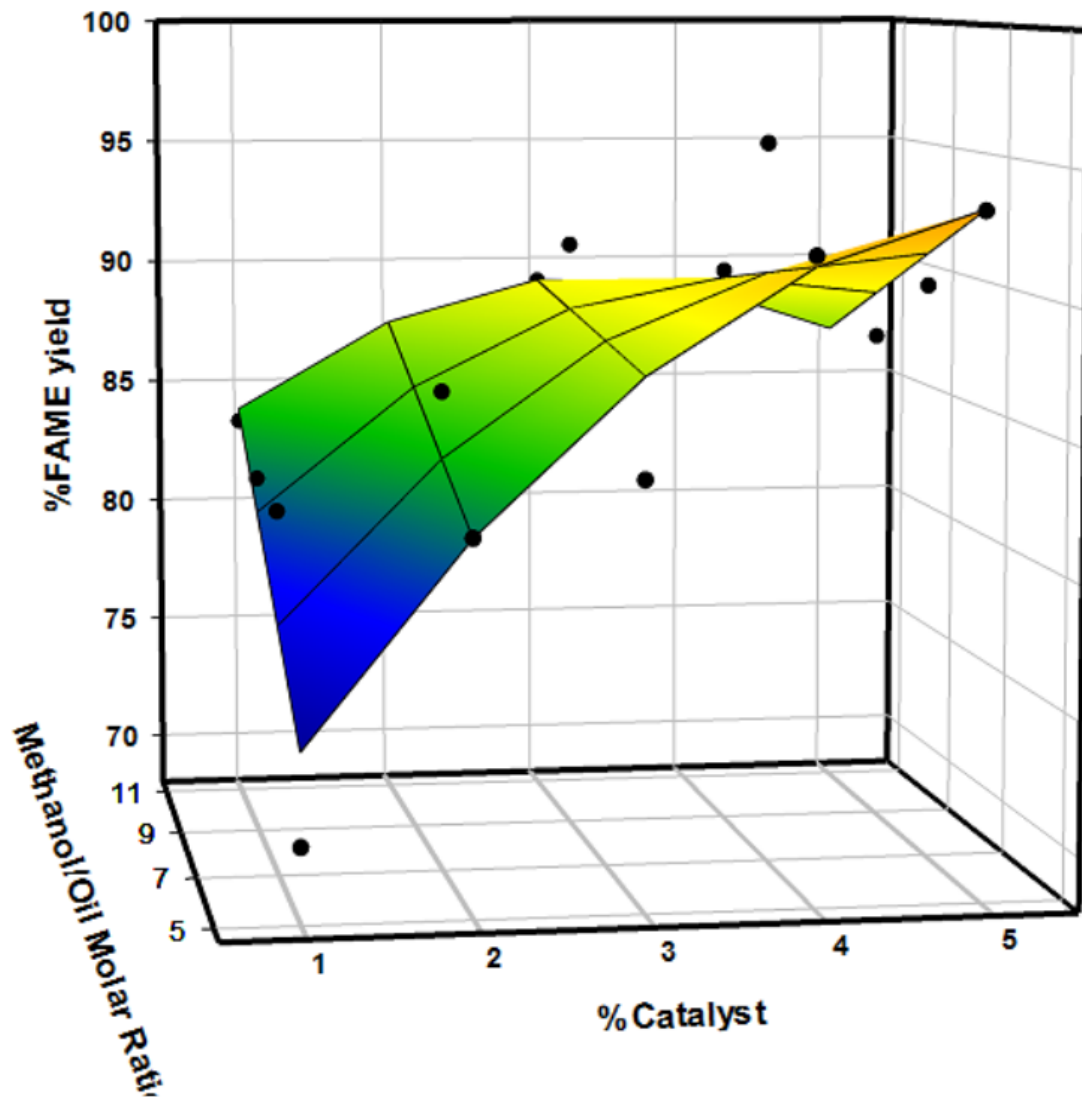
**Table 4.** Backward elimination of non-significant terms in the quadratic model.

Terms <sup>1</sup>	Original		Modified	
	Coefficient	$p$ -value ( $\alpha = 0.05$ )	Coefficient	$p$ -value ( $\alpha = 0.05$ )
$X_1^2$	-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

<sup>1</sup> $X_1$  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:

$$\% \text{ FAME yield} = -0.9259 \times \% \text{ Catalyst}^2 + 13.7328 \times \% \text{ Catalyst} + 2.2870 \times \text{Methanol / oil molar ratio} - 0.06741 \times \% \text{ Catalysts} \times \text{Methanol / oil molar ratio} + 53.4386$$

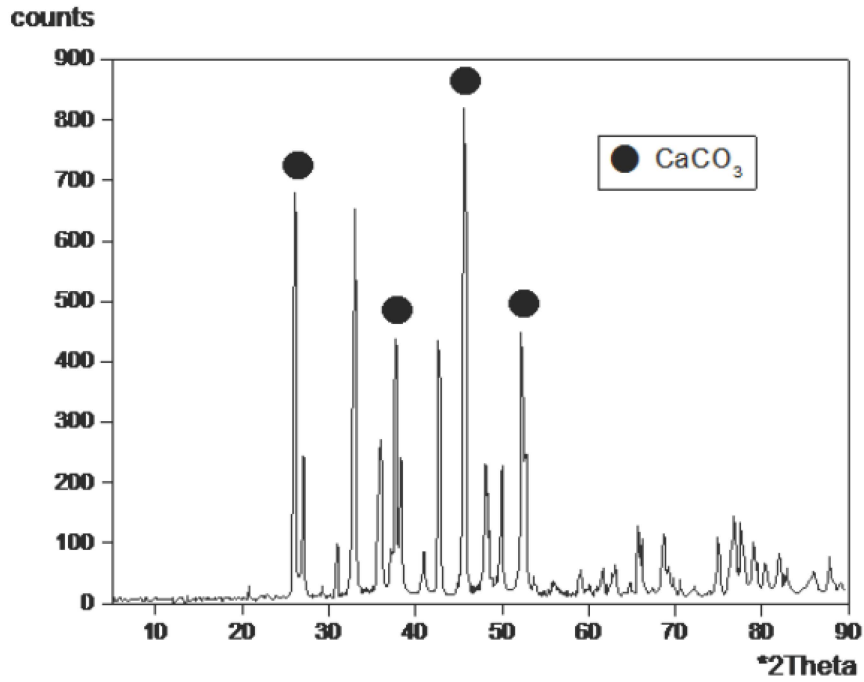


Fig. 3. X-Ray structure of apple snail shell (adapted from Margaretha *et al.*, 2012).

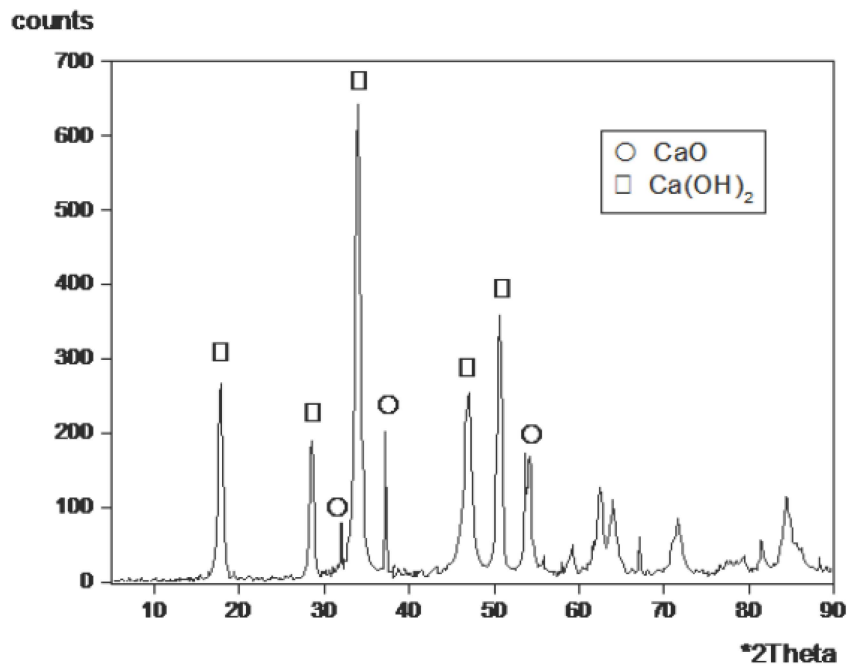


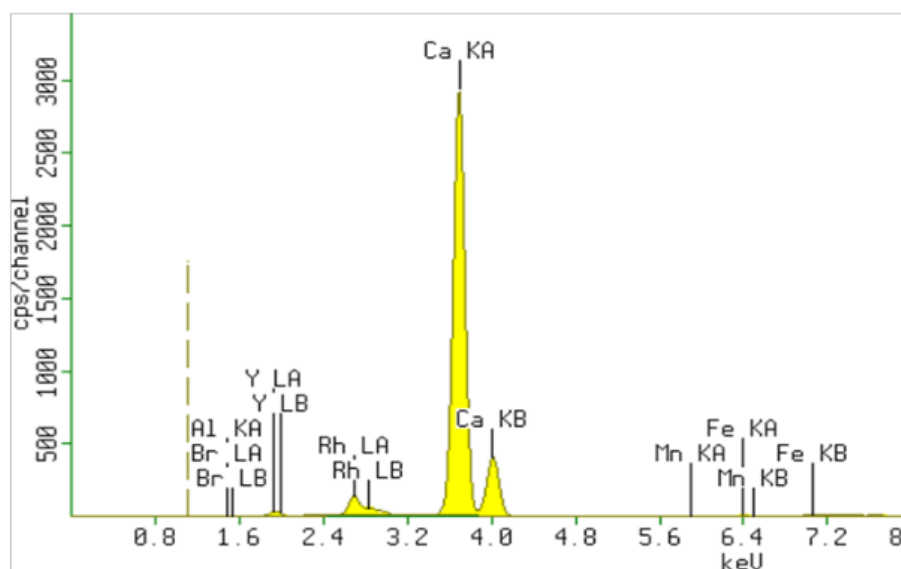
Fig. 4. X-Ray structure of CaO catalyst (adapted from Margaretha *et al.*, 2012).

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%.

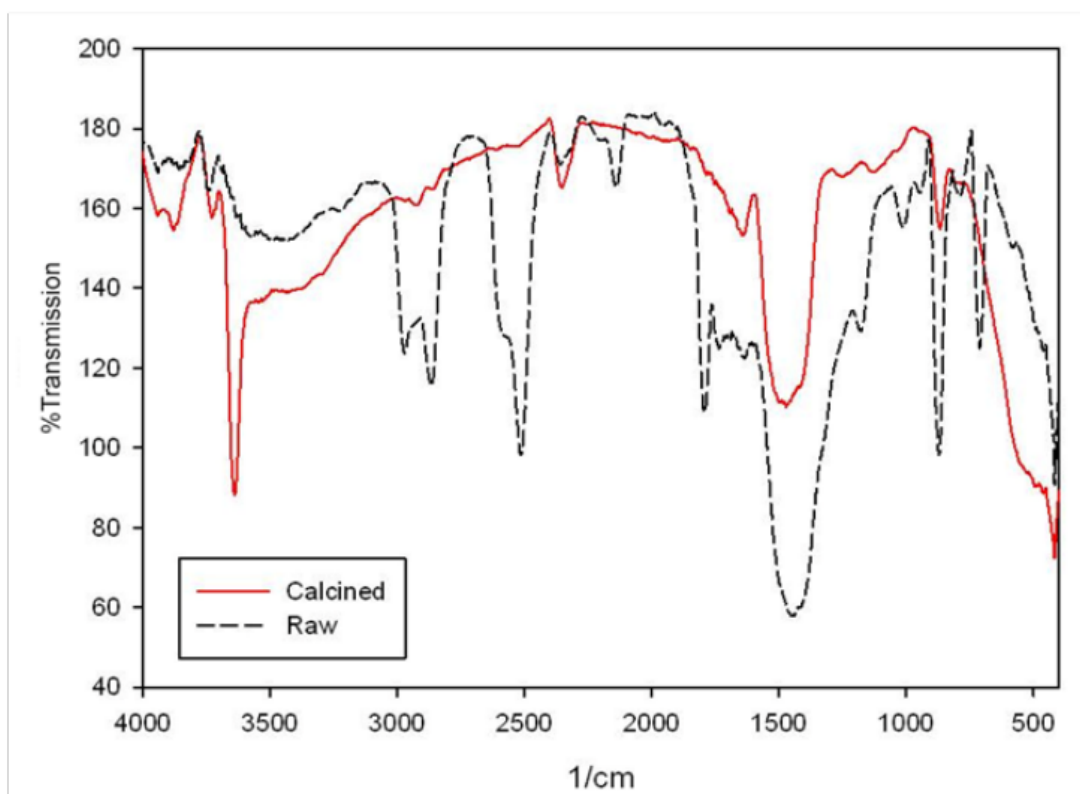
### ***Characterization of catalyst and apple snailshell***

The presence of  $\text{CaCO}_3$  and  $\text{CaO}$  in the apple snail shell and the catalyst are indicated by the XRD patterns of both materials (Figs. 3 and 4). The presence of  $\text{CaCO}_3$  is indicated by  $2\theta$  at 26.2, 33.1, 37.8, 45.8 and 52.4°, while  $\text{CaO}$  is indicated at 32.2, 37.3 and 53.8°. The presence of  $\text{Ca(OH)}_2$  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  $\text{CaO}$  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  $\text{Ca(OH)}_2$  because of the reaction of  $\text{H}_2\text{O}$  in the air with its highly basic surface (Margaretha *et al.*, 2012).

Fig. 5 depicts the XRF analysis of the bulk composition of the  $\text{CaO}$  catalyst. It consists mainly of calcium oxide (96.8 %), with some other metal impurities. The FTIR spectra of both snailshell and  $\text{CaO}$  are depicted in Fig. 6. The characteristic peaks of the  $\text{C=O}$  bond stretching and bending modes of  $\text{CaCO}_3$  are observed at  $3117\text{ cm}^{-1}$ ,  $2513\text{ cm}^{-1}$ ,  $1420\text{ cm}^{-1}$  and  $867\text{ cm}^{-1}$  for the combined shell catalyst and  $\text{CO}_2$



**Fig. 5.** XRF spectra of  $\text{CaO}$  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. <sup>2</sup> During the calcination process,  $\text{CaCO}_3$  is decomposed into  $\text{CaO}$  and  $\text{CO}_2$ . This is reflected by the FTIR spectra of the catalyst, which indicates the decreasing intensity of the characteristic peaks representing  $\text{CaCO}_3$ . After the calcination process, a new peak appears at  $3620\text{ cm}^{-1}$ , indicating the formation of basic  $-\text{OH}$  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 in accordance 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 <sup>2</sup> /g	17
Pore volume, cm <sup>3</sup> /g	0.04
Mean pore size, nm	3.2
Particle size, mesh	80/100
CaO content, %	96.83
SiO <sub>2</sub> , %	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.



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