November 2021

Regional Expansion and Evaluation of Potential Chemical Control for Invasive Apple Snails (Pomacea maculata) in Southwest Louisiana

Julian M. Lucero
Louisiana State University and Agricultural and Mechanical College

REGIONAL EXPANSION AND EVALUATION OF POTENTIAL CHEMICAL CONTROL FOR INVASIVE APPLE SNAILS (*POMACEA MACULATA*) IN SOUTHWEST LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Julian Martin Lucero
B.S., Louisiana State University, 2018
December 2021

Acknowledgements

The past two years conducting this research have been a very challenging and rewarding experience. However, completing this journey would not have been possible without the support of my family and friends. Their words of encouragement and advice have helped me push forward in times of uncertainty and have helped me accomplished everything that I have today. My gratitude for their presence in my life goes beyond words could express. I would like to dedicate this thesis to my parents Julian and Letty Lucero, my sisters Diana and Vanessa, and the several other family and friends that have helped me reached this accomplishment.

I would like to thank my major advisor Dr. Blake Wilson for giving me the opportunity to pursue a master's degree in his lab. Without him, none of this research would have been possible, and to say that he has been an amazing mentor and inspiration is the least that can be said to explain the person he has been to not only me, but to everyone in his lab. His knowledge and passion for agricultural research in entomology have inspired me and helped me prepare myself for the next steps in my career. I would also like to thank my committee members, Dr. Michael Kaller, Dr. Thanos Gentimis, and Dr. Rodrigo Diaz, for not only improving my research but also being among the most interesting and genuine professors I have had during my education. I would like give further thanks to the Head of the Entomology Department at LSU, Dr. Michael Stout, for allowing me to use his lab to conduct experiments and for his words of encouragement.

My gratitude is extended to Kim Landry and John Sonnier at the LSU AgCenter Rice Research Station for providing lab space to conduct toxicity assays and help in data collection. I would also like to give appreciation to Ross Hebert, Kevin Landry, and Christian Richard for allowing me to conduct on-site experiments at their farms. They were among the nicest and most

cooperative people I could have worked with and were willing to offer all their services in order for us to complete our research objectives.

Much appreciation is also expressed to my lab mates James Villegas, Megan Mulcahy, Leonardo Salgado, and Ethan Doherty. Without them, this research would have been difficult to complete. They brought me into the Wilson Lab and mentored me throughout this entire journey. They created a lab that only one could wish to be a part of, and I hope that life can bring them even more accomplishments. I would like to thank Michael Becker and Darrius Davis for introducing me to the Entomology Department at LSU and helping me establish myself in the department. I started out as a student worker in the same entomology lab as them, and they were also great mentors but even better friends.

Lastly, I would like to extend gratitude to the funding agencies that provided the financial support for my research. This research was funded by the Louisiana Rice Research Board and the LSU College of Agriculture.

Table of Contents

List of Tables

List of Figures

Abstract

The integration of monitoring and chemical control is an efficient strategy for managing invasive apple snails, *Pomacea maculata*, in the rice (*Oryza sativa* L.) and crawfish systems of southwest Louisiana. However, their current distribution, expansion rates, and susceptibility to chemical control methods in this area are not well known. This study evaluated the expansion of *P. maculata* in southwest Louisiana and assessed potential chemical control for *P. maculata* among toxicity assays using various application rates. The effects of potential chemical control were also assessed on a non-target species, the red swamp crawfish (*Procambarus clarkii*). *P. maculata* was recorded to have area expansion occur at approximately 880 km^2 per year and a northward expansion at a rate of 6 km per year with the majority of presence recorded in southern areas. *P. maculata* had a lower probability of being found in aquatic habitats with decreasing levels of dissolved oxygen. The treatment of 8 ppm using a liquid form of copper sulfate and the treatment of 12 ppm using a crystal form of copper sulfate each caused nearly 50% average *P. maculata* mortality and were the lowest effective treatments. No treatment of either form of copper sulfate had a significant effect on *P. clarkii* mortality. *P. maculata* egg mass hatching success and hatchlings were highly affected by a low treatment (5%) and a high treatment (10%) of crop oil adjuvant with no significant difference between the two treatments. The results suggest continuous *P. maculata* expansion in southwest Louisiana as well as copper sulfate treatments that are effective to *P. maculata* mortality and not effective to *P. clarkii* mortality. *P. maculata* egg masses can also be potentially controlled with crop oil. Future studies should attempt to find new factors associated with *P. maculata* occurrence in southwest Louisiana. More research should be conducted on the levels of copper sulfate that are actually ingested by *P. maculata* after initial exposure as well as new methods for crop oil applications on *P. maculata* egg masses.

Introduction and Justification

Rice (*Oryza sativa* L.) is one of the world's major food crops that is considered the staple food for more than half the human population. It is grown in every continent except Antarctica with the highest production and consumption occurring in Asia (Huggan 1995; Bandumula 2017). Although the United States is not one of the highest rice-producing countries in the world, it is still ranked among the most reliable and timely suppliers of high-quality rice. In the U.S., rice is mainly cultivated in the south with Louisiana being among the country's top rice producers (McBride et al. 2018).

Unfortunately, Louisiana farmers come across devastating rice pests, such as the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) and a complex of stem-boring lepidopteran pests (Lepidoptera: Crambidae), that reduce annual yields by feeding on rice (Harrell et al. 2019). A more recent invasive pest is the apple snail, *Pomacea maculata* (Gastropoda: Ampullariidae). Apple snails impact rice by consuming seedlings and causing significant decreases in total production (Litsinger and Estano 1993). *P. maculata* is native to South America but is an invasive pest in Asia, Europe, and Central America (Wilson et al. 2020). Its current presence in these rice-producing regions has caused serious economic losses, estimated up to $2.1 billion per year in the Philippines, Thailand, and Vietnam combined (Arfan et al. 2015). Apple snails can also be a health risk as vectors for toxic parasitic *Angiostrongylus* nematodes (Arfan et al. 2015). Their modes of introduction have varied from transportation through attachment to boats to intentional release to establish a food market or through the aquarium fish trade. In recent decades, apple snails have been found in a new region, the southeastern United States. In the U.S., apple snails were first observed in Texas in 1989 and later seen in Florida in the 1990s (Rawlings et al. 2007). However, early confusion with species identification between *P. maculata* and the similar *P. canaliculata*

left uncertainty towards which species of apple snail arrived first (Burks et al. 2013). Additionally, the confusion between the species caused researchers to mix the two species mistakenly when conducting past studies (Hayes et al. 2012; Burks et al. 2013). Once the snails were introduced, isolated populations were observed in various southern states including Louisiana. In Louisiana, the first report of *P. maculata* was in Gretna in 2006 and has since spread to other southern regions of the state (Benson 2019).

Over the last decade, *P. maculata* has established itself in Louisiana, but has only recently begun infesting rice and crawfish (*Procambarus* spp.) farms (Wilson et al. 2020). Observations suggest rapid expansion from natural waterbodies into rice and crawfish production systems occurred as a result of the 2016 flooding event in south Louisiana (Wilson et al. 2020). However, the snails' current distribution, rates of expansion, impacts to rice and crawfish production, and management strategies in Louisiana are unknown. The main objectives of this research were to: (1) determine how rapidly *P. maculata* populations are expanding and identify factors conducive to their establishment and (2) evaluate potential chemical control strategies to reduce *P. maculata* populations in rice and crawfish systems.

Chapter 1. Literature Review

1.1. *P. maculata* biology and life cycle

The apple snail, *Pomacea maculata*, is a large freshwater snail that has a polyphagous feeding behavior, amphibious respiration, and high fertility (Arfan et al. 2015). The males and females mate regularly with different partners (Burela and Martín 2011). Females lay spherical, vividly pink eggs in clutches that average about 1,500–2,100 eggs but can reach up to 4,700 eggs (Barnes et al. 2008; Burks et al. 2010; Kyle et al. 2011; Hayes et al. 2012). This is much higher than the number of eggs laid in one clutch for the similar *P. canaliculata* (200–300 eggs), and single egg diameter for *P. maculata* is also smaller (Barnes et al. 2008; Hayes et al. 2012). Females that have copulated once are capable of laying the same number of total eggs and egg masses as the females that have copulated frequently with a male showing that a female can store enough viable sperm for the rest of her fertile life after a single copulation (Burela and Martín 2011). Once the eggs are ready to be laid, the female waits until nightfall to crawl out of the water and lay her clutch onto a stable surface (Barnes et al. 2008). As the eggs get closer to hatching, the calcium hardens causing the pink eggs to fade to white (Hayes et al. 2012). Clutches are laid on emergent vegetation or other structures just above the water surface during the warmer months of the year (Kyle et al. 2011). In rice fields, egg masses are typically laid on firm stems of rice plants or on irrigation structures (Snyder and Snyder 1971). They remain dry for at least 6–9 days to maximize hatching success (Burks et al. 2017). Female age, non-fertilization, and water damage can all be explanations towards low hatching success or total clutch failure (Burks et al. 2017). In a study conducted in Florida, apple snails older than 3 years showed to have lower hatching success than younger snails (Burks et al 2017).

After the eggs hatch, the hatchlings drop directly into the water below (Barnes et al. 2008). One-day old hatchlings have shells that are roughly 1.2 mm tall and have a whorl about 0.8 mm wide (Hayes et al. 2012). They also have a semi-translucent operculum (calcareous trapdoor-like plate that is attached to the upper surface of the snail's foot) and can have a width of approximately 1.1 mm (Hayes et al. 2012; Xu et al. 2019). During the initial 6 weeks, shell height can increase from 10 to 20 mm and after an additional 6 weeks, the shell may reach a height of about 28 mm (Kyle et al. 2009). As the snails develop, they mostly feed on various soft, aquatic vegetation, such as rice seedlings, duckweed (*Lemna minor*), taro (*Colocasia esculenta*), and algae, but they are also known to consume other smaller snails, decomposing organic matter, snail eggs, and amphibian eggs (Mohan 2002; Carter et al. 2018). Various life history traits of *P. maculata* remain unstudied. In outdoor mesocosms, maturation and copulation were first observed at 3 to 4 months after hatching (Bernatis and Warren 2014). Adults have shells that can weigh more than 200 g and reach heights of 165 mm (Hayes et al. 2012). Adult shells are thick and spherical and tend to have yellowish-brown to greenish-brown coloration with 5–6 whorls (Kyle et al. 2009).

Although aquatic, evidence has shown that apple snails are able to survive extensive periods without water by closing their shells with the operculum and burrowing into muddy substrate (Ramakrishnan 2007). A stable isotope analysis conducted on *P. maculata* shells suggested 1-to-3-year lifespans for apple snails (Arnold et al. 2014). They survive short periods out of water by using their lung instead of their gill to breathe (Ramakrishnan 2007). These methods are used when apple snails overwinter in rice fields (Ito 2002). During the winter, rice farmers drain their fields to work the land and prepare for the next growing season (LSU AgCenter 2014). In warmer southern regions, apple snails are able to hibernate by remaining on the surface or burrowing themselves into the soil (Ito 2002). However, most snails, specifically those in

northern regions, prefer to overwinter in the limited shallow areas of drainage canals that contain water with high dissolved oxygen and low pH levels (Ito 2002). If the water in the shallow areas reach very low temperatures, the snails sink to the bottom of deeper areas, close their operculum, and go into hibernation. These overwintering snails cause the most damage to rice, because they establish stable populations that emerge and begin feeding on the new rice plants once farmers begin to irrigate their fields (Ito 2002).

1.2. Distribution and impact

P. maculata is native to South America being found in parts of Uruguay, Paraguay, Argentina, and several areas of Brazil (Hayes et al. 2012). Over time, *P. maculata* has been introduced to other parts of the world. Once it is introduced, natural expansion can occur through floatation on moving water, attachment to mobile objects, and crawling up and down water ways. (Levin et al. 2006; EFSA Panel on Plant Health 2012). In the 1980s, apple snails were transported to Asia as a potential human food source through the aquaculture industry, but markets failed to develop resulting in the release and escape of snails (Mochida 1991; Naylor 1996; Wada 1997). Soon after their introduction, the apple snails became major rice pests causing large decreases in rice production, such as 41–50% yield loss in the Ifugao Rice Terraces of the Philippines (Joshi et al. 2001). However, misidentification of *Pomacea* spp. and false assumptions led to uncertainty in the introduction and distribution of *P. maculata* within Southeast Asia. *P. canaliculata* was listed as one of the world's 100 worst invasive species, but a few studies that contributed to this listing probably involved *P. maculata* as well (Lowe et al. 2004). In 2009, *P. maculata* was introduced to Spain via the aquarium trade (EFSA Panel on Plant Health 2012). After only 5 months, they expanded their range to cover more than 9,500 hectares, which included several rice fields (López et al. 2010). This prompted the Spanish Ministry of Environment, and Rural and Marine Affairs

to prepare a pest risk analysis for *P. maculata* the following year of their introduction (EFSA Panel on Plant Health 2012).

P. maculata populations are thought to be the result of multiple introductions to the southern U.S. through the aquarium trade (Karatayev et al. 2009). In the U.S., pet stores receive various freshwater snails, but store owners are unable to identify the snail species (Karatayev et al. 2009). One apple snail species, *Pomacea diffusa*, is not known as an agricultural threat making it easily available in the aquarium trade (Burks et al. 2013). Without any identification skills, juvenile *P. maculata* can be perceived as *P. diffusa* and unintentionally sold to the public. Mature *P. maculata* can reach large sizes and excrete high quantities of mucus and egg masses making them undesirable in aquariums, so pet owners tend to release them into the environment (Burks et al. 2013). Since their initial introduction to Texas in 1989, *P. maculata* managed to establish populations across several states, including Mississippi, Alabama, and Florida though it is unknown whether these populations resulted from new introductions or spread within the U.S. (Rawlings et al. 2007; Byers et al. 2013). Despite being mainly found in warm locations, *P. maculata* have been observed in more northern areas in the U.S., such as Charleston, South Carolina. Suitable temperatures in regions further north have also been indicated through climatic modelling (Byers et al. 2013).

After arriving in Louisiana in 2006, *P. maculata* began to populate the Atchafalaya Basin and other bayous connected to the Gulf Intracoastal Waterway (Burks et al. 2017). In the last few years, farmers in the southwestern region of Louisiana have noticed high populations of apple snails in their rice and crawfish farms (Wilson et al. 2020). In 2018, initial snail sightings were reported by a farmer on his rice and crawfish farm in the Indian Bayou near the Mermentau River. Around the same time, another farmer reported high numbers of snails on his rice and crawfish

farm located in Abbeville near the Vermillion River. The increase of snail sightings on these farms may have been mostly due to the severe 2016 floods that helped move snail populations from infested waterbodies, such as the Vermillion River and Mermentau River, into the southwestern region of Louisiana (Wilson et al. 2020). Farmers that use surface water connected to infested water ways for field irrigation also facilitate snail introduction onto new farms (Wilson et al. 2020). The first report of apple snail damage to rice in Louisiana occurred in 2020 after a 50-acre field of water-seeded rice was destroyed and had to be replanted (Schultz 2020). Crawfish production has been negatively impacted by the high populations of apple snails as well (Wilson et al. 2020).

During crawfish season, farmers use bait to attract crawfish that also attracts the omnivorous apple snails to their crawfish traps (Wilson et al. 2020). When doing so, large apple snails may block the trap entrances, and smaller snails can accumulate inside the traps in large quantities reducing crawfish capture. Sorting trap capture is also impeded as farmers have to remove all the snails that are caught (Wilson et al. 2020). On some farms located in Vermillion and Jefferson Davis Parishes, apple snails were so abundant that farmers were forced to stop harvesting and had to drain their ponds early in the season (Wilson et al. 2020). Apple snails not only injure crop production but field infrastructure as well. They do so by burrowing into field levees during periods of aestivation. Large populations of apple snails can weaken levees through constant burrowing causing high maintenance costs for farmers (Burlakova et al. 2009). Farmers must repair these levees before beginning their crop farming (McClain et al. 2007).

Apple snails can also pose as a big threat to important wetland ecosystems. Wetlands are one of the most productive ecosystems that provide numerous services and economic benefits, such as erosion control, maintenance of high animal and plant species diversity, protection against storms and floods, and sources of human nutrition (Mitsch and Gosselink 2007; Horgan et al.

2012). An important determinant of wetland form and function is the macrophyte community found within these ecosystems (Horgan et al. 2012). Macrophytes improve wetlands by providing food and microhabitats for aquatic organisms, recycling nutrients, providing supplementary food for people and livestock, purifying water, and providing physical structures that determine sedimentation levels, water flow patterns, and light and temperature gradients through the water body (Horgan et al. 2012). As apple snails move into wetlands, larger quantities of macrophytes are consumed resulting in striking effects on water turbidity and chemistry, lowered amounts of food and habitat sources for natural species, and the overall degradation of the wetlands (Horgan et al. 2012).

1.3. Rice and crawfish production in Louisiana

In Louisiana, rice was grown as early as 1718 (Babineaux 1967). The crop was initially limited to the lowland region connecting with the Mississippi River, but after technological advancements that took place in the 1880s, such as the completion of the Southern Pacific Railroad and the mechanization of rice farming, the modern rice industry in Louisiana was formed (Babineaux 1967). With the development of the Louisiana rice belt, whose center can be found on the Southwest prairie where Crowley is located, production has been able to increase throughout the years (Babineaux 1967). In 2020, the total acreage of rice planted was 480,000 acres with 474,000 acres being harvested, and yield was at approximately 6,820-lb per acre (NASS 2021). An estimated 32,306,000 cwt of rice was produced with a value of $397,364,000. Overall, the value of rice production had increased by 28% from the previous year in Louisiana (NASS 2021). Rice is best produced when grown on nearly level fields under flooded conditions (LSU AgCenter 2014). In order to maximize rice yields, flooding and draining rice fields in a timely manner is important. High yields can be reached if fields are flushed in 2–4 days, flooded in 4–5 days and

drained, dried, and then re-flooded in 2 weeks after initial planting (LSU AgCenter 2014). In southwest Louisiana, it is recommended to plant rice towards the end of winter into early spring (LSU AgCenter 2014). Earlier planting dates can help farmers by giving them the opportunity to produce a ratoon, or second, crop. Ratooning occurs when farmers harvest grain from tillers derived from the stubble of a previously harvested crop (LSU AgCenter 2014). However, rice that is planted early in colder conditions may have slow emergence and poor seedling vigor resulting in stand reduction and seedling disease, and if planted late in the season, rice yield may be damaged by pests and higher temperatures (LSU AgCenter 2014). The main rice crop can be harvested from the end of July to September depending on planting dates and weather conditions, but if a ratoon crop is grown, the main crop should be harvested by mid-August to make sure the ratoon rice has adequate time to grow (LSU AgCenter 2014).

Rice production in Louisiana can be conjoined with crawfish cultivation. Louisiana's commercial sales of crawfish began in the late 1800s, when crawfish were harvested from natural waters in the southern region of the state (McClain et al. 2007). Although harvests of farmed crawfish occur in other states, Louisiana is the largest producer of crawfish in the U.S. accounting for 90–95% of total U.S. annual production (McClain et al. 2007). In 2018, farm-raised crawfish was Louisiana's most valuable aquaculture sector with 151.8 million pounds in total crawfish production and $209 million in gross farm value (LSU AgCenter 2018). Two species of crawfish compose Louisiana's crawfish harvest, which are the red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus zonangulus*) (McClain et al. 2007). Both species can be found within the same ponds, but the red swamp crawfish is more common and desired. Annual crawfish harvest in Louisiana is usually composed of 70–80% red swamp crawfish (McClain et al. 2007). The red swamp crawfish produces small eggs and can spawn year-around in the south. It

can survive high nutrient rich waters with low dissolved oxygen content and will feed at temperatures higher than 30°C (McClain et al. 2007). Eggs are mostly laid during the fall and winter months. Juveniles thrive in systems that are flooded early in the fall, and they mainly appear from September through December and mature from April to June (McClain et al. 2007). Mature red swamp crawfish have a red pigment and short and flat claws. When high temperatures persist in late spring and early summer, they mate then create burrows where they survive until the fields are reflooded in the fall, and the new eggs hatch (McClain et al. 2007). High stress for these crawfish can be due to habitats with low oxygen, elevated temperatures, poor nutrition, and overcrowding (high competition). Crawfish ponds are usually between 10 to 40 acres but can include more than 1,000 acres in areas where water levels are controlled in natural habitats for production (McClain et al. 2007). Farmers flood the ponds with at least 20 to 30 cm of water, and may stock mature crawfish to produce young naturally or depend on reproduction by unharvested crawfish from the previous year to stock their ponds (McClain et al. 2007). The ponds are flooded in autumn as spawning and emergence of females with hatchlings or eggs from burrows begin to occur. The crawfish feed on rice, sorghum-sudangrass [*Sorghum bicolor* (L.) Moench], and natural vegetation that is grown in the summer when the ponds are drained (McClain et al. 2007). They are usually caught in pyramid traps baited with gizzard shad, menhaden, or manufactured baits (McClain et al. 2007). These traps may be employed as early as November to check for juvenile production and continue to the following June. Most crawfish in Louisiana are harvested from March to June when they are most active and densities are higher (McClain et al. 2007).

Since rice and crawfish cultivation have similar settings, Louisiana farmers use two strategies that incorporate both crops into the production of the other in order to maximize variety of production. One strategy is monocropping, in which one crop is harvested, and production

usually occurs in the same location for multiple production cycles (McClain et al. 2007). A pond may be stocked with mature crawfish anywhere from April to early July (McClain et al. 2007). The pond is drained about 2 to 3 weeks after stocking. Rice is planted during the summer when the pond is completely drained and serves as the main nutrition for the crawfish. Once the pond is reflooded in autumn, farmers monitor the crawfish population using baited traps and begin harvesting when the catch can be economically justified (McClain et al. 2007). Harvesting continues until the next summer when the ponds are drained, and the cycle restarts. This strategy allows farmers to manage for maximum production without the concerns that come with growing other crops, including seasonal limitations and pesticide exposure (McClain et al. 2007).

The second strategy is a crop rotational system, which is composed of two basic systems (McClain et al. 2007). One system is rice-crawfish-rice, in which rice is grown and harvested in the summer followed by the cultivation of crawfish in autumn, winter, and early spring in the same field every year (McClain et al. 2007). An initial stock of crawfish is introduced to the rice after it has been planted for about 4 to 7 weeks. Once the rice is harvested, the remaining rice is fertilized with a nitrogen-based fertilizer and irrigated in order to obtain a ratoon crop. (McClain et al. 2007) The field is flooded in the fall, and the growth and harvest season of crawfish begins. In this system, the crawfish season is shortened to accommodate for planting of the subsequent rice crop (McClain et al. 2007). Although two crops can be obtained in one year, this farming method is less common, because farmers are unable to produce maximum yields for either crop and can potentially expose their crawfish to pesticides used in rice (McClain et al. 2007). The other system is rice-crawfish-fallow or rice-crawfish-soybean. This structure uses similar practices to those used in the rice-crawfish-rice system except rice is not grown in the same field during successive years in order to control for rice disease and weeds. Since crawfish cultivation follows rice culture,

crawfish is also not grown and produced in the same field during consecutive years (McClain et al. 2007). Once the crawfish season ends, farmers can plant a different crop, such as soybean or hay, or leave the field fallow. This cropping system is preferred, because three crops per field can be obtained in two years, each crop is easier to manage, and the crawfish season can be extended for higher production (McClain et al. 2007). Unfortunately, rice and crawfish cultivation require fields to stay flooded for most of the year making them optimal habitats with abundant sources of food for invasive apple snails.

1.4. Management of *P. maculata*

1.4.1. Monitoring

Monitoring can play a key role in mitigating invasive species by providing nonbiased information that can be used to make well-informed management decisions (FWS 2009). The results can be used to show where management actions are successful and where there is a need for modification to the actions that are ineffective (FWS 2009). Although there is current apple snail monitoring that is being conducted based on citizen reports, there is no monitoring being done that is specific to *P. maculata* expansion into the rice and crawfish systems of southwestern Louisiana. Monitoring and analyzing the rates of expansion and factors that influence apple snail establishment in these crop systems is an effective initial step in creating a management plan for *P. maculata*. One method that can achieve this monitoring and analysis is by developing a predictive model. In a study conducted by Byers et al. (2013), the Maximum Entropy (MaxEnt) model was used to predict the distribution of *P. insularum* (currently known as *P. maculata*) and the environmental factors that contribute to its distribution in the southeastern U.S. Byers et al. (2013) conducted site surveys at locations based off *P. maculata* distribution data gathered from the USGS Nonindigenous Aquatic Species database. Bioclimatic variables, such as annual mean

temperature and annual precipitation, were obtained from a global climate data source (WorldClim) to be used for the modelling. Abiotic factors, including pH, salinity, and temperature, were retrieved to determine the apple snails' tolerance towards these different abiotic factors. The results showed that *P. insularum* should remain within the southeastern U.S. where factors, such as temperature and precipitation, limit expansion outside this range. Low pH levels in blackwater swamps were predicted to prevent apple snail establishment within these areas of the southeastern region, even though these areas have a suitable climate. The model did an efficient job at using current climate and environmental variables to predict potential distribution. However, there were potential biases with false negatives, such as a habitat may have become suitable but the species had yet to colonize it, or the species may have been in an area but was not discovered yet (Mackenzie 2006; Beale et al. 2008).

1.4.2. Biological control

Various animals found throughout southwestern Louisiana are known to prey on *P. maculata*, such as limpkins (*Aramus guarauna*), American alligators (*Alligator mississippiensis*), raccoons (*Procyon lotor*), redear sunfish (*Lepomis microlophus*), ducks (Anseriformes: Anatidae), turtles (Testudines), rats (*Rattus* spp.), and fire ants (*Solenopsis invicta* Buren) (Mohan 2002; Fasulo 2004; Elsey et al. 2018). However, due to the apple snails' high reproductive rates, natural predation does not appear to be sufficient in controlling snail populations. Two biocontrol systems that have been studied to decrease apple snails in rice fields are rice-duck and rice-fish systems (Carvalho de Brito and Joshi 2016). In a study conducted by Teo 2001, a variety of ducks, including mallard (*Anas platyrhynchos* L.) and Muscovy (*Cairina moschata* L.), were used to control for *P. canaliculata* in direct-seeded and transplanted rice cultivation. The results varied by duck species and time of release but ultimately showed that ducks in groups of 5 to 10 per hectare

were able to decrease pest density from 5 to less than 1 snail per square meter. The ducks fed on more *P. canaliculata* in the transplanted rice, but there was no statistical significance in the difference of feeding activity between the two planting types. This form of control has been effective in some Asian and Latin American rice systems, but it may be costly (Carvalho de Brito and Joshi 2016). The common carp (*Cyprinus carpio* L.) can adapt well to different farming environments (Teo 2006), and a density of 10 fish per plot or 2,041 fish per hectare is recommended for apple snail control in rice (Carvalho de Brito and Joshi 2016). In a separate study conducted by Halwart et al. 2014, common carp and Nile tilapia (*Oreochromis niloticus* L.) were used in replicated field plot trials to control *P. canaliculata* in transplanted rice during dry and wet seasons. They were able to show that 58–87% of *P. canaliculata* populations were suppressed by common carp, and 48–87% were suppressed by Nile tilapia throughout both seasons. Both fish species showed greater suppression than the control plots that contained no fish. However, larger *P. canaliculata* were not fed on by either fish species allowing for continual infestation.

Parasitic organisms can also help in mitigating apple snail populations on agricultural systems. Maketon et al. (2009) studied the efficacy of the parasitic fungus *Paecilomyces lilacinus* on *P. canaliculata* eggs and hatchlings. They hypothesized that enzyme hydrolysis would help fungal mycelial penetration through egg and juvenile cell walls. Their results showed that after eggs were treated with the fungus, there was only 12% hatching success (100% in the controls), and 100% mortality of 1-day old juveniles, susceptible to the conidia. However, increased development in the juveniles resulted in lower susceptibility to the fungal enzymatic activity. The pathogenicity of the nematode *Heterorhabditis bacteriophora* on *P. canaliculata* was evaluated in another study and showed that the inoculation of up to 16,000 nematodes for each snail led to septicemia and 100% snail mortality within 96 hours (Salcedo 2013).

1.4.3. Cultural control

P. maculata consumes rice by rasping the soft stems of young rice plants and eating the leaves during crop establishment (Horgan 2017). Three main factors that make seedlings vulnerable to apple snail predation are the depth of rice paddy water, stem thickness, and snail population density and age structure (Teo 2003). Large adult snails can consume between 7 to 24 rice seedlings per day and are capable of feeding on seedlings of various ages (Litsinger and Estano 1993; Horgan 2018). However, when water depth is at approximately shell height, snail movement is limited. This allows farmers to manage larger snail damage by lowering the depth of rice paddy water (Litsinger and Estano 1993; Teo 2003). Smaller snails can only feed on the younger seedlings, but they still have high feeding rates (Teo 2003). Pre-germinated rice seed and young rice seedlings are substantially damaged by these smaller snails even at low water depth (Teo 2003). As the seedlings grow, increases in their stem thickness, hardness, and weight make them more resistant to apple snails (Horgan 2018).

Rice that is transplanted as older seedlings is generally more resistant to high snail population densities than direct-seeded rice, but due to its lower labor costs, direct-seeding appears to be a more ideal practice for managing the snails (Litsinger and Estano 1993; Horgan 2018). Dry-seeding (i.e., drill-seeding) is the preferred direct-seeding practice for snail control, because the planting and initial development of rice are done under dry conditions when apple snails are dormant (Wada 2002). Drill seeding is the most common practice of planting in Louisiana, and is thought to be a key factor in limiting snail impacts to rice to date. For efficient snail control in wet-seeding, draining the field after sowing can highly decrease snail damage. A drainage period of 2–3 weeks after sowing has been shown to almost eliminate snail damage in direct-seeded paddies with snails (Wada 2002).

Chapter 2. Range expansion and distribution of *P. maculata* in southwestern Louisiana

2.1. Introduction

The apple snail, *Pomacea maculata*, has been in Louisiana for approximately 15 years. However, not much is known about its current locations or rates of expansion in the agricultural systems of the southwestern region. With the first report of *P. maculata* consuming rice in Louisiana in 2020, there is much concern over the potential future decrease in the state's rice production. In addition, large populations of snails have begun to infest crawfish farms making fishing harder for farmers and reducing their yield. As *P. maculata* continues to expand its range in southwest Louisiana, monitoring snail expansion in rice and crawfish fields and water ways as well as surveying the snail's population densities in areas where they are already established are critical starting approaches in creating an integrated management plan. These techniques will help in detecting current *P. maculata* movement and allow us to inform farmers when the snails are in the area. Monitoring locations where *P. maculata* have already colonized will provide data on current population densities and the factors that make these certain locations suitable for their establishment. Therefore, the specific research objectives of this study were to: 1) determine current distribution in rice and crawfish systems, 2) measure rate of spread, and 3) assess factors associated with high populations.

2.2. Materials and methods

Site surveys. Site surveying is an efficient method in locating and monitoring current *P. maculata* populations. A total of 46 sites were established in rice and crawfish fields and adjacent water ways located in Iberia, Vermilion, Jefferson Davis, Lafayette, Acadia, Calcasieu, St. Landry, Allen, Evangeline, and Cameron Parishes from September to October 2019. Sites were chosen based off previous *P. maculata* sightings at rice and crawfish farms in southwestern Louisiana.

Sites were separated by an average of 16 km apart from one another. Each site consisted of a 1.5-m PVC pipe hammered approximately 30 cm into the ground. The PVC pipe was used as a structure for egg masses to be laid on to provide targeted-monitoring sites. Coordinates for each site were tracked using a Garmin Etrex 20x GPS (Garmin Ltd., Olathe, KS, USA). All sites were checked monthly from 2019 to 2021 giving an estimation of two years to complete monitoring. Sites were added and adjusted to accommodate for new expansion. The mapping of sites was conducted with QGIS 3.14.15 to estimate current *P. maculata* distribution in southwest Louisiana. QGIS (formerly known as Quantum GIS) is a free and open-source geographic information system (GIS) that provides spatial analysis tools for determining feature statistics and performing geoprocessing activities as data interpolation (QGIS Development Team 2021).

Survey date, daily temperature, environment type, and *P. maculata* presence were recorded for each survey session. Environment type consisted of non-crop (rivers, canals, and bayous) and crop (rice and crawfish fields) habitats. Water parameters, such as water level, water temperature, pH level, conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO), were also recorded in 2021 using a portable pH/EC/TDS/temperature meter (Model HI9813–6, Hanna Instruments, Woonsocket, RI, USA) and a portable dissolved oxygen meter (Model HI9146, Hanna Instruments). Water levels were measured in four measurements: None (0 cm), Low (0–10 cm), Medium (10–20 cm), and High (>20cm) using a 16-ft tape measure (Table 2.1).

Table 2.1. Sample of collected parameters in southwestern Louisiana, 2021.

ID	Latitude	Longitude	Date	Temp.	Env. Type	Snail Presence	Water Level	pH	Water Temp.	TDS (ppm)	EC (mS/cm)	DO (mg/L)
4	30.02173	-92.411976	Jun-2021	30.6	NC	0	H	5.8	27.4	62	0.08	3.33
5	30.03813	-92.575050	Jun-2021	30.6	C	1	M	5.7	29.9	63	0.08	3.39
6	29.98773	-92.700933	Jun-2021	30.6	C	1	H	6.6	31	132	0.18	2.55
7	30.14207	-92.749150	Jun-2021	28.9	NC	0	H	6.3	30	194	0.27	1.15
8	30.15148	-92.507000	Jun-2021	31.7	C	0	H	7.8	29.4	197	0.28	2.85

An initial map of the original 46 sites was constructed using an Excel spreadsheet containing the sites' coordinates. The first step in creating the map was to import a terrain Google Map layer as an XYZ tile and add it to the new QGIS canvas. A dataset containing Louisiana Parish boundaries was then imported to the canvas and used as a template for the digitizing of the project boundary. Digitizing is the process of transforming analog information into digital information (Campbell and Shin 2012). The digitization of spatial information can be done through three primary methods: tablet digitizing, heads-up digitizing, and vectorization (Campbell and Shin 2012). The project boundary for the map was constructed using heads-up digitizing. This method consists of manually tracing the outlines of features from another dataset on a computer screen (Campbell and Shin 2012). Once the project boundary was completely digitized, it was saved and exported as a polygon shapefile to the canvas with the proper coordinate system for Louisiana (WGS 84/UTM zone 15N). The Excel datasheet that contained the coordinates of each site was saved as a CSV (comma separated values) file and then imported to QGIS. It was then exported onto the canvas with the same coordinate system creating a vector layer of points. Once a site turned positive for snail presence, it was replaced with a new negative site and added to the map.

Rate of expansion. The rate of *P. maculata* expansion was based on distance between a previous positive site and a new positive site and survey date. Spatial interpolation of positive sites was conducted with QGIS 3.14.15 to estimate potential spread across rice and crawfish systems and water ways in southwest Louisiana. Spatial interpolation can be used to calculate data such as precipitation, population density, elevation, water table, temperature, and snow accumulation (QGIS Development Team 2021). There are several interpolation methods, but for this research, the Inverse Distance Weighted (IDW) method was used. Compared to other interpolation methods,

IDW is a simpler non-geostatistical approach that uses random exponential weighting of influence that each sample has according to distance with the assumption that nearby sample data points are more similar than data points that are more distant (Lu and Wong 2008; Zarco-Perello and Simões 2017). Weighting is inversely proportional to the power of distance (Lu and Wong 2008). As sites turned positive, the IDW method assisted by showing potential positive areas in relation to the distance from a previous positive site, which were checked in the following month's survey session.

The IDW spatial interpolation was conducted using a grid resolution of 1.5 km with a distance coefficient (p-value) of 3. The grid resolution was related to the distance between the sampled points (Boots and Getis 1988) and followed the Nyquist frequency concept, which states that the grid resolution should not be more than half the average spacing between the closest point pairs ($p \leq \frac{\bar{h}_{ij}}{2}$) where $\bar{h}_{ij}$ is the mean shortest distance, or average distance between the two closest point pairs (Hengl 2006). For this interpolation, the mean shortest distance was 3,077.53 m, so $p \leq \frac{3{,}077.53}{2}$. Thus, $p \leq 1{,}538.765$ m resulting in a grid resolution of 1500 m, or 1.5 km. The distance coefficient of 3 was used for the interpolation to decrease the influence of distant points (Menke et al. 2016). A scale of 0 to 1 was used to show the probability of apple snail presence in an area based on whether a site turned positive or stayed negative.

Analysis of factors associated with positive sites. Daily temperature, environment type, and snail presence were recorded from each site. Water parameters, such as pH level, conductivity, total dissolved solids, and dissolved oxygen, were also collected. Data were analyzed with generalized linear models (PROC LOGISTIC, SAS® Institute 1999). Snail presence was the binary response variable and the other collected parameters were the predictor variables. The DESCENDING option was used to order a value of 1 for snail presence in order to model the

probability of snail presence (SAS® Institute 1999). The stepwise selection was used to remove any insignificant effects from the model and leave the significant predictor variables. Variables with a significance level of 0.15 or lower (SLENTRY = 0.15) were allowed into the model, and variables with a significance level of 0.05 or lower (SLSTAY = 0.05) were allowed to stay in the model (SAS® Institute 1999). The LACKFIT option was used to compute the Hosmer and Lemeshow Goodness-of-Fit test, which showed how well the final selected model fit the data (SAS® Institute 1999; Hosmer and Lemeshow 2000).

2.3. Results

Site surveys. Among the original 46 sites, a total of 24 sites were in rice fields, 1 in a crawfish pond, and 21 in adjacent water ways (Figure 2.1). As sites were adjusted for *P. maculata* presence, the locations of 11 sites changed from when they were first placed at the end of 2019 to

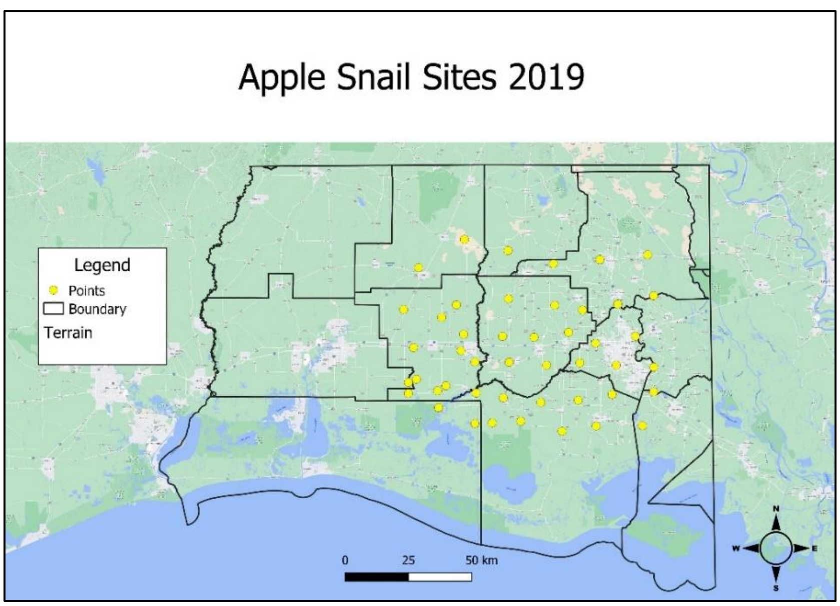


Figure 2.1. Terrain map of original 46 sites and parish boundaries in southwestern Louisiana, 2019.

the end of 2020. This resulted in 17 in rice fields, 3 in crawfish ponds, and 26 in adjacent water ways (Figure 2.2). Only 2 site locations were changed from when they were placed at the end of

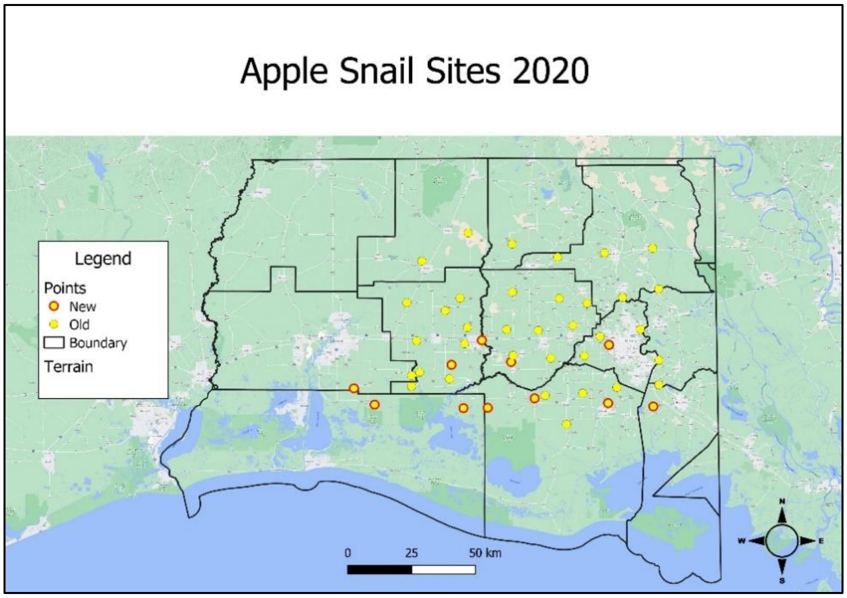


Figure 2.2. Terrain map of adjusted 46 sites, including 11 new site locations, and parish boundaries in southwestern Louisiana, 2020.

2020 to the end of 2021. Site 1 was relocated to another canal, and site 5 was moved from a canal to a rice field (Figure 2.3). Sites were moved at an average distance of 7 km north from the

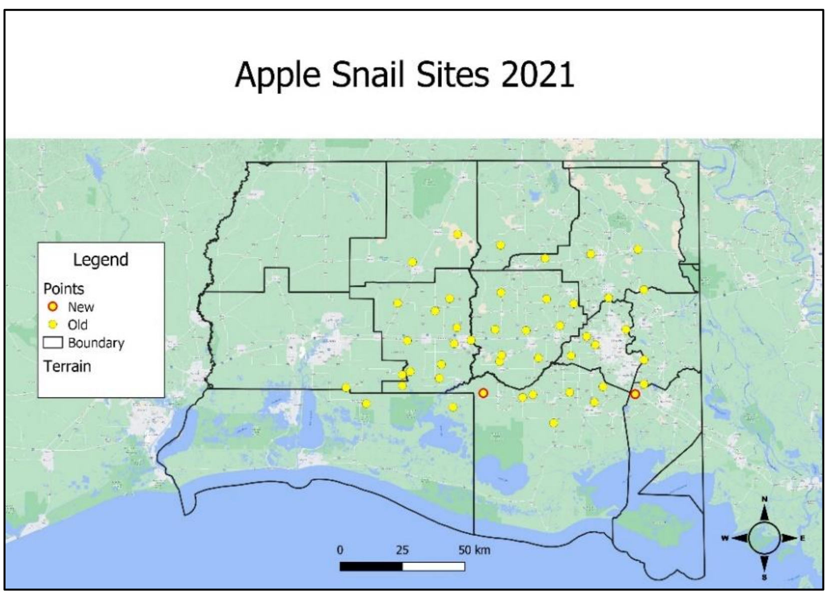


Figure 2.3. Terrain map of current 46 sites with two new site locations and parish boundaries in southwestern Louisiana, 2021.

previous positive site except for sites 43 and 44. These sites were moved further west at an average distance of 30 km. The new locations of sites were based on detecting future expansion deeper into the rice and crawfish production region of southwestern Louisiana. Higher *P. maculata* detection

in water ways and inaccessibility to farms caused more sites to be placed in non-crop habitats. These monitored water ways filtered through rice and crawfish producing areas and could facilitate *P. maculata* expansion onto farms once infested. According to the figures, new sites were mainly adjusted among the southern region of the project area where new detections occurred. Location changes to sites in more northern areas were sufficient indicators on how far north *P. maculata* had expanded their range in southwest Louisiana.

Rate of expansion. Observations from the initial site survey showed *P. maculata* occurrence in five parishes: Jefferson Davis, Lafayette, Iberia, Vermilion, and Cameron Parishes (Figure 2.4). Over the last two years, monthly site surveying recorded expansion of *P. maculata*

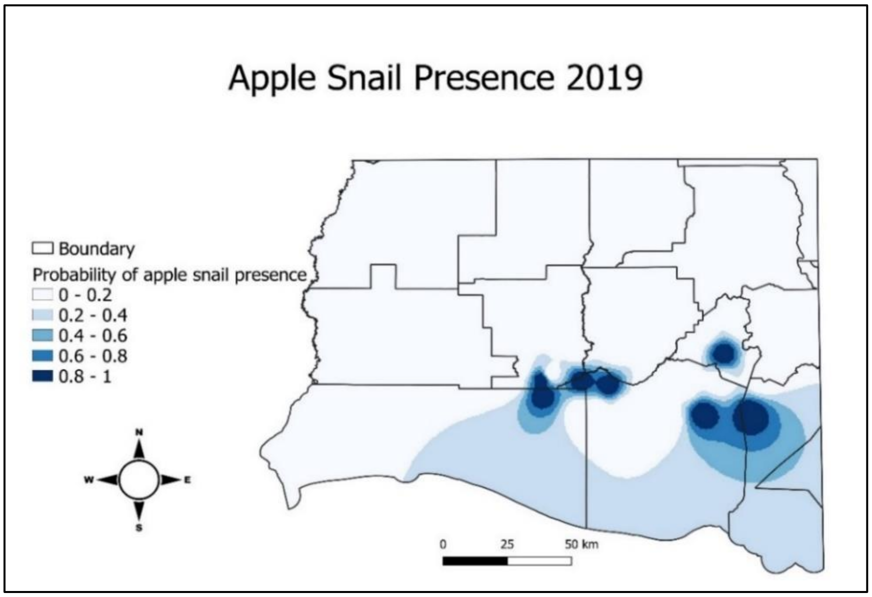


Figure 2.4. Distribution map based on spatial interpolation of site surveying data from original 46 sites showing the probability of *P. maculata* presence in southwestern Louisiana, 2019. Six sites were found positive throughout five southern parishes.

within these parishes as well as documenting snail presence in Acadia Parish. *P. maculata* appeared to be expanding its range at a rate of approximately 880 km^2 per year, while having a northward expansion at an average rate of approximately 6 km per year. Spatial interpolation

through QGIS helped depict a constant probability of 0–0.2 apple snail presence along the majority of the southern border of the map. As more southern sites were invaded, this probability increased to 0.4–0.6. The probability of apple snail presence decreased in a few areas due to the addition of new negative sites. Isolated populations occurred along the Vermilion River with *P. maculata* being found on one southern site in Abbeville, Vermilion Parish, and then reoccurring approximately 25 km north on a site in Lafayette, Lafayette Parish. From the four sites placed in Cameron Parish and Iberia Parish, three sites were positive in rice, or crawfish, farms connected to Grand Lake and Bayou Carlin, respectively. Vermilion Parish had the most sites with 13 in total and among these, 54% were found to be positive (Figure 2.5). These positive sites were mostly

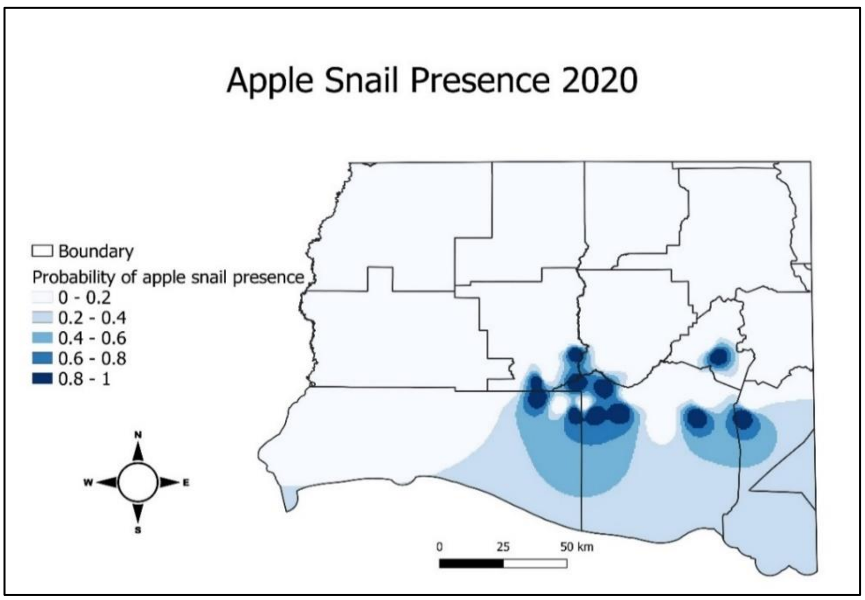


Figure 2.5. Distribution map based on spatial interpolation of annual site surveying data from adjusted 46 sites showing the probability of *P. maculata* presence in southwestern Louisiana, 2020. Five sites were found positive with three becoming positive from May to July 2020.

located in the northwest corner of the parish near Grand Lake and Lake Arthur. The most northern location reached by apple snails was in Jennings, Jefferson Davis Parish, with high numbers of egg masses being found on the edges of Bayou Nezpique in 2021 (Figure 2.6).

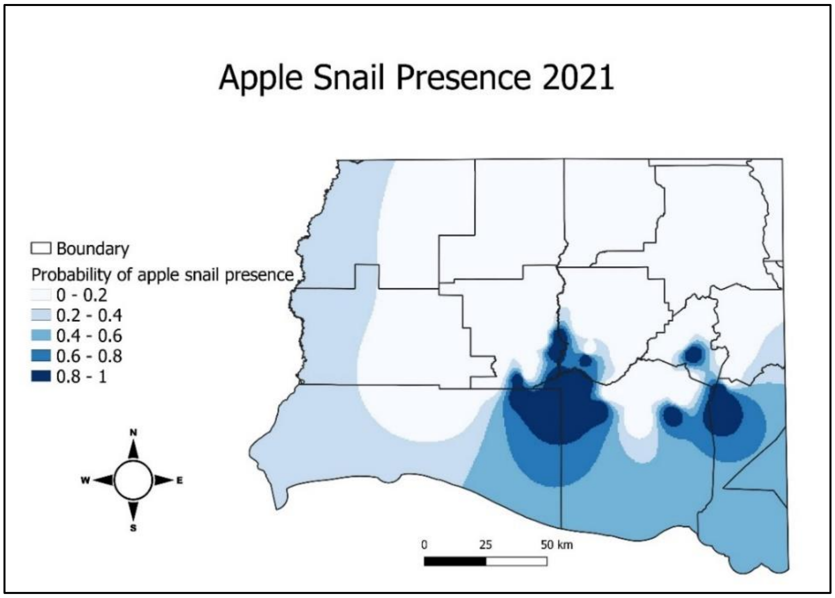


Figure 2.6. Distribution map based on spatial interpolation of annual site surveying data from current 46 sites showing the probability of *P. maculata* presence in southwestern Louisiana, 2021. Three more sites were found positive in areas near Lake Arthur and the Vermilion River.

Analysis of factors associated with positive sites. A total of 8 crop sites out of 129 (6.2%) and 8 non-crop sites out of 146 (5.5%) were positive for *P. maculata* presence. The Hosmer and Lemeshow Goodness-of-Fit test showed to have a high *P*-value of 0.2897, which meant that the model was fitting the data well. The stepwise selection model showed to initially have two potential effects that met the SLENTRY = 0.15 significance level: conductivity (df = 1, 0.1033) and dissolved oxygen (df = 1, *P* = 0.0853). However, since the *P*-value for conductivity highly exceeded the SLSTAY = 0.05 significance level, it was removed from the model. This resulted in dissolved oxygen (df = 1, *P* = 0.0437) being the only significant predictor for the probability of apple snail presence (Table 2.2). According to the statistical analysis, a decrease in dissolved oxygen decreased the probability of apple snail presence in an area.

Table 2.2. Analysis of maximum likelihood estimates for resulting model using a SLENTRY=0.15 and a SLSTAY=0.05 to predict *P. maculata* expansion in southwestern, Louisiana.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.5449	0.6014	6.5991	0.0102
DO	1	-0.3077	0.1526	4.0669	0.0437

2.4. Discussion

Over the last two years, *P. maculata* has established itself within 10 parishes using major water ways, such as the Mermentau River and Bayou Carlin, as sources for expansion. Our *P. maculata* site surveying showed the majority of new positive sites occurred in the south near Lake Arthur in Vermilion Parish and Cameron Parish. The rate of expansion of *P. maculata* into rice and crawfish ponds varied from 2019 to 2021 and was relatively slow as only nine new detections were made. However, the new detections of *P. maculata* is still a large concern for rice and crawfish farmers in areas such as Acadia Parish, because fishing has had to be terminated in a growing number of crawfish ponds in the region due to high apple snail infestations since 2018 (NOAA 2020). New detections were mainly recorded during the summer when temperatures were increasing. This observation is supported with published literature, which has shown that apple snail growth, activity, and reproduction increase with temperature (Carlsson 2017). Water with temperatures more than 15°C have shown high survival for *P. maculata* (Seuffert and Martín 2013). *P. maculata* have also been documented surviving short periods of exposure to temperatures of 10 and 5°C, but as colder water temperatures moderate due to global warming, the potential for apple snails to expand more north increases (Deaton et al. 2016). According to the site surveying and spatial analysis, *P. maculata* appeared to be expanding at a rate of approximately 880 km^2 per year and was expanding north at an average rate of approximately 6 km per year. One of the most recent occurrences was recorded in July 2021 off of Bayou Nezpique near a boat ramp. This waterbody connects to the heavily infested Lake Arthur, so there is possibility of *P. maculata* naturally moving upstream, or human transportation through attachment to boats. The spatial interpolation indicated for stable probability of apple snail presence along the south border of Louisiana, but isolated populations more inland have connected and extended to new areas during

the last two years. A possible surveying and expansion rate error may have been placing sites too close to one another creating error in the IDW interpolation.

Among the parameters used to predict the probability of *P. maculata* occurrence, the logistic regression analysis showed that dissolved oxygen was the most significant predictor. The resulting model indicated that as levels of dissolved oxygen decreased, so did the probability of apple snail presence. This result is supported by previously published literature that suggests apple snails prefer to remain in aquatic habitats of high dissolved oxygen levels (Ito 2002). However, they can survive waters with low dissolved oxygen levels due to their amphibious respiration. Apple snails have a lung that is used for aerial respiration and gills that allow for aquatic respiration (McClary 1964; Andrews 1965; Berthold 1991; Seuffert and Martín 2009). As dissolved oxygen levels decrease to low levels (1–2 ppm), apple snails extend their retractable siphon to the water surface to breathe air through their lung, and as dissolved oxygen levels increase to high levels (5–6 ppm), they remain submerged using their ctenidium, or gill, to exchange gases (San Martíns et al. 2009). This characteristic also allows apple snails to live in warmer conditions, since lower dissolved oxygen levels occur at higher temperatures (Aldridge 1983). Similarly, *P. clarkii* is also tolerant of relatively low dissolved oxygen levels but prefer aquatic habitats with increased levels of dissolved oxygen (Bonvillain 2012). This shared living condition allows apple snails to continuously survive in similar habitats as crawfish and makes them a constant potential pest for rice and crawfish farmers. Possible biases with the parameter data collection could have been similar to those experienced in past studies, such as Byers et al. (2013). Data could have been collected from a habitat suitable for apple snails but they had yet to colonize it.

As *P. maculata* expands its range in Louisiana, more freshwater habitats that may seem unsuitable may be infested. Therefore, continuous site surveying and prediction analysis are

needed in order to get a better understanding of current *P. maculata* distribution, rates of expansion, and factors that make areas conducive for their establishment. In order to slow *P. maculata* expansion, farmers should resort to using well water in order to irrigate their farms instead of using potentially infested surface water. They should also check equipment regularly in order to reduce the potential of transporting infested equipment and attempt to stock their ponds from safe sources of water. Future studies should focus on looking at other parameters, such as salinity, that may be associated with *P. maculata* presence.

Chapter 3. Chemical control of *P. maculata* in rice and crawfish systems

3.1. Introduction

Biological and cultural controls have been relatively successful at lowering *P. maculata* populations in rice culture, and mechanical controls, such as hand-picking snails and destroying egg masses, have also shown to be effective. However, even with the implementation of these types of control, *P. maculata* still persist at high levels in infested rice and crawfish systems. A more efficient solution may be integrating potential chemical control with one, or more, of these control methods. Chemical controls are essential in the development of an aquatic integrated pest management plan. They are often economical, highly effective, and readily available (Fredricks et al. 2019). In many cases, chemical controls offer the only management option for immediate control of severe infestations. However, they can also pose risks of negative health and environmental effects (Nicolopoulou-Stamati et al. 2016). Many chemicals used for pest control pollute waterbodies and are lethal to non-target organisms (Joshi 2005). They may also remain in soil and contaminate groundwater, which can become harmful to plants, animals, microorganisms, and humans (Tomašević and Gašić 2012). When implementing chemical controls, several factors must be considered, such as properties of the chemical and the application site and effects on non-target species (Fredricks et al. 2019).

Ideally, pesticides are used with the intention of being highly toxic toward their target species while also being nontoxic toward non-target species (Duke et al. 2010). In a pilot study conducted by Olivier et al. (2015), two molluscicides, a tea (*Camellia sinensis*) seed derivative (TSD) and niclosamide monohydrate (Pestanal®, 2’,5-dichloro-4’-nitrosalicylanilide), were evaluated for their efficacy on apple snails (*P. maculata*) and two biocontrol agents, the red swamp crawfish and redear sunfish. The results revealed that the TSD was completely lethal to the snails,

not lethal to the crawfish, and sunfish were only killed at high concentration. The data suggested that both molluscicides could potentially cause negative environmental impacts to non-target fish species and native mollusks. In another study, apple snails (*P. canaliculata*) were exposed to powder extracts of soap nut pericarp and seven isolated saponins from Indian soapberry, *Sapindus mukorossi* (Sapindaceae). The results proved that both crude soap nut extract and isolated saponins were lethal to apple snails (Huang et al. 2003). However, currently, farmers still prefer to use conventional mulluscicides, such as niclosamide and metaldehyde, because botanical molluscicides have not been produced commercially (Joshi 2007).

A more available and lower cost option that is already registered for rice in the U.S. is copper sulfate. Copper can be toxic to snails and other aquatic invertebrates, but when used at low rates, there is reduced potential for chronic non-target effects (Leslie 1992; Dummee et al. 2015). Other crop pesticides that have lethal effects on apple snail eggs and are environmentally friendly when used at proper dosages are oil-based adjuvants. Applications of methylated seed oil have shown to be lethal to apple snail egg masses (Estate Management Services 2021). The aim of this study was to identify effective chemical control strategies that provide the lowest risk to crawfish. Therefore, the objectives were to: (1) evaluate environmentally safe molluscicide chemistry (copper sulfate) for control of *P. maculata* in rice and crawfish systems, (2) determine the susceptibility of *P. clarkii* to copper sulfate, and (3) assess oil-based control of *P. maculata* eggs.

3.2. Materials and methods

***P. maculata* copper sulfate toxicity assays.** Laboratory toxicity assays investigating potential chemical control of liquid copper sulfate (AgriTec®, copper sulfate pentahydrate, Rogers, AR, USA) and crystal copper sulfate (Chem One, copper sulfate pentahydrate, Houston, TX, USA) on apple snails were conducted at the LSU Rice Entomology Lab. Apple snails were

hand collected from Hebert Farms in Abbeville, Louisiana. A total of three toxicity assays using the liquid copper sulfate and three toxicity assays using the crystal copper sulfate were conducted. Each experiment used three copper sulfate dosages, which were selected as Low, Medium, and High doses according to the toxicity results from previous assays. Initial dosages using the liquid copper sulfate consisted of 0.63 ppm [L], 1.25 ppm [M], and 2.5 ppm [H] where 2.5 ppm was the labeled rate used for algae and tadpole shrimp (*Triops longicaudatus*) control in recreational bodies of water, and a non-treated control in a completely randomized design with seven replications. Initial dosages using the crystal copper sulfate consisted of 4 ppm [L], 10 ppm [M], and 14 ppm [H] where 4 ppm was the labeled rate for schistosome-infected freshwater snail control in recreational bodies of water, and a non-treated control in the same experimental design. These initial rates were selected, because there was no labeled rate for apple snail control. Each toxicity assay was individually analyzed due to the different low, medium, and high dosages used for each assay. A total of 28 6-L clear plastic containers (Sterilite®, Fitchburg, MA, USA) were used. The containers were divided among the four treatments with three snails per container and seven reps for a total of 84 snails. Holes were inserted towards the top of the container to allow for the entry of an aeration tube, and each container was covered with a lid. Each container contained 4 L of water and 1 mL of water conditioner (Tetra®, Melle, Germany). The containers were kept in a laboratory under a photoperiod of light (14 hours): dark (10 hours) at 23 ± 2°C and were washed with dish soap and water after every toxicity assay. There was an acclimation period of 48 hours, and mortality was assessed during a 72-hour period after initial snail exposure to the copper sulfate. An apple snail was considered dead when the snail was out of its shell, and there was no movement from the snail after its flesh was gently probed with a scalpel. Dead snails were taken out of the containers after every daily check. Data was corrected using the Abbott’s formula to neutralize

mortality in the controls (Abbott 1925). Data was analyzed using generalized linear mixed models (PROC GLIMMIX, SAS® SAS Institute 2013). The Kenward-Roger function was used to estimate the denominator degrees of freedom (Kenward and Roger 1997), and LS-Means were separated using Tukey's HSD (α=0.05). The model consisted of treatment, time, and interaction between treatment and time as fixed effects and rep as a random effect. Differences in effects were tested based on the response variable average mortality, which consisted of the average mortality among the three snails in each container. The experiments were repeated until an effective low dosage resulting in 90–100% average *P. maculata* mortality was reached in the 72-hour period in order to reduce risk to *P. clarkii*.

***P. clarkii* copper sulfate toxicity assays.** Liquid copper sulfate (AgriTec®, copper sulfate pentahydrate) and crystal copper sulfate (Chem One, copper sulfate pentahydrate) were evaluated in a series of toxicity assays conducted at the LSU AgCenter Crawfish Laboratory in Crowley, Louisiana. Crawfish were collected from the Rice Research Station South Farm in Crowley. A total of three toxicity assays using the liquid copper sulfate and two toxicity assays using the crystal copper sulfate were conducted. Each experiment used the same dosages that were used for the *P. maculata* toxicity assays except for one liquid copper sulfate set of dosages and one crystal copper sulfate set of dosages. The liquid copper sulfate dosages of 0.625 ppm [L], 1.25 ppm [M], and 2.5 ppm [H] used on *P. maculata* were substituted for the dosages of 7.5 ppm [L], 12 ppm [M], and 22.5 ppm [H] used on *P. clarkii*. The crystal copper sulfate dosages of 12 ppm [L], 25 ppm [M], and 35 ppm [H] used on *P. maculata* were not used for *P. clarkii*. Each toxicity assay was individually analyzed due to the different low, medium, and high dosages used for each assay. The experiments were conducted in a completely randomized design with 16 replications. The initial dosages were determined in similar manner to those used in for the crawfish toxicity assays. A

total of 64 30-L plastic bins were used. The bins were divided among the four treatments with one crawfish per bin and 16 reps for a total of 64 crawfish. Each bin contained 30 quarts of water and was cleaned with ammonia and water after every toxicity assay. Aeration tubes were inserted at the top of the bin. The containers were kept in a laboratory under a photoperiod of light (14 hours): dark (10 hours) at 25 ± 2°C. There was an acclimation period of 120 hours, and mortality was assessed during a one-week period after initial snail exposure to the copper sulfate. A crawfish was considered dead when no movement was observed after gently brushing the ventral portion with a wooden stick. Statistical analysis previously conducted for the *P. maculata* toxicity assays was used for the *P. clarkii* toxicity assays. The experiments were repeated in correspondence to finding an effective low dosage resulting in 90–100% *P. maculata* mortality within the 72-hour period in order to reduce risk to *P. clarkii*.

***P. maculata* egg toxicity assay.** The lethal effect of crop oil (Prime Oil® adjuvant, phytobland paraffinic oil, St. Paul, MN, USA) on *P. maculata* eggs was assessed in a toxicity assay trial at the LSU Rice Entomology Lab. *P. maculata* egg masses were hand collected from Hebert Farms in Abbeville, Louisiana. The experiment was conducted using 60 120.5-g foam cups (Walmart, Bentonville, AK, USA), 2 62.5-L clear plastic storage bins, and a 240-mL adjustable spray bottle (VWR®, Radnor, PA, USA). Each cup was punctured through the bottom by the object the egg mass was attached to and was placed just under the egg mass to catch any falling hatchlings. Each bin was filled with approximately 5 cm of sediment and 2 cm of water. The spray bottle contained 200 mL of a crop oil and water solution. The toxicity assay had a completely randomized design with three treatments, one egg mass per cup, and 20 replications per treatment for a total of 60 egg masses. The three treatments consisted of a control (0), low (5%), and high (10%). The low and high percentages were calculated based on a ratio of crop oil to water. The

low concentration contained 10 mL of crop oil (5%) and 190 mL of water. The high concentration contained 20 mL of crop oil of crop oil (10%) and 180 mL of water. Each individual egg mass was sprayed and fully coated with approximately 3.75 mL of the solution. The containers were kept in a laboratory under a photoperiod of light (14 hours): dark (10 hours) at 25 ± 2°C. There was an acclimation period of 24 hours, and mortality was assessed during a two-week period after initial egg mass exposure to the crop oil. The number of hatchlings inside the cups was counted for each egg mass at the end of the two-week period. An egg mass was considered dead if less than 10 eggs hatched per egg mass due to the high mortality in treated egg masses. Data were analyzed using generalized linear mixed models (SAS, PROC GLIMMIX). Hatching success per egg mass data was analyzed with a binomial distribution and logit link function (Stroup 2015). The model consisted of treatment as a fixed effect and replication as a random effect. Hatchling data was analyzed with Gaussian distributions. The model consisted of treatment as a fixed effect and replication as a random effect. The Kenward-Roger method was used to estimate denominator degrees of freedom, and Tukey' HSD ($\alpha = 0.05$) was used to separate LS-Means.

3.3. Results

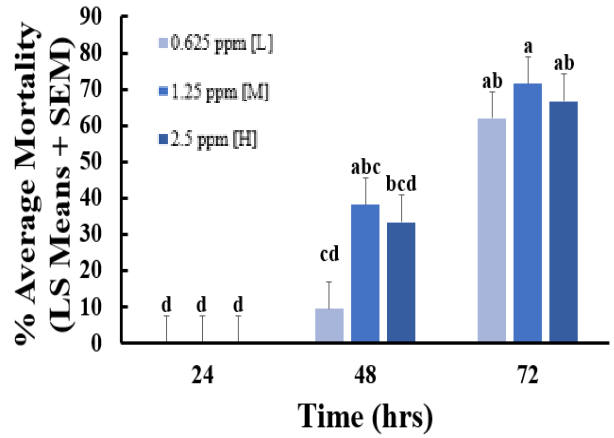
***P. maculata* copper sulfate toxicity assays**. Treatment, time, and the interaction affected *P. maculata* mortality in each toxicity assay using the liquid copper sulfate. In the first toxicity assay, mortality across time was 23.81%, 36.52%, and 33.35% ± 4.46 [SE] for 0.625 ppm, 1.25 ppm, and 2.5 ppm, respectively. Across treatments, mortality was 0%, 20.14%, and 49.92% ± 4.01 [SE] for 24 hours, 48 hours, and 72 hours, respectively. All treatments had more than 60% average snail mortality at 72 hours with the treatment of 1.25 ppm causing the highest snail mortality (71.46%) at 72 hours (Figure 3.1). However, there was no mortality after 24 hours, so the treatments were increased. In the second toxicity assay, mortality across time was 39.68%,

30.16%, and 39.68% ± 7.03 [SE] for 5 ppm, 10 ppm, and 20 ppm, respectively. Across treatments, mortality was 9.52%, 26.19%, and 47.62% ± 6.60 [SE] for 24 hours, 48 hours, and 72 hours, respectively. The treatment of 5 ppm was highly effective at 48 hours by causing 42.86% average snail mortality. Although higher, the treatments of 10 ppm and 20 ppm were not as effective and only caused 28.57% and 33.33% average snail mortality at 48 hours, respectively (Figure 3.1). Only the treatment of 24 ppm caused higher mortality at 48 hours than the treatment of 5 ppm. In the third toxicity assay, mortality across time was 44.47%, 49.22%, and 63.51% ± 6.06 [SE] for 8 ppm, 14 ppm, and 24 ppm, respectively. Across treatments, mortality was 20.75%, 33.86%, and 61.25% ± 5.70 [SE] for 24 hours, 48 hours, and 72 hours, respectively. Among all treatments used, the treatment of 24 ppm affected average snail mortality the most, but this treatment was still not significantly different from the treatments of 8 ppm and 14 ppm (Figure 3.1). Although the 90–100% average snail mortality was reached at 72 hours using the treatment of 24 ppm, there were several inconsistencies with the treatments used for the liquid copper sulfate, so a crystal form of copper sulfate was used.

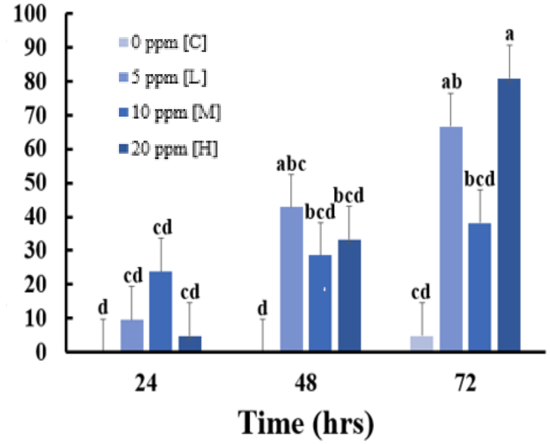
Treatment and time significantly affected average *P. maculata* mortality in all of the toxicity assays using the crystal copper sulfate. The interaction between treatment and time was only significant in the toxicity assay containing the 12 ppm [L], 25 ppm [M], and 35[H] treatments (F = 5.79; df = 6, 62.2; P = <.0001), but the interaction between treatment and time was still compared for these toxicity assays. Based on the first conducted toxicity assay, mortality across time was 20.62%, 23.81%, and 25.38% ± 9.35 [SE] for 4 ppm, 10 ppm, and 14 ppm, respectively. Mortality throughout the treatments was 0%, 14.99%, and 32.85% ± 9.04 [SE] for 24 hours, 48 hours, and 72 hours, respectively. The treatment of 10 ppm caused no mortality at 24 hours but caused the highest mortality (57.16%) at 72 hours within this toxicity assay (Figure 3.1). There

were no significant differences among the treatments of 4 ppm [L], 10 ppm [M], and 14 ppm [H], so the treatments were increased. In the second toxicity assay, mortality across time was 9.50%, 17.47%, and 26.99% ± 7.72 [SE] for 8 ppm, 16 ppm, and 24 ppm, respectively. Across treatments, mortality was 1.38%, 8.51%, and 24.01% ± 7.54 [SE] for 24 hours, 48 hours, and 72 hours, respectively. The treatment of 8 ppm had the lowest effect out of all the crystal copper sulfate treatments. There was no significant difference among these treatments, and the interaction between treatment and time for this toxicity assay was highly insignificant (Figure 3.1). Based on the last toxicity assay using the crystal copper sulfate, mortality across time was 47.65%, 60.34%, and 39.70% ± 6.19 [SE] for 12 ppm, 25 ppm, and 35 ppm, respectively. Across treatments, mortality was 15.67%, 29.96%, and 62.15% ± 5.85 [SE] for 24 hours, 48 hours, and 72 hours, respectively. The treatments of 12 ppm and 25 ppm reached the highest mortality at 72 hours by each causing 85.78% average snail mortality (Figure 3.1). The treatment of 25 ppm was significantly different to the treatment of 35 ppm but not to the treatment of 12 ppm throughout all 72 hours.

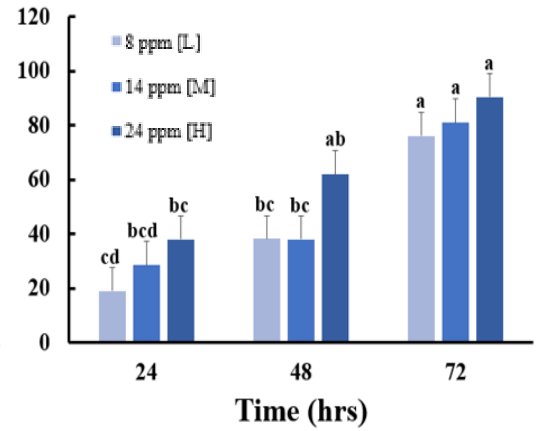
After analyzing the effects of both forms of copper sulfate at 72 hours, the results showed a positive linear relationship between the treatments and average *P. maculata* mortality. Potential outliers appear throughout both regressions. However, the R^2 values are not low indicating that much of the variance of average snail mortality is described by the treatment. This is further supported by the significance of the treatments for the liquid copper sulfate ($F = 17.3$; df = 9, 65.6; $P = <0.001$) and crystal copper sulfate ($F = 7.81$; df = 9, 61.9; $P = <0.001$), which were both highly significant. Lower treatments of the liquid copper sulfate appeared to have more effect on average snail mortality when compared to the effects of the low treatments of the crystal copper sulfate (Figure 3.2).



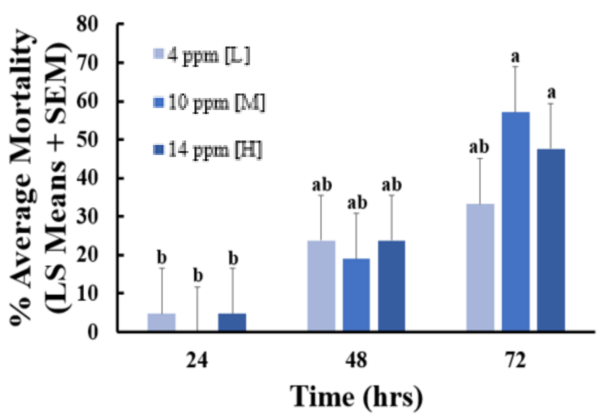
Treatment: F = 13.93; df = 3, 63.7; P = <0.001
Time: F = 45.85; df = 2, 62.8; P = <0.001
Trt x Time: F = 5.51; df = 6, 62.8; P = <0.001



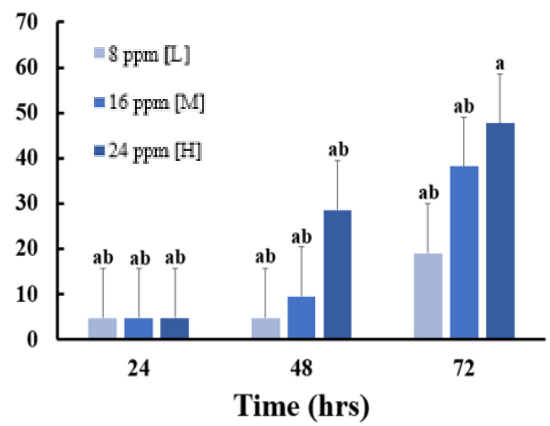
Treatment: F = 14.01; df = 3, 66.0; P = <0.001
Time: F = 20.97; df = 2, 66.0; P = <0.001
Trt x Time: F = 4.35; df = 6, 66.0; P = <0.001



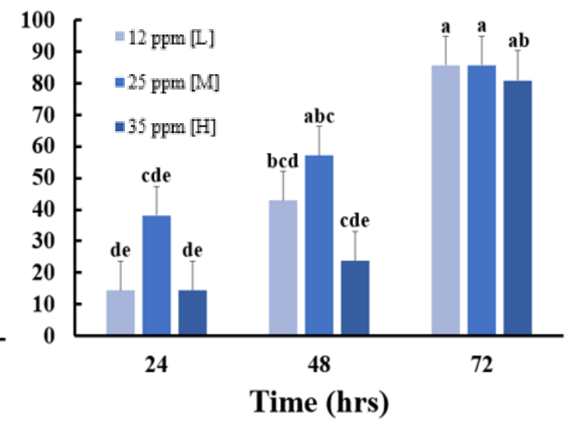
Treatment: F = 36.43; df = 3, 63.0; P = <0.001
Time: F = 27.71; df = 2, 62.6; P = <0.001
Trt x Time: F = 3.07; df = 6, 62.6; P = 0.012



Treatment: F = 7.61; df = 3, 62.9; P = <0.001
Time: F = 13.83; df = 2, 62.8; P = <0.001
Trt x Time: F = 2.13; df = 6, 62.8; P = 0.062



Treatment: F = 5.22; df = 3, 57.7; P = <0.001
Time: F = 5.09; df = 2, 56.7; P = 0.009
Trt x Time: F = 0.96; df = 6, 56.7; P = 0.459



Treatment: F = 23.70; df = 3, 60.2; P = <0.001
Time: F = 28.01; df = 2, 59.2; P = <0.001
Trt x Time: F = 3.32; df = 6, 59.2; P = 0.007

Figure 3.1. Average *P. maculata* mortality based on the interaction between treatment and time for three toxicity assays using liquid copper sulfate (top) and three toxicity assays using crystal copper sulfate (bottom) during a 72-hour period. Bars that share a letter are not significantly different (Tukey's HSD, α=0.05).

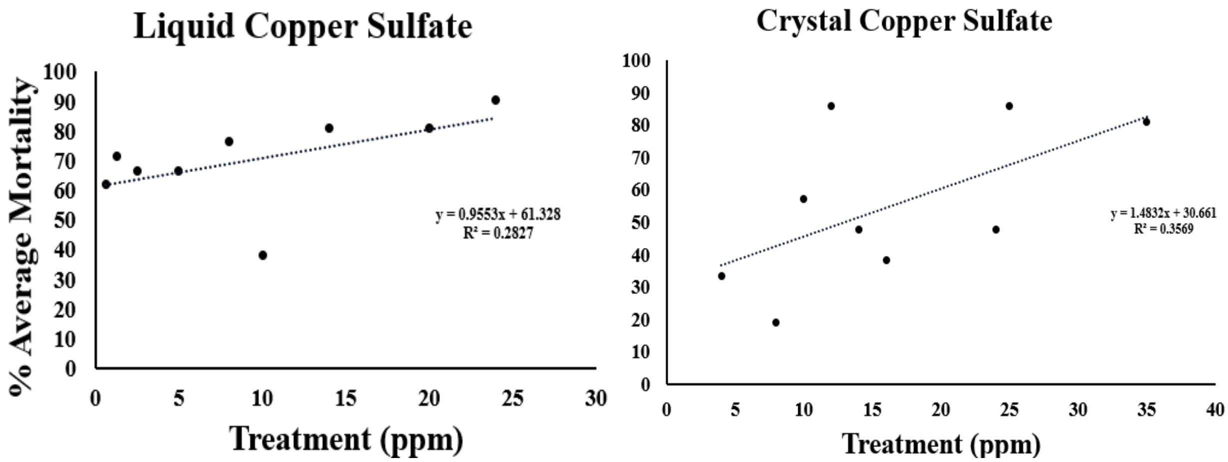


Figure 3.2. Linear regression relationship between treatments of copper sulfate and average *P. maculata* mortality at 72 hours.

***P. clarkii* copper sulfate toxicity assays.** Among the three toxicity assays that incorporated the liquid copper sulfate, there was no significant effect on *P. clarkii* mortality from treatment, time, or interaction between treatment and time. Only three crawfish died after the one-week period. Application rates of 14 and 20 ppm caused one crawfish mortality each after 6 days, and one crawfish mortality was recorded in treatment of 5 ppm after 7 days. Treatment, time, and interaction between treatment and time also had no significant effect on *P. clarkii* mortality among the two toxicity assays using the crystal copper sulfate. No mortality was recorded within these treatments during the 7-day period. There were no deaths recorded at the highest treatment used for either form of copper sulfate used, which was 24 ppm.

***P. maculata* egg mass toxicity assay.** Throughout the experiment, a total of 14 egg masses (70% ± 10.3 [SE]) hatched in the control, 9 egg masses (45% ± 11.1 [SE]) hatched in the low treatment, and 3 egg masses (15% ± 8.0 [SE]) hatched in the high treatment. Treatment (F = 5.30; df = 2, 38.0; P = 0.009) had a significant effect on hatching success per egg mass. The control contained the highest hatching success per egg mass and was significantly different to the high treatment but not to the low treatment. Treatment (F = 7.96; df = 2, 38.0; P = 0.001) also had a

significant effect on the number of hatchlings, which was approximately 8.7-fold greater in the control than in the low or high treated egg masses (Figure 3.3).

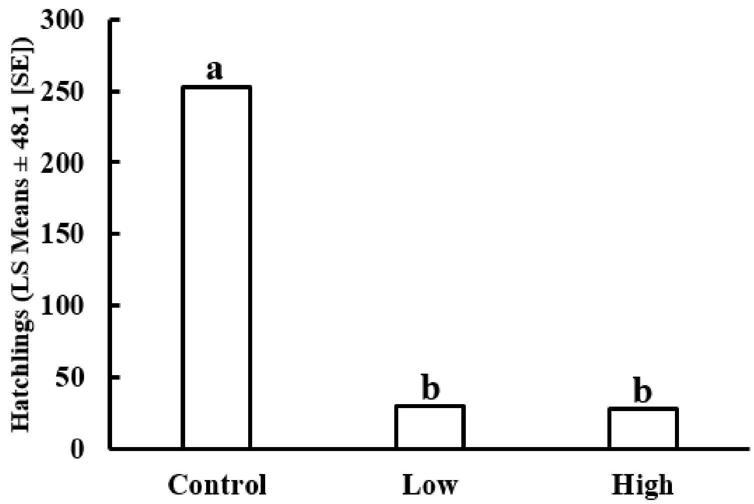


Figure 3.3. Mean *P. maculata* hatchling count among three treatments of a crop oil adjuvant after a 2-week period. Bars that share a letter are not significantly different (Tukey's HSD, α=0.05).

3.4. Discussion

Our work presents a new understanding towards potential chemical solutions that can control *P. maculata* in rice and supports the non-lethal affect copper sulfate has on a non-target species, *P. clarkii*. Results from the apple snail toxicity assays using copper sulfate are consistent with previous studies showing that this chemical is effective in controlling *P. maculata* (Yahaya et al. 2017). This study provides new potential rates of copper sulfate and crop oil that can be used in conjunction with other practices for the management of *P. maculata.*

The results of this study show a range of effective treatments that can cause high *P. maculata* mortality without lethal effects to *P. clarkii*. When applying the liquid copper sulfate, the standard dosage of 2.5 ppm used for algae and tadpole shrimp control can still be used to control *P. maculata,* since lower treatments of 0.625 ppm and 1.25 ppm showed high snail mortality at 72 hours and 48 hours, respectively. However, the treatment of 8 ppm was among the

three most effective rates for the liquid copper sulfate during the 72-hour period and had no lethal effect on *P. clarkii* after one week. The highest rate applied was 24 ppm, which presented the most significant effects to average snail mortality and no lethal effects to crawfish. However, applications of such high rates greatly increase costs as well as the potential for negative impacts to the environment. The results indicated that the low rates were moderately efficient in causing snail mortality but that high rates were required to achieve a high level of control. Those high rates are riskier, but they did not cause mortality in the crawfish toxicity assays. Rates of 10 ppm or lower have shown to achieve appreciable snail control with little risk. There appeared to be a positive linear relationship between the treatments used for both forms of copper sulfate and average snail mortality. The results indicated that with an increase in treatments, there was an increase in average snail mortality. The liquid copper sulfate seemed to be more effective than the crystal copper sulfate. Variation in the effectiveness among similar treatments and inconsistency in snail mortality suggests that there were possible influential outliers. Factors, such as snail behavior and prior health conditions, could have caused error in average snail mortality. Various snails were also recorded enclosing themselves tightly during the 72 hours of the experiment, which potentially could have caused lower amounts of *P. maculata* mortality in the treatments. Apple snails can endure harsh environmental conditions by closing their operculum tightly (Cowie 2002). Since they have this ability, the actual dose that the snails were exposed to during each toxicity assay remains unknown. Another possible explanation for error was could have been that the number of snails used for the experiments was too low. Larger observation numbers could have shown more effective differences among the treatments. The results of the *P. clarkii* toxicity assays suggested that *P. clarkii* was able to withstand high dosages of the liquid and crystal copper sulfate throughout a one-week period. However, accumulation of copper in *P. clarkii* can occur quickly

causing them to become toxic and have adverse health conditions if consumed (Qoraychy et al. 2015). The insignificant difference between the low (5%) and high (10%) treatment effects of crop oil adjuvant on *P. maculata* egg mass hatching success and hatchlings showed that the low treatment was an efficient dosage for crop oil applications on *P. maculata* egg masses. These results indicated that crop oil appears to be a viable control strategy to reducing egg hatching.

Although there were several positive results with the analyses conducted, there is much uncertainty that needs to be addressed if copper sulfate and crop oil are to be used for future *P. maculata* control. When conducting toxicity assays, further research should attempt to determine the actual copper sulfate dosages that are ingested by the apple snails to define what an actual lethal dose is for *P. maculata*. If the effects of copper sulfate are also being tested on *P. clarkii* mortality, future research should examine potential sub-lethal effects the chemical may have, such as negative effects on crawfish growth rates or fecundity. Since the crop oil treatments appeared to be effective on egg mass hatching success and hatchling count, more studies should be conducted to determine which application methods work best and at what frequencies in order to determine the lowest rates possible needed for effective *P. maculata* egg mass control. This data could then be used to examine the potential effects egg control could have on adult *P. maculata* populations.

Summary and Conclusions

The introduction of invasive *P. maculata* into the rice and crawfish systems of southwestern Louisiana has created a need for an efficient monitoring and management plan. Our results indicated that *P. maculata* have established several populations in major southern water ways, such as the Vermilion River and Mermentau River, and they are using these water ways to expand their range into new crop regions at a rate of 880 km^2 per year. Factors, such as increasing temperatures and water dissolved oxygen levels, were shown to be conducive for *P. maculata* establishment. The copper sulfate toxicity assays indicated that a rate of 8 ppm using liquid copper sulfate and a rate of 12 ppm using crystal copper sulfate were the lowest effective rates. The toxicity assays also showed that the various treatments of copper sulfate used in this study had no significant effect on *P. clarkii* mortality. The crop oil toxicity assay showed a low rate of 5% crop oil to water was highly effective for *P. maculata* egg mass control.

This study indicates that *P. maculata* is increasing in southwest Louisiana, and copper sulfate and crop oil can potentially be used in pest management programs. The liquid copper sulfate appeared to be more effective, but further research is needed for a more thorough understanding of efficient application rates. In order to increase *P. maculata* and reduce the potential for negative environmental impacts, chemical control should be integrated with other management strategies. Controlling *P. maculata* at different life stages has shown to be very effective. Future research should continue to evaluate new areas with *P. maculata* populations and determine other factors conducive to their establishment. More studies should also assess lower effective rates of copper sulfate and crop oil adjuvant that can be used to control *P. maculata* at all life stages in order to minimize the variety of chemical output and increase the efficiency of this potential IPM strategy for *P. maculata*.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265–267.

Aldridge, D. W. 1983. Physiological ecology of freshwater prosobranchs. Pages 329–358 *in* W. D. Russell-Hunter, editor. The Mollusca. Volume 6. Ecology. Academic Press, London, England.

Andrews, E. B. 1965. The functional anatomy of the mantle cavity, kidney and blood system of some pilid gastropods (Prosobranchia). Journal of Zoology 146:70–94.

Arfan, A. G., R. Muhamad, D. Omar, A. A. Nor Azwady, and G. Manjeri. 2015. Comparative life cycle studies of *Pomacea maculata* and *Pomacea canaliculata* on rice (*Oryza sativa*). Pakistan Journal of Agricultural Sciences 52(4):1075–1079.

Arnold, T. E., M. Brenner, J. H. Curtis, A. Dutton, S. M. Baker, J. H. Escobar, and C. A. Ortega. 2014. Application of stable isotopes ($\delta^{18}O$) to determine growth patterns of the invasive gastropod *Pomacea maculata* in Florida Lakes. The Florida Scientist 77:126–143.

Babineaux, L. P., Jr. 1967. A history of the rice industry of Southwestern Louisiana. Thesis, University of Southwestern Louisiana, Lafayette, USA.

Bandumula, N. 2017. Rice production in Asia: key to global food security. Proceedings of the National Academy of Sciences, India – Section B: Biological Sciences. DOI 10.1007/s40011-017-0867-7.

Barlett, M. S. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences 160:268–282.

Barnes, M. A., R. K. Fordham, R. L. Burks, and J. J. Hand. 2008. Fecundity of the exotic applesnail, *Pomacea insularum*. Journal of the North American Benthological Society 27(3):738-745

Beale, C. M., J. J. Lennon, and A. Gimona. 2008. Opening the climate envelope reveals no macroscale associations with climate in European birds. Proceedings of the National Academy of Science USA 105:14908–14912.

Benson, A. J. 2019. *Pomacea maculata*. USGS Nonindigenous Aquatic Species Database, Gainesville, Florida. <https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2633>. Accessed 26 Nov 2019.

Bernatis, J. L., and G. L. Warren. 2014. Effectiveness of a hand removal program for management of nonindigenous apple snails in an urban pond. Southeastern Naturalist 13:607–618.

Berthold, T. 1991. Vergleichende anatomie, phylogenie und historische biogeographie der Ampullariidae (Mollusca, Gastropoda). Abhandlungen des Naturwissenschagftlinchen Vereins in Hamburg (NF) 29:1–256 [in German].

Bonvillain, C. P. 2012. Red swamp crayfish *Procambarus clarkii* in the Atchafalaya River Basin: biotic and abiotic effects on population dynamics and physiological biomarkers of hypoxic stress. Dissertation, Louisiana State University, Baton Rouge, USA.

Boots, B., and A. Getis. 1988. Point pattern analysis. Sage Publications, Newbury Park, California, USA.

Burela, S., and P. R. Martín. 2011. Evolutionary and functional significance of lengthy copulations in a promiscuous apple snail, *Pomacea canaliculata* (Ampullariidae, Caenogastropoda). Journal of Molluscan Studies 77:54–64.

Burlakova, L. E., D. K. Padilla, A. Y. Karatayev, D. N. Hollas, L. D. Cartwright, and K. D. Nichol. 2009. Differences in population dynamics and potential impacts of a freshwater invader driven by temporal habitat stability. Biological Invasions 12:927–941.

Burks, R. L., C. H. Kyle, and M. K. Trawick. 2010. Pink eggs and snails: field oviposition patterns of an invasive snail, *Pomacea insularum*, indicate a preference for an invasive macrophyte. Hydrobiologia 646:243–251.

Burks, R. L., A. E. Miller, and A. L. Hill. 2013. *Pomacea maculata*. CABI Invasive Species Compendium. <http://www.cabi.org/isc/datasheet/116486>. Accessed 26 Nov 2019.

Burks, R. L., J. Bernatis, J. E. Byers, J. Carter, C. W. Martin, W. G. McDowell, and J. V. Dyke. 2017. Identity, reproductive potential, distribution, ecology and management of invasive *Pomacea maculata* in the southern United States. Pages 293–333 *in* R. C. Joshi, R. H. Cowie, and L. S. Sebastian, editors. Biology and management of invasive apple snails. Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija 3119.

Byers, J. E., W. G. McDowell, S. R. Dodd, R. S. Haynie, L. M. Pintor, and S. B. Wilde. 2013. Climate and pH predict potential range of the invasive apple snail (*Pomacea insularum*) in the southeastern United States. PloS ONE 8:e56812.

Campbell, J. E., and M. Shin. 2012. Geographic Information System Basics. Saylor Academy, Washington, D.C., USA.

Carlsson, N. O. L. 2017. Invasive apple snails are threatening natural ecosystems in Southeast Asia, Europe, and North America. Pages 45–62 *in* R. C. Joshi, R. H. Cowie, and L. S. Sebastian, editors. Biology and management of invasive apple snails. Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija 3119.

Carter, J., D. Johnson, and S. Merino. 2018. Exotic invasive *Pomacea maculate* (Giant Apple Snail) will depredate eggs of frog and toad species of the southeastern U.S. Southeastern Naturalist 17(3):470–475.

Carvalho de Brito, F., and R. C. Joshi. 2016. The golden apple snail *Pomacea canaliculata*: a review on invasion, dispersion and control. Outlooks on Pest Management. DOI: 10.1564/v27_aug_03.

Cowie, R. H. 2002. Apple snails as agricultural pests: their biology, impacts, and management. Pages 145–192 *in* G. M. Baker, editor. Molluscs as Crop Pests. CABI, Wallingford, Oxfordshire, UK.

Deaton, L. E., W. Schimdt, B. Leblanc, J. Carter, K. Mueck, and S. Merino. 2016. Physiology of the invasive apple snail *Pomacea maculata*: tolerance to low temperatures. Journal of Shellfish Research 35(1):207–210.

Duke, S. O., C. L. Cantrell, K. M. Meepagala, D. E. Wedge, N. Tabanca, and K. K. Schrader. 2010. Natural toxins for use in pest management. Toxins 2:1943–1962.

Dummee, V., P. Tanhan, M. Kruatrachue, P. Damrongphol, and P. Pokethitiyook. 2015. Histopathological changes in snail, *Pomacea canaliculata*, exposed to sub-lethal copper sulfate concentrations. Ecotoxicology and Environmental Safety 122:290–295.

EFSA Panel on Plant Health. 2012. Scientific opinion on the evaluation of the pest risk analysis on *Pomacea insularum,* the island apple snail, prepared by the Spanish Ministry of Environment and Rural and Marine Affairs. EFSA Journal 10:2552.

Elsey, R. M., E. Ledet, M. D. Kaller, B. Landry, and M. Miller. 2018. *Alligator mississippiensis* (American Alligator) tolerance of potentially toxic non-native prey. Herpetological Review 49:532–534.

Estate Management Services. 2021. Solutions to invasive apple snails. <https://www.ponds.org/solutionstoinvasiveapplesnails.html>. Accessed 18 Sep 2021.

Fasulo, R. T. 2004. Featured creatures. Common name: applesnails of Florida scientific name: *Pomacea* spp. (Gastropoda: Ampullariidae). <http://entnemdept.ufl.edu/creatures/misc/gastro/apple_snails.htm#:~:text=In%20Florida%2C%20some%20of%20the,immature%20snails%20(FFWCC%202006).>. Accessed 5 July 2020.

Fredricks, K. T., T. D. Hubert, J. J. Amberg, A. R. Cupp, and V. K. Dawson. 2019. Chemical controls for an integrated pest management program. North American Journal of Fisheries Management. DOI: 10.1002/nafm.10339.

Halwart, M., J. A. Litsinger, M. C. Viray, and G. Kaule. 2014. Efficacy of common carp and Nile tilapia as biocontrol agents of the golden apple snail in the Philippines. Philippine Journal of Science 143(2):25–136.

Harrell D. L., R. Zaunbrecher, K. A. Fontenot, E. P. Webster, J. P. Hebert, B. E. Wilson, S. Brown, J. H. Oard, D. E. Groth, M. Kongchum, and A. Famoso. 2019. 2019 rice varieties &management tips. Louisiana State University Agricultural Center, Baton Rouge, USA.

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

Hengl, T. 2006. Finding the right pixel size. Computers and Geosciences 32(9):1283–1298.

Horgan, F. G., A. M. Stuart, and E. P. Kudavidanage. 2012. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. Acta Oecologica 54:90–100.

Horgan, F. G. 2017. Ecology and management of apple snails in rice. Pages 393–418 *in* B. S. Chauhan, K. Jabran, and G. Mahajan, editors. Rice Production Worldwide. Springer, Switzerland.

Horgan, F. G. 2018. The ecophysiology of apple snails in rice: implications for crop management and policy. Annals of Applied Biology 172:245–267.

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

Hosmer, D. W., and S. L. Lemeshow. 2000. Applied Logistic Regression. Second edition. Wiley-Interscience, Hoboken, NJ, USA.

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

Huang, H. C., S. C. Liao, F. R. Chang, Y. H. Kuo, and Y. C. Wu. 2003. Molluscicide saponins from *Sapindus mukorossi*, inhibitory agents of golden apple snails, *Pomacea canaliculata*. Journal of Agricultural and Food Chemistry 51:4916–4919.

Huggan, R. D. 1995. Co-evolution of rice and humans. GeoJournal 35:262–265.

Ito, K. 2002. Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan. Applied Entomology and Zoology 37(4):655–661.

Joshi, R. C., M. S. Delacruz, E. C. Martin, J. C. Cabigat, R. G. Bahatan, A. D. Bahatan, E. H. Abayao, J. Choy-Awon, N. P. Chilagan, and A. B. Cayong. 2001. Current status of the golden apple snail in the Ifugao Rice Terraces, Philippines. Journal of Sustainable Agriculture 18:71–90.

Joshi, R. C. 2005. Managing invasive alien mollusc species in rice. International Rice Research Notes 30:5–13.

Joshi, R. C. 2007. Problems with the management of the golden apple snail *Pomacea canaliculata*: an important exotic pest of rice in Asia. Pages 257–264 *in* M. J. B Vreysen, A. S. Robinson, and J. Hendrichs, editors. Area-wide control of insect pests. Springer, Netherlands.

Karatayev, A. Y., L. E. Burlakova, V. A. Karatayev, and D. K. Padilla. 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. Hydrobiologia 619:181–194.

Kenward, M. G., and J. H. Roger. 1997. Small sample interference for fixed effects from restricted maximum likelihood. Biometrics. 53:983.

Kyle, C. H., M. K. Trawick, J. P. McDonough, and R. L. Burks. 2009. Population dynamics of an established reproducing population of the invasive apple snail (*Pomacea insularum*) in suburban southeast Houston, Texas. Texas Journal of Science 61:323–327.

Kyle, C. H., A. W. Kropf, and R. L. Burks. 2011. Prime waterfront real estate: apple snails choose wild taro for oviposition sites. Current Zoology 57:630–641.

Leslie, A. J. 1992. Copper-herbicide use patterns in Florida water. Florida Department of Natural Resources, Tallahassee, USA.

Levin, P., R. H. Cowie, J. M. Taylor, K. A. Hayes, K. M. Burnett, and C. A. Ferguson. 2006. Apple snail invasions and the slow road to control: ecological, economic, agricultural, and cultural perspectives in Hawaii. Pages 325–335 *in* R. C. Joshi and L. S. Sebastian, editors. Global advances in ecology and management of golden apple snails. Philippine Rice Research Institute (PhilRice), Los Baños, Philippines.

Litsinger, J. A., and D. B. Estano. 1993. Management of the golden apple snail *Pomacea canaliculata* (Lamarck) in rice. Crop Protection 12:363–370.

López, M. A., C. R. Altaba, K. B. Andree, and V. López. 2010. First invasion of the apple snail *Pomacea insularum* in Europe. Tentacle 18:26–28.

Louisiana State University Agriculture Center [LSU AgCenter]. 2014. Louisiana rice production handbook. Louisiana State University, Baton Rouge, USA.

Louisiana State University Agricultural Center [LSU AgCenter]. 2018. Louisiana Summary, Agricultural and Natural Resources, 2010. Louisiana State University, Baton Rouge, USA.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2004. 100 of the world's worst invasive alien species. Invasive Species Specialist Group, Auckland.

Lu, G. Y., and D. W. Wong. 2008. An adaptive inverse–distance weighting spatial interpolation technique. Computers and Geosciences 34:1044–1055.

Mackenzie, D. I. 2006. Modeling the probability of resource use: the effect of, and dealing with, detecting a species imperfectly. Journal of Wildlife Management 70:367–374.

Maketon, M., K. Suttichart, and J. Domhom. 2009. Effective control of invasive apple snail (*Pomacea canaliculata* Lamarck) using *Paecilomyces lilacinus* (Thom) Samson. Malacologia 51(1):181–190.

Martin, C. W., K. M. Bayha, and J. F. Valentine. 2012. Establishment of the invasive island apple snail *Pomacea insularum* (Gastropoda: Ampullaridae) and eradication efforts in Mobile, Alabama, USA. Gulf of Mexico Science 30:30–38.

McBride, W. D., S. R. Skorbiansky, and N. Childs. 2018. U.S. rice production in the new millennium: changes in structure, practices, and costs. USDA Economic Research Service, USA.

McClain, R. W., R. P. Romaire, C. G. Lutz, and M. G. Shirley. 2007. Louisiana crawfish production manual. Louisiana State University, Baton Rouge, USA.

McClary, A. 1964. Surface inspiration and ciliary feeding in *Pomacea paludosa* (Prosobranchia: Mesogastropoda: Ampullariidae). Malacologia 2:87–104.

Menke, K., R. Smith Jr., L. Pirelli, J. Van Hoesen. 2016. Mastering QGIS. Second edition. Packt Publishing, Birmingham, UK.

Mitsch, W. J., and J. G. Gosselink. 2007. Wetlands. Fourth edition. John Wiley & Sons, New Jersey, USA.

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

Mohan, N. 2002. Introduced species summary project apple snail (*Pomacea canaliculata*). <http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/Pomacea_canaliculata.html#Taxonomy>. Accessed 17 June 2020.

Morrison, W., and M. Hay. 2011. Feeding and growth of native, exotic and non-exotic alien apple snails (Ampullariidae) in the United States: exotics eat more and grow more. Biological Invasions 13:945–955.

National Oceanic and Atmospheric Administration [NOAA]. 2020. Our next invasive species is a “snail” of a story. Sea Grant Louisiana Newsroom. <https://www.laseagrant.org/2020/snail-of-a-story/>. Accessed 12 August 2021.

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

Nicolopoulou-Stamati, P., S. Maipas, C. Kotampasi, P. Stamatis, and L. Hens. 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front Public Health 4:148. DOI: 10.3389/fpubh.2016.00148.

Olivier, H. M., J. A. Jenkins, M. Berhow, and J. Carter. 2015. A pilot study testing a natural and a synthetic molluscicide for controlling invasive apple snails (*Pomacea maculata*). Bulletin of Environmental Contamination and Toxicology. DOI 10.1007/s00128-015-1709-z.

Osborne, J. W., and E. Waters. 2002. Four assumptions of multiple regression that researchers should always test. Practical Assessment, Research, and Evaluation (8). DOI: https://doi.org/10.7275/r222-hv23.

QGIS Development Team. 2021. QGIS Geographic Information System, open source geospatial foundation project. <https://qgis.org/en/site/>. Accessed 5 July 2020.

Qoraychy, I. El., M. Fekhaoui, A. El Abidi, R. Benakame, A. Bellaouchou, and A. Yahyaoui. 2015. Accumulation of copper, lead, chrome, cadmium in some tissues of *Procambarus clarkii* in Rharb Region in Morocco. Journal of Geoscience and Environment Protection 3:74–81.

Ramakrishnan, V. 2007. Salinity, pH, temperature, desiccation and hypoxia tolerance in the invasive freshwater snail Pomacea insularum. Dissertation, University of Texas, Arlington, USA.

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

Salcedo, G. A. 2013. Acción patogénica de *Heterorhabditis bacteriophora* (Poinar) sobre el caracol manzana (*Pomacea canaliculata* Lamarck), plaga de los cultivos de arroz (*Oriza sativa*) en la cuenca baja del río Daule, Guayas, Ecuador. Avances en Investigación Agropecuaria 17(2):53–56.

San Martíns, R., C. Gelmi, J. V. de Oliveira, J. L. Galo, and H. Pranto. 2009. Use of a saponin based molluscicide to control *Pomacea canaliculata* snails in southern Brazil. Natural Product Communications 4(10):1327–1330.

SAS Institute Inc. 1999. SAS/STAT® User's Guide, Version 8. SAS Institute Inc., Cary, NC, USA.

SAS Institute Inc. 2013. SAS® 9.4 Procedures Guide: Statistical Procedures, 2nd ed. SAS Institute Inc., Cary, NC, USA.

Schultz, B. 2020. First instance of apple snails damaging rice crop reported. <https://www.lsuagcenter.com/profiles/rbogren/articles/page1585862797099>. Accessed 11 Nov 2020.

Seuffert, M. E., and P. R. Martín. 2009. Influence of temperature, size and sex on aerial respiration of *Pomacea canaliculata* (Gastropoda: Ampullariidae) from southern Pampas, Argentina. Malacologia 51(1):191–200.

Seuffert, M. E., and P. R. Martín. 2013. Juvenile growth and survival of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) reared at different constant temperates. SpringerPlus 2:312.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591.

Snyder, N. F. R., and H. A. Snyder. 1971. Defenses of the Florida apple snail *Pomacea paludosa*. Behavior 40:175–215.

Stroup, W. W. 2015. Rethinking the analysis of non–normal data in plant and soil science. Argon. J. 107:811–827.

Teo, S. S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. Crop Protection 20(7):599–604.

Teo, S. S. 2003. Damage potential of the golden apple snail *Pomacea canaliculata* (Lamarck) in irrigated rice and its control by cultural approaches. International Journal of Pest Management 49(1):49–55.

Teo, S. S. 2006. Evaluation of different species of fish for biological control of golden apple snail *Pomacea canaliculata* (Lamarck) in rice. Crop Protection 25:1004–1012.

Tomašević, A.V., and S. M. Gašić. 2012. Photoremediation of carbamate residues in water. Pages 39–60 *in* S. Soloneski and M. Larramendy, editors. Insecticides – Basic and Other Applications. InTech, Rijeka, Croatia.

US Environmental Protection Agency [EPA]. 2018. What is a pesticide? <https://www.epa.gov/minimum-risk-pesticides/what-pesticide#:~:text=Pesticide%20law%20defines%20a%20%E2%80%9Cpesticide,regulator%2C%20defoliant%2C%20or%20desiccant.>. Accessed 28 Oct 2020.

US Fish and Wildlife Service [FWS]. 2009. Managing invasive plants: concepts, principles, and practices. Monitoring. <https://www.fws.gov/invasives/stafftrainingmodule/assessing/monitoring.html#part2>. Accessed 10 Nov 2020.

USDA National Agricultural Statistics Service [NASS]. 2021. 2020 State agriculture overview. <https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=LOUISIANA>. Accessed 17 January 2020.

Wada, T. 1997. Introduction of the apple snail *Pomacea canaliculata* and its impact on rice agriculture. Pages 170-180 *in* E. Yano, K. Matsuo, and D. A. Andow, editors. Proceedings of international workshop on biological invasions of ecosystems by pests and beneficial organisms. Tsukuba, Japan.

Wada, T. 2002. Influences of changing from transplanting to direct seeding on the status of some pest species. Pages 341–348 *in* S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, editors. Direct seeding: research strategies and opportunities. International Rice Research Institute, Metro Manila, Philippines.

Wilson, B., J. M. Lucero, and M. Shirley. 2020. Invasive apple snails threaten Louisiana rice, crawfish production. <https://www.lsuagcenter.com/profiles/lbenedict/articles/page1584029414317>. Accessed 15 June 2020.

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

Xu, X., J. Wu, K. Wang, Y. Yang, and S. Yan. 2019. Locking of the operculum in a water snail: theoretical modeling and applications for mechanical sealing. Journal of Theoretical Biology 464:104–111.

Zarco-Perello, S., and N. Simões. 2017. Ordinary kriging vs inverse distance weighting: spatial interpolation of the sessile community of Madagascar reef, Gulf of Mexico. PeerJ 5:e4078. DOI: 10.7717/peerj.4078.

Yahaya, H., A. Badrulhadza, A. Sivapragasam, M. Nordin, M. N. Muhamad Hisham, and H. Misrudin. 2017. Invasive apple snails in Malaysia. Pages 169–195 *in* R. C. Joshi, R. H. Cowie, and L. S. Sebastion, editors. Biology and Management of Invasive Apple Snails. Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija 3339.

Vita

Julian Martin Lucero was born in Marrero, Louisiana, and received his Bachelor's Degree in Natural Resource Ecology and Management with a concentration in Wildlife Ecology at Louisiana State University (LSU). He began working as a student worker in the Veterinary Entomology Lab at LSU, which ultimately led him to join the Wilson Lab to begin his Master's Degree in Entomology with a Minor in Applied Statistics in August 2019. For his Master's thesis, Julian evaluated the regional expansion and potential chemical control for invasive apple snails (*Pomacea maculata*) in southwest Louisiana through conducting site surveys on rice and crawfish farms and adjacent water ways as well as conducting a series of laboratory toxicity assays. He also worked on projects that examined resistance mechanisms of sugarcane (*Saccharum* spp. L.) to stem borers *Diatraea saccharalis* (F.) and *Eoreuma loftini* (Dyar).

Julian intends to receive his Master's Degree in December 2021 and plans to begin working in environmental consulting right after graduating.