Life History and Phenological Characteristics of the Invasive Island Apple Snail *Pomacea maculata* (Perry, 1810) in Stormwater Retention Ponds in Coastal South Carolina, USA

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LIFE HISTORY AND PHENOLOGICAL CHARACTERISTICS OF THE INVASIVE ISLAND APPLE SNAIL *POMACEA MACULATA* (PERRY, 1810) IN STORMWATER RETENTION PONDS IN COASTAL SOUTH CAROLINA, USA

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ABSTRACT The impacts of non-native species on native ecosystems can be substantial, and effective management strategies often require a comprehensive understanding of species biology and ecology within the invaded range. The island apple snail *Pomacea maculata* is an invasive species known to alter the structure and function of wetland habitats. Researchers first reported island apple snails in the United States in Tallahassee, FL, in 2002 and subsequently observed this species in South Carolina (SC) in 2008. The objectives of this study were to document the spatial distribution, phenology and life history, and habitat preference of island apple snails, as well as its association with co-occurring gastropods in SC. Populations were surveyed in stormwater retention ponds throughout coastal SC, where surveyors documented the numbers of live specimens, sex ratios, and the substrate types on which island apple snails deposited egg clutches. The high abundances and year-round presence of egg clutches observed in this study indicate that these populations are successfully reproducing throughout the year, although egg clutch abundance was positively correlated with air temperature. Overall, this study found higher numbers of female *P. maculata* than males, and that females preferred to lay egg clutches on culverts as opposed to other available substrates. In addition to *P. maculata*, four other non-native gastropods were documented in stormwater retention ponds. Among these, *Melanoides tuberculata* and *Pyrgophorus parvulus* are potential vectors for multiple human diseases and had never before been reported in SC. Understanding the current distribution and life history traits of *P. maculata* is important for determining the potential for further spread and providing opportunities to protect healthy, natural ecosystems from the impacts of non-native species.

KEY WORDS: gastropod, stormwater retention pond, non-native, distribution, freshwater, *Melanoides tuberculata*, *Pyrgophorus parvulus*, *Pomacea maculata*

INTRODUCTION

Non-native species can often have detrimental effects on native flora and fauna, potentially reducing biodiversity, disrupting food webs, and altering ecosystem processes (Parker et al. 1999, Carlsson 2006, Boland et al. 2008, Baker et al. 2010). Effective management of these species requires a more comprehensive understanding of their ecology, including their distribution and dispersal, life history and life cycle phenology, and relationships with environmental factors and co-occurring species; however, this information is not always available from the invaded range. Therefore, it is important to understand the ecology of non-native species within invaded ranges. The island apple snail *Pomacea maculata* (Perry, 1810) (synonymous with *Pomacea insularum*) (Gastropoda: Ampullarioidea: Ampullariidae) is a non-native species in both Asia and North America and is known to have substantial effects on both natural wetlands and agricultural rice fields (Horgan et al. 2014).

The native range of *Pomacea maculata* spans across South America, including Argentina and Brazil (Hayes et al. 2008, 2012, 2015). Various vectors, such as the aquarium and food trades, introduced *P. maculata* to parts of Asia, including Thailand, Vietnam, Korea, Malaysia, Singapore, Cambodia, and Japan (Matsukura et al. 2013). Fog (2002) formally documented island apple snails in the United States in 2002 from Lake Munson in Tallahassee, FL, but because of the widespread taxonomic confusion with *Pomacea canaliculata* (Hayes et al. 2012), reports of *P. canaliculata* in Florida and Texas before 2002 likely also refer to *P. maculata* (Neck & Schultz 1992, Cowie et al. 2006, Rawlings et al. 2007). The earliest records of *P. canaliculata* or *P. maculata* in the USGS Nonindigenous Aquatic Species database include Florida (1989), Texas (2000), Alabama (2003), Georgia (2005), Louisiana (2006), Mississippi (2008), and South Carolina (SC) (2008) (Benson 2016).

Numerous vectors may have introduced *Pomacea maculata* into the United States where it negatively impacts native species (Cattau et al. 2010), although the aquarium trade is most likely (Cowie 2002, Howells et al. 2006, Karatayev et al. 2009). Ampullariids are often kept as domestic animals, partly because of their ability to consume excess algae in tank systems (Perera & Walls 1996). Various *Pomacea* spp. have also been imported for human consumption and as biological control agents in other parts of the world, including, but not limited to, Japan, China, and the Caribbean (Okuma et al. 1994, Pointier & Jourdane 2000). As biological controls, *Pomacea* spp. have effectively consumed non-native or unwanted macrophytes (Wada 2006, Burlakova et al. 2008, 2010); however, the species can feed relatively indiscriminately (Baker et al. 2010). More often, *Pomacea* spp. serve as agricultural pests, consuming rice, taro, and lotus (Okuma et al. 1994, Naylor 1996, Cowie 2002). Introduced populations of island apple snails can negatively impact native plant abundance, density, and diversity (Boland

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et al. 2008, Baker et al. 2010) and can, therefore, alter the balance of natural ecosystems. For example, introduced *Pomacea* spp. in southeastern Asia caused the transition of a wetland from a macrophyte-dominated ecosystem to a phytoplankton-dominated ecosystem (Carlsson et al. 2004).

The high fecundity of *Pomacea maculata* also contributes to its success as an invasive species and can escalate the impacts that this species can have within its invaded range (Barnes et al. 2008). Multiple sources suggest that high fecundity is the best indicator of molluscan invasive potential in the United States (Baker 1974, Kolar & Lodge 2001, McMahon 2002, Marchetti et al. 2004, Keller et al. 2007). Although additional studies are warranted, Teo (2004) suggested that island apple snails can become sexually mature in as few as 3 mo, whereas others determined a time to maturity of up to 2 y (Martin et al. 2001, Cowie 2002). After internal fertilization, adult female island apple snails emerge from the water and deposit bright pink egg clutches on natural or man-made substrates above the waterline (Barnes et al. 2008, Burks et al. 2010). During the reproductive period, beginning as early as April, female island apple snails in Texas can deposit a new egg clutch each week, with an average of 2,064 eggs (range 211–4,751) per clutch (Barnes et al. 2008). After 1–3 wk of desiccation, hatchlings emerge and fall into the water (Barnes et al. 2008, Burks et al. 2010). Hatching success varies, but it is estimated that approximately 10% of hatchlings survive to adulthood (Barnes et al. 2008).

Native gastropod species exhibit lower feeding rates than *Pomacea* spp. (Kwong et al. 2010). This characteristic may present a competitive threat to the native gastropod community and to other grazing macroinvertebrates (Dillon 2000). According to Morrison and Hay (2011), the feeding rates of *Pomacea maculata* and *Pomacea canaliculata* exceeded those of all the other ampullariid species tested, including the North American native *Pomacea paludosa*, with no food preferences observed. In addition, Burlakova et al. (2008) found that *P. maculata* consumed many native plant species at a greater rate than invasive plant species.

Modeling efforts, based on bioclimatic data, including temperature, precipitation, isothermality, pH, and salinity, predicted a restricted distribution to coastal SC (Byers et al. 2013). At the commencement of this study, the South Carolina Department of Natural Resources (SC DNR) had reported island apple snails from three locations in SC, all associated with stormwater retention ponds: Socastee (Horry County) in 2008, Mount Pleasant (Charleston County) in 2010 and 2011, and the West Ashley area of Charleston (Charleston County) in 2013 (Knott, D. unpublished data).

The purpose of this study was to gain a broader understanding of the biology and ecology of *Pomacea maculata* near the northern end of its invaded range in North America. Specifically, the following questions were addressed: (1) What is the spatial distribution of *P. maculata* within stormwater retention ponds across the coastal region of SC? (2) What are the phenological, life history, and reproductive patterns of *P. maculata*? (3) How does temperature influence the life history characteristics of *P. maculata*? (4) Does *P. maculata* show a preference for certain substrate types for the deposition of its egg clutches? (5) What gastropod species co-occur with *P. maculata*? This study provides important information relevant to the life history and ecology of *P. maculata* at the northern edge of its current distribution.

MATERIALS AND METHODS

Four types of surveys were conducted to determine the spatial extent of the invasion by island apple snails within stormwater retention ponds in coastal SC to understand the dispersal capacity, environmental correlates, and life history characteristics of the species. The types of surveys were as follows: (1) a broad-scale statewide survey; (2) fine-scale targeted (FST) surveys in areas with known populations of *Pomacea maculata*; (3) a biweekly survey of a single pond with a well-established population of *P. maculata*; and (4) a haphazard survey of stormwater retention ponds on Hilton Head Island, SC. The first three surveys used the same methods. At each pond surveyed, available substrate types (culverts, rocks, or plastic) and vegetation (grass, trees, vertical vegetation, or floating vegetation) were recorded. The perimeter length of each pond determined the number of "rake sites" at each location where surveyors raked, using a clam rake and a garden rake, along the top 3 cm of the pond surface approximately every 100 m around the pond to collect sediment and detritus. The clam rake sampled denser, deeper substrate, with the aim of collecting buried snails, whereas the garden rake collected surface vegetation and associated fauna. Depth profiles of ponds were not recorded, but vegetation type was recorded. For the fourth survey, substrate and vegetation were not recorded, but rake sampling was implemented. Material collected from rakes were thoroughly examined for the presence of *P. maculata* and other gastropod species, and all observed *P. maculata* were retained.

In addition, a visual survey was performed for island apple snail egg clutches around the entire perimeter of each pond. Because female island apple snails deposit bright pink egg clutches above the waterline, they serve as the best indicator of species presence during periods of reproductive activity. All accessible egg clutches were counted and destroyed by scraping remnants into the water. All collected snails were transferred to the laboratory at the SC DNR Marine Resources Research Institute in Charleston, SC, for future analysis and preservation.

Spatial Extent and Dispersal Capacity

A broad-scale statewide survey was conducted to determine the spatial extent of *Pomacea maculata* throughout the coastal counties of SC. The survey occurred from May 2015 to August 2015, coinciding with the predicted peak of the spawning season of island apple snails, to use the high visibility of egg clutches as a secondary indicator of the presence of the gastropod. The survey consisted of a stratified random sample of 100 stormwater retention ponds from Horry to Jasper County. Because of the uneven distribution of ponds across the eight coastal counties (Horry, Georgetown, Berkley, Charleston, Dorchester, Colleton, Beaufort, and Jasper), five zones were created to divide the coastline evenly (Fig. 1). Ponds were randomly selected using an ArcGIS layer of ponds digitized from 2006 to 2013. Each month, four to six ponds from each of these five zones were sampled to avoid a seasonal effect, such as that created by sampling from north to south, or *vice versa* over time.

In September and October 2015, four FST surveys were conducted, one in West Ashley and three in Myrtle Beach, to determine the degree to which *Pomacea maculata* dispersed from target ponds with previously reported populations of *P. maculata*. The FST surveys consisted of sampling the target pond and all other

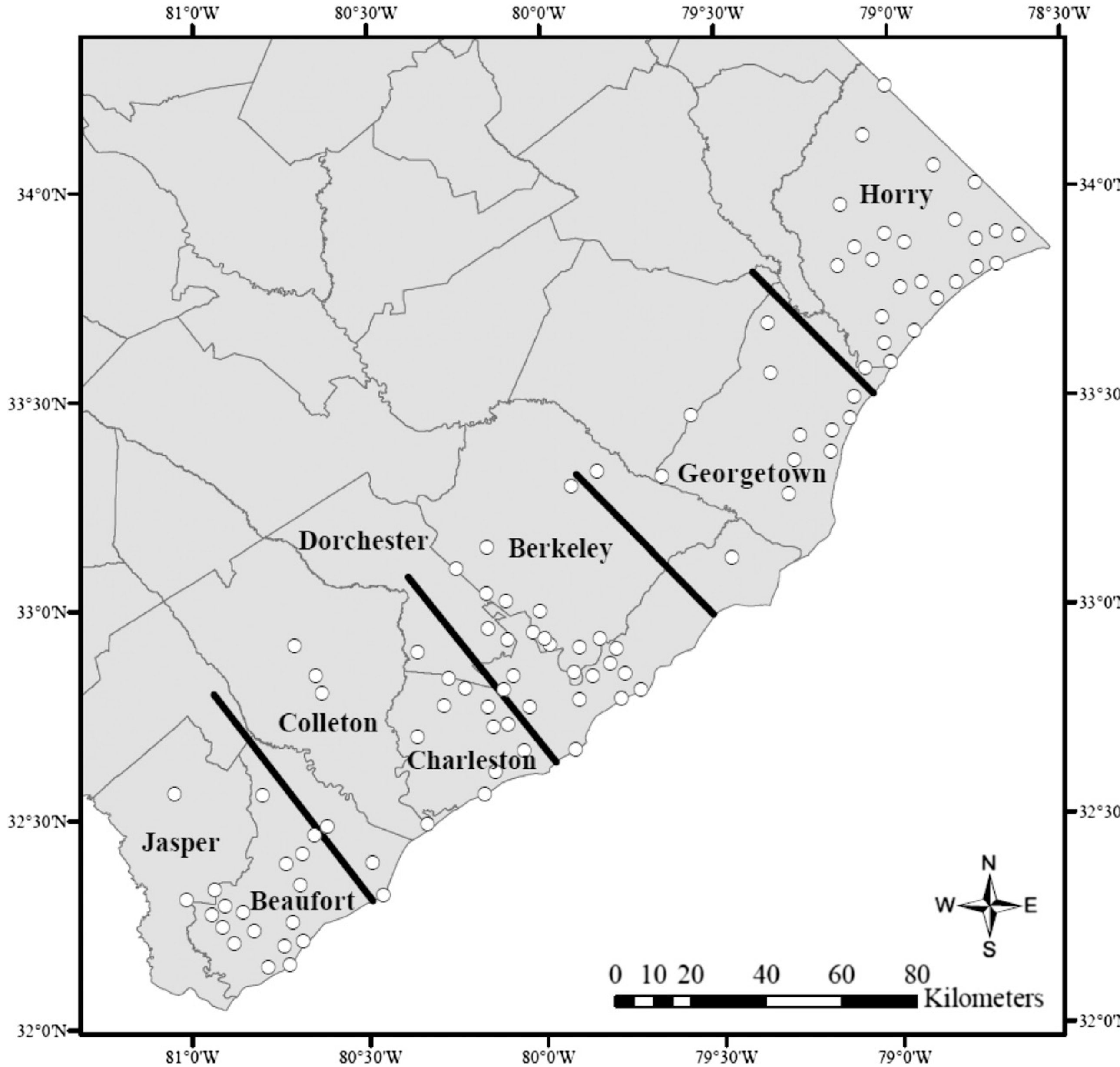

Figure 1. Equal divisions of the coastal zone in SC established to distribute sampling effort across the state to avoid seasonal effects when surveying 100 randomly selected stormwater retention ponds. Circles indicate the locations of stormwater retention ponds sampled during the broad-scale statewide survey.

retention ponds within a 0.8-km radius of the target pond (Table 1). The 0.8-km radius of the first two Myrtle Beach target ponds overlapped, and 18 survey ponds from these two target areas fell within both surveys.

Phenology, Life History, and Reproductive Ecology

Temporal patterns of abundance, size distribution, and sex ratios of an established population of island apple snails were surveyed biweekly at a single pond in West Ashley, SC (32.850425, -80.086864) from May 2015 to April 2016. After each survey, all island apple snails were immediately frozen. Sex was determined for 205 adult (or near-adult) snails collected from West Ashley between June and October 2015. Similarly, 125 individuals from the Myrtle Beach FST survey were examined to calculate sex ratio. Sex was determined by the presence of a penis sheath for males or a large pink ovary for females. In the cases in which snails lacked either structure [$n = 14$, 24–40 mm shell length (SL)], they were considered to be sexually immature, following Burks et al. (2011). During each biweekly survey, all recently-deposited egg clutches were counted and destroyed. In addition, the number of copulating pairs observed was recorded.

Temperature Relationships

The biweekly survey in West Ashley was used to investigate whether temperature correlated with the abundance of island apple snails and the number of egg clutches. Because of the shallow depth of the pond, air temperatures were assumed to be

TABLE 1.

Results of *Pomacea maculata* fine-scale targeted survey sampling sites in West Ashley, SC, and Myrtle Beach, SC, conducted between September 2015 and October 2015.

Date	Location name	Site location (latitude°, longitude°)	Number of ponds surveyed	Number of ponds with *P. maculata* observed	Number of ponds with only egg clutches observed	Percentage of ponds in survey area with *P. maculata*
September 2015	West Ashley	32.850425, –80.086864	24	7	2	37.5
October 2015	Myrtle Beach (1)	33.664417, –79.000375	20	1	0	5.0
October 2015	Myrtle Beach (2)	33.660253, –79.004783	25	3	0	12.0
October 2015	Myrtle Beach (3)	33.647075, –78.984397	29	12	3	51.7

correlated with water temperatures (Byers et al. 2013). Snail abundance was compared to air temperatures (data collected by Weather Underground; www.wunderground.com, Creekside Carolina Bay KSCCHARL91) recorded both on the day of sampling and 2 wk before sampling. Salinity was also recorded using a YSI Pro 2030 probe.

Substrate Preference for Egg Deposition

The biweekly survey in West Ashley was used to determine substrate preference for depositing egg clutches. The types of substrates on which island apple snails deposited egg clutches were recorded. Substrates available included lawn grass, various trees, vertical vegetation along the bank, floating vegetation, culverts, rocks, and plastic material. Only newly laid, bright pink egg clutches were counted to avoid double counting (Fig. 2). To determine substrate preferences for egg clutch deposition of *Pomacea maculata*, the percentages of egg clutches observed on specific substrates was compared with the percentages of each substrate available at the pond, estimated based on visual observations of pond perimeter. If *P. maculata* has no preference, the percentages of available substrates should equal the percentages of egg clutches observed on those corresponding substrate types.

Figure 2. Egg clutches deposited by *Pomacea maculata* in West Ashley (Charleston County), SC. This illustration demonstrates the approach taken to counting newly laid egg clutches during the surveys. Newly laid egg clutches are bright pink. As the clutches begin to desiccate, they fade to a lighter pink color. Eleven egg clutches were recorded as newly laid for the culvert pictured here.

Co-Occurring Gastropods

During all surveys, the presence of all gastropod species, both native and non-native, was recorded. Data were collected on the presence of these gastropod species during all four surveys and summarized at the county level.

Statistical Analyses

Correlations were conducted among abundances of *Pomacea maculata*, egg clutches, and copulating pairs and environmental conditions using Pearson product-moment correlations in the "lmodel2" package of R (Legendre 2014, R Core Team 2016). Conditional on significant correlations between factors, standard major axis (SMA) linear regressions were conducted to determine the slope and intercept of the linear relationships (Quinn & Keough 2002). Standard major axis regressions were used to account for the variability present in both the x and y values of these regressions. Sex ratios were calculated and tested for deviation from 1:1 ratio using chi-square goodness-of-fit tests. Shell length was compared between sexes within and among sites using Student's t-tests. Binomial test with confidence intervals was used to determine whether the number of egg clutches laid on each substrate deviated significantly from what is expected based on the relative availability of each substrate type.

RESULTS

Spatial Extent and Dispersal Capacity

No populations of island apple snails were documented during the broad-scale statewide survey of 100 ponds encompassing the entire SC coast. After completing the FST surveys in West Ashley and Myrtle Beach, it appears that the populations have not spread extensively from the targeted ponds. In the FST survey, populations of island apple snails were observed in 9 of the 24 ponds within 0.8 km of the West Ashley target pond (Table 1). Salinity of these ponds ranged from 0.0 to 0.2 during the study. In the first of three FST surveys in Myrtle Beach, only 1 of 20 ponds yielded *Pomacea maculata* and associated egg clutches (Table 1). In the second FST survey in Myrtle Beach, 3 of 25 ponds yielded *P. maculata* and associated egg clutches (Table 1). Of the 29 stormwater retention ponds in the third survey area in Myrtle Beach, island apple snails and associated egg clutches were observed in 12 ponds and observed only egg clutches, but no adults, in an additional three ponds (Table 1).

Phenology, Life History, and Reproductive Ecology

Although a seasonal pattern in the number of *Pomacea maculata* and the number of egg clutches is evident (Fig. 3A, C), the relationship between the number of *P. maculata* and the number of egg clutches on a given sampling date was not significant ($r = 0.38$, $P = 0.07$). The number of *P. maculata*, however, was significantly related to the number of egg clutches found on the previous sampling day ($r = 0.47$, $P = 0.03$, $y = 0.25x + 2.84$, not shown). The greatest number of copulating pairs were collected on September 15, 2015 and September 30, 2015 (21 and 20 pairs, respectively). The number of copulating pairs was significantly correlated with the number of live *P. maculata* collected ($r = 0.87$, $P < 0.001$, $y = 5.75x + 13.69$, not shown). Copulating pair abundance was also significantly correlated with the number of egg clutches ($r = 0.53$, $P = 0.007$, $y = 22.32x + 49.61$, not shown).

From May 2015 to April 2016, 1,064 specimens of *Pomacea maculata* and 4,050 egg clutches were observed in the West Ashley pond that was sampled biweekly. The number of island apple snails collected during an individual survey ranged from 4 (April 2016) to 126 individuals (September 2015). The number of newly laid egg clutches observed ranged from 0 (February 2016) to 462 (May 2015) (Fig. 3C). Newly laid egg clutches occurred on every sampling day with the exception of 1 day in mid-February. Peak abundances of *P. maculata* and egg clutches occurred during the warmer months, between August and October 2015, with an additional peak of egg clutches observed in late April 2016 (Fig. 3A, C).

The sample of adult or subadult island apple snails from West Ashley demonstrated a sex ratio highly skewed toward females (female:male = 2.6:1; $P < 0.001$), as did the sample of 125 snails from Myrtle Beach (female:male = 2.2:1; $P < 0.001$). In West Ashley, sex ratios did not change over time; at least twice as many females as males were consistently observed. Females also demonstrated significantly greater SL in the West Ashley population ($t_{203} = 3.15$, $P < 0.001$), although this was not apparent in Myrtle Beach ($t_{123} = 0.94$, $P = 0.174$). There were significant differences in SL between sites for both males ($t_{96} = -5.92$, $P < 0.001$) and females ($t_{227} = 12.76$, $P < 0.001$), with larger males and females collected from West Ashley compared with Myrtle Beach. Minimum SL declined markedly in May 2015 and remained low through June 2015 and July 2015 as a new cohort entered the population (Fig. 4). A sharp decrease in minimum SL was observed in January 2016 (Fig. 4).

Temperature Relationships

Pomacea maculata was observed on every sampling date during the biweekly survey, even through the winter months when air temperatures dropped to –5°C in January 2016. The daily average air temperatures during the sampling period ranged from –1°C to 32°C. Average air temperature on the day of sampling and egg clutch abundance were significantly correlated (Fig. 3F), as was the relationship between egg clutch abundance and average air temperature over the previous 2 wk before sampling (Fig. 3F). The lowest temperature at which *P. maculata* was observed moving occurred on January 5, 2016, at 8.4°C. There was no significant relationship between average air temperature on the sampling day and the number of island apple snails observed. The relationship between the abundance of island apple snails and average air temperature over the previous 2 wk before sampling was marginally significant ($r = 0.41$, $P = 0.05$, not shown). The number of copulating pairs observed and the average air temperature on the sampling day were positively and significantly correlated (Fig. 3E).

Substrate Preference for Egg Deposition

Island apple snails laid egg clutches on seven different types of substrate, specifically lawn grass, trees, vertical vegetation, floating vegetation, culverts, rocks, and plastic. Female island apple snails demonstrated a preference for laying egg clutches on culverts ($P < 0.001$), with 46.6% of the observed egg clutches deposited on this substrate type, even though culverts only comprised approximately 13% of the pond perimeter (Table 2). By contrast, grass surrounded about 70% of the West Ashley pond surveyed, but only 35.5% of egg clutches observed at this location were laid on this substrate type, demonstrating a preference against grass ($P < 0.001$) (Table 2). There was not a preference for or against any of the other substrate types encountered.

Co-Occurring Gastropods

During the broad-scale statewide survey, three other non-native gastropod species were found, specifically the Japanese mystery snail (*Bellamya japonica*), the ghost rams-horn (*Biomphalaria havanensis*), and the red-rim melania (*Melanoides tuberculata*), on Hilton Head Island (Beaufort County), SC. Additional surveys revealed two other non-native gastropods, namely, the crownsnail (*Pyrgophorus parvulus*) and the Florida apple snail (*Pomacea paludosa*) in southern SC. This is the first report of *P. parvulus* and *M. tuberculata* in SC. No trend in county-level latitudinal diversity of native gastropods was observed during this study (Table 3).

DISCUSSION

The results of this study provide a better understanding of the biology and ecology of *Pomacea maculata* in SC. Based on these surveys, *P. maculata* appears restricted to the previously known localities, where they inhabit discrete systems of mostly interconnected stormwater retention ponds in coastal counties, generally located in residential communities. The occurrence of island apple snails in residential areas is not surprising, because the species was likely introduced through the aquarium trade. A variety of mechanisms for spread, including stormwater retention pond connectivity, large flood events, and dispersal by predators, may be facilitating secondary invasions by island apple snails, such that additional surveys in larger streams or rivers in the vicinity of the ponds surveyed in this study may be warranted.

The high abundances of adult island apple snails and egg clutches, along with the broad size range of island apple snail specimens collected, suggest that these populations are multigenerational and successfully reproducing in these stormwater retention pond habitats. The reproductive capacity of this species contributes to its success as an invader. In this year-long biweekly study, reproductive behavior was observed throughout the year, supported by both the deposition of newly laid egg clutches and the observation of copulating pairs, making this survey the first to document reproductive effort by *Pomacea maculata* year-round in SC. Despite this year-round reproductive effort, juvenile island

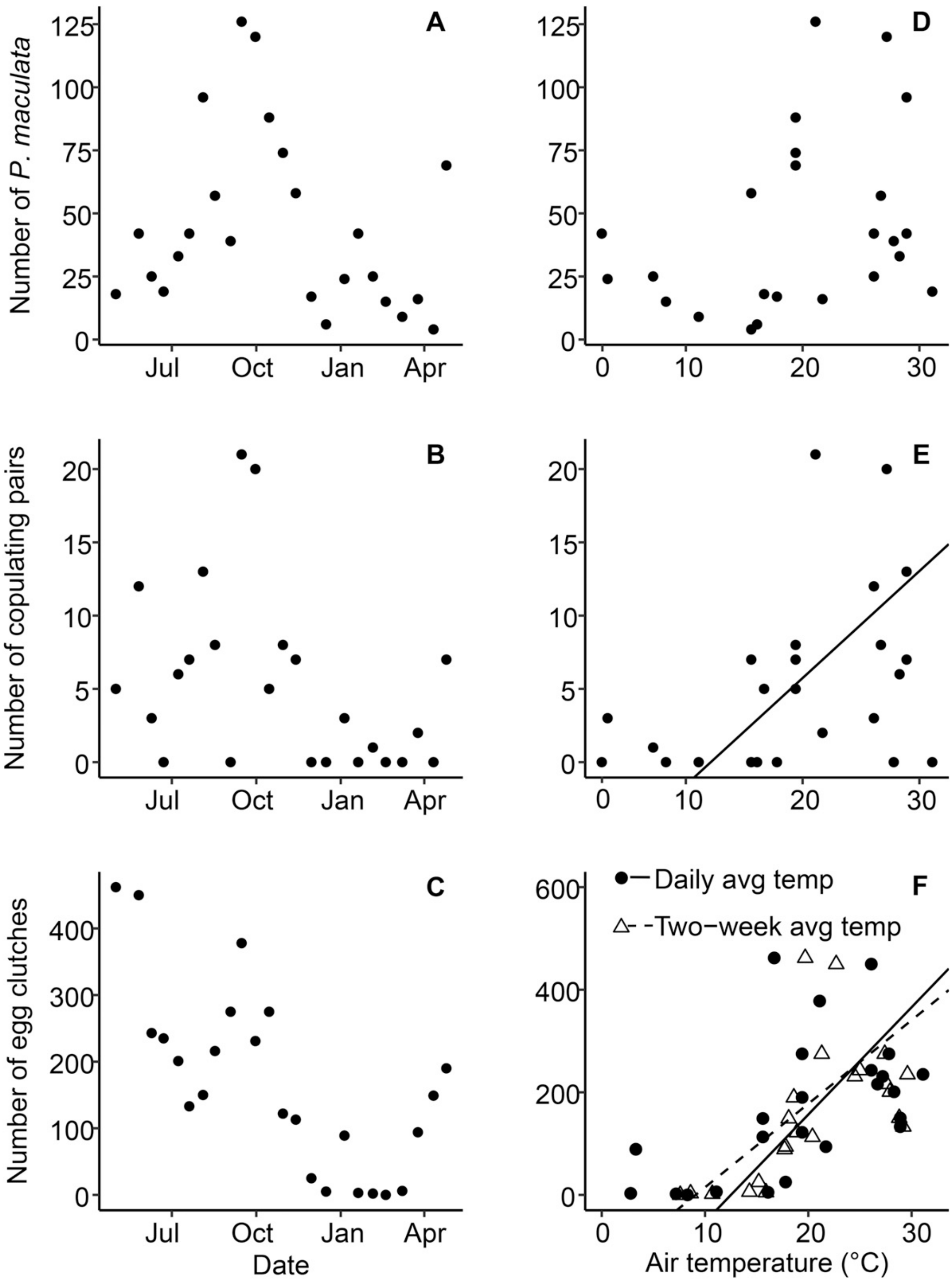

Figure 3. **Results from a biweekly survey in a retention pond in West Ashley, SC, from May 1, 2015 to April 25, 2016. Abundance of *Pomacea maculata* (A), copulating pairs (B), and egg clutches (C). Linear SMA regressions depicting the nonsignificant relationship between *P. maculata* abundance and average air temperature on the day of sampling ($r = 0.33$, $P = 0.12$) (D), the significant relationship between copulating pairs and average air temperature on the day of sampling ($y = 0.73x - 8.86$; $r = 0.42$, $P = 0.04$) (E), and the significant relationship between egg clutch abundance and average air temperatures, both during the day of sampling (solid line; $y = -261.6x + 20.9$; $r = 0.55$, $P = 0.005$) and for the 2 wk before sampling (dashed line; $y = -148.1x + 16.3$; $r = 0.61$, $P = 0.001$) (F) from May 1, 2015 to April 25, 2016 in a stormwater retention pond located in West Ashley, SC.**

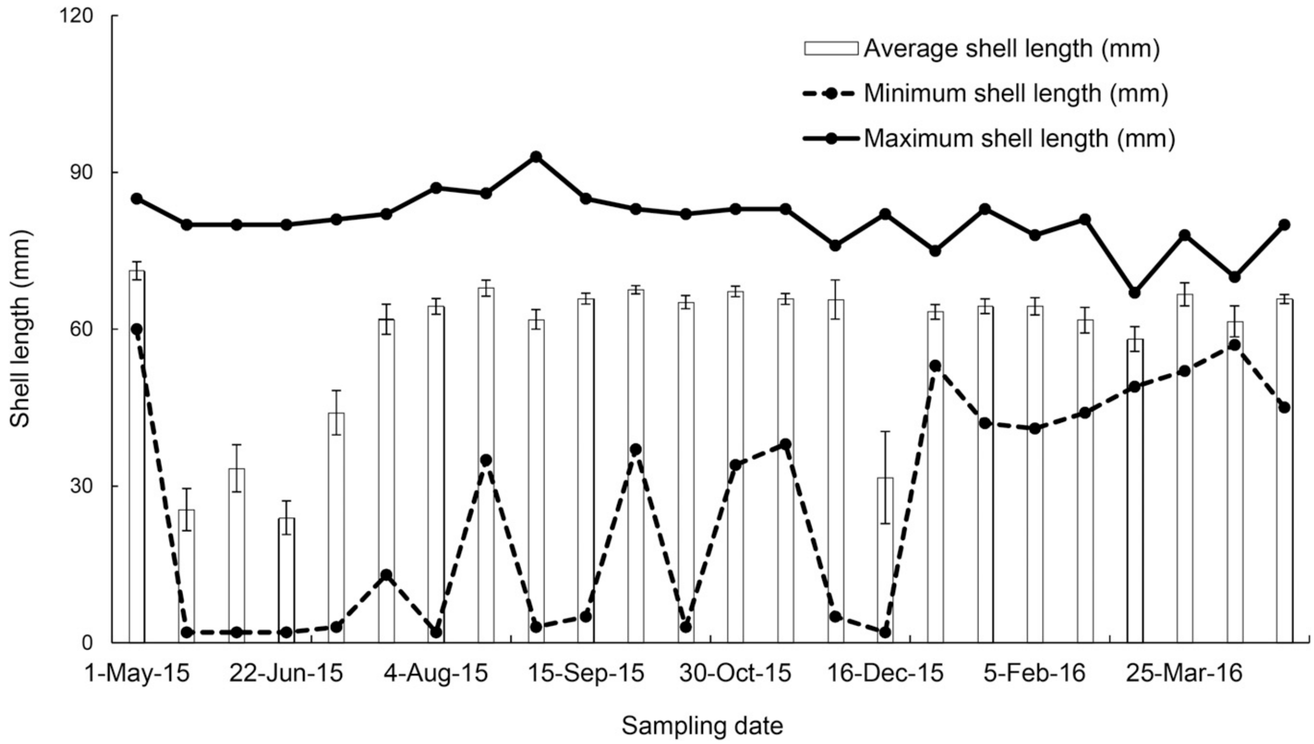

Figure 4. Minimum, maximum, and average SL of live *Pomacea maculata* collected in the biweekly study in West Ashley, SC.

apple snails were not collected from January to April. The presence of juvenile *P. maculata* during only part of the year suggests that juveniles grow and reach maturity within a single year with iteroparous reproduction in the West Ashley population (Dillon 2000). These sampling methods, however, are not sufficiently quantitative to rule out a 2-y maturation time.

During the biweekly survey, significantly greater frequencies of females compared to males were observed in both the West Ashley and Myrtle Beach stormwater retention ponds, a pattern that was consistent throughout the year. Recent research on *Pomacea canaliculata* suggests that sex determination is multigenic and biparental, leaving great potential for natural sex ratio variance (Yusa 2006, 2007). Given these sampling methods, there may be bias in the calculated sex ratio because females may appear on pond edges more frequently than males to deposit egg clutches during the reproductive season, rendering them more likely than males to be collected by targeted sampling at the perimeter of ponds. Despite this potential bias, significant sexual size dimorphism was not observed in the Myrtle Beach population. Both males and females from the Myrtle Beach population were significantly smaller than those from the West Ashley population. Such interpopulation differences in size may be because of food availability or levels of intraspecific competition for resources (Berrie & Visser 1963). Observed density of *Pomacea maculata* was much greater at the Myrtle Beach location where 345 live island apple snails were observed in one small pond (approximately 2,200 m^2), compared with 128 live island apple snails observed from the much larger pond (approximately 6,600 m^2) in West Ashley. The relatively high density of island apple snails at the Myrtle Beach location could have been an important factor leading to the differences in population size structure observed between these two study sites.

When considering potential spread of this species, it is important to determine suitable habitat in areas surrounding known populations of island apple snails in relation to the physiological tolerances of the species. Island apple snails withstand water temperatures as low as 4°C in their native range in Buenos Aires (Rawlings et al. 2007, Hayes et al. 2008). In the laboratory, Ramakrishnan (2007) found that *Pomacea maculata* from Texas could survive water temperatures between 15°C and 36°C; however, the trials generally lasted 30 days, suggesting that the temperature tolerance range of island apple snails may be broader when exposed for shorter periods of time.

TABLE 2.

Percentage of *Pomacea maculata* egg clutches ($n = 4{,}050$) deposited in West Ashley, SC, between May 1, 2015 and April 25, 2016 in relation to the percentage of specific substrates available compared using a binomial test with confidence intervals.

Substrate type	Percentage of egg clutches observed	Percentage of substrate type available	*P*-value
Grass	35.5	70.0	<0.0001
Trees	0.9	1.5	0.626
Vertical vegetation	0.9	3.0	0.216
Floating vegetation	0.02	1.0	0.325
Culvert	46.6	13.0	<0.0001
Rock	16.0	11.0	0.109
Plastic	0.02	0.5	0.496

TABLE 3.

Freshwater gastropod distribution (presence/absence) observed during the broad-scale statewide survey of 100 retention ponds completed from May 2015 to August 2015 across the eight coastal counties of SC, ordered from north to south.

	Horry	Georgetown	Berkley	Charleston	Dorchester	Colleton	Beaufort	Jasper
Bellamya japonica*	–	–	–	–	–	–	X	–
Biomphalaria havanensis*	–	–	–	–	–	–	X	–
Gyraulus parvus	X	X	X	X	–	–	X	–
Helisoma trivolvis	–	X	X	X	–	–	X	–
Littoridinops tenuipes	–	–	–	X	–	X	X	–
Lymnaea columella	X	–	X	X	–	–	–	–
Melanoides tuberculata*	–	–	–	–	–	–	X	–
***Physa* sp.**	X	X	X	X	X	X	X	X
Pomacea paludosa*	–	–	–	–	–	–	X	–
Pyrgophorus parvulus*	–	–	–	–	–	–	X	–

Species denoted with an asterisk are invasive to SC.

Similarly, Deaton et al. (2016) observed no mortality in Louisiana populations of *P. maculata* held for 10 days at 20°C or 15°C, but they found a significant increase in mortality at 10°C and 5°C and the highest mortality at 0°C. Although mortality was not assessed in this study, the number of observed individuals in relation to temperature was recorded, and no significant relationship with temperature recorded on the day of sampling or the preceding 2-wk average temperature was found. This suggests that factors other than temperature are likely important in determining abundances of island apple snails observed in a given survey. For example, weather conditions on the sampling day may affect the turbidity of the pond, thus impacting the number of island apple snails observed during the visual survey. Although the total number of individuals observed was not related to temperature, both the number of copulating pairs and the number of observed egg clutches were significantly correlated, indicating that reproductive activity may be more sensitive to temperature than dispersal and feeding activity in this species.

Island apple snails withstood salinities up to 13.6 for hatchlings and adults in Texas (Ramakrishnan 2007) and up to approximately 15 for Alabama hatchlings (Martin & Valentine 2014), suggesting that salinity tolerance may be a plastic trait at the population level in this species. In the current study, *Pomacea maculata* was observed in salinities up to 0.2, although these surveys were limited to stormwater retention ponds. The salinity tolerance of this invasive species is important for understanding the potential habitat range of island apple snails, should this species enter more natural systems. The extent to which introduced *P. maculata* can survive if introduced into natural, estuarine systems after flooding events, for example, will be influenced by their salinity tolerance. A better understanding of the temperature and salinity tolerances of this species will improve predictions regarding the potential for *P. maculata* to spread and its eventual spatial distribution.

Burks et al. (2010) found that shallow lakes and ponds surrounded by emergent vegetation and available hard substrate are ideal habitats for island apple snails, especially in regard to oviposition. Previous research suggests that egg clutches that were occasionally inundated yielded similar hatching rates to those that were unsubmerged or periodically spritzed, whereas constantly submerged egg clutches failed to hatch and tended to disintegrate (Martin & Valentine 2014). In the current study, *Pomacea maculata* preferred to deposit egg clutches on culverts, along with an avoidance of the use of grass as an oviposition substrate. Island apple snails may prefer the culverts and rocks in the West Ashley pond because they are generally hard, dry substrates and are less likely to become inundated. Females deposit one egg at a time, ensuring that the eggs attach to either the substrate or other eggs to form a contiguous clutch of eggs (Gooding, pers. obs.). On slick substrates, the integrity of the clutch may be compromised, causing the eggs to fall into the water, where they are no longer viable, before hatching (Martin & Valentine 2014). In addition to being smoother and wetter, grassy fringes of these ponds are typically at lower elevations than the rocks and culverts, increasing the likelihood that eggs laid on grass may experience inundation after increases in water level after heavy rain events. Although the data in this study show a preference by *P. maculata* for depositing egg clutches on culverts and a preference against grass, island apple snails deposited eggs on a broad diversity of available substrate materials during this study, including emergent pipes, old rusty tires, and floating plastic bottles (Gooding, personal observation).

In addition to determining the spatial extent, life history characteristics, and habitat preferences of island apple snails, possible impacts of *Pomacea maculata* on native gastropod fauna was also investigated. In addition to *P. maculata*, this study identified four additional nonindigenous gastropod species, two of which previously had not been documented in SC, namely, the crownsnail (*Pyrgophorus parvulus*) and the red-rim melania (*Melanoides tuberculata*). The red-rim melania is of particular concern given that this species serves as an intermediate host for nematode and trematode parasites known to infect fish and mammals, including humans (Derraik 2008, Dorta-Contreras et al. 2011). Among these trematodes is *Opisthorchis viverrini*, a liver fluke that can cause cholangiocarcinoma, a cancer of the bile ducts, in humans (Sripa et al. 2010) and *Clonorchis sinensis* that causes clonorchiasis disease in humans (Lim et al. 2006). In addition, *M. tuberculata* is known to serve as an intermediate host for the trematode *Centrocestus formosanus*. Although rare, *C. formosanus* is known to infect humans (De & Le 2011) and is documented from *M. tuberculata* and fish in western Texas, USA (McDermott et al. 2014).

It is challenging to predict the impacts that island apple snails will have on native species, especially because eradication of *Pomacea maculata* is difficult to achieve. In the West Ashley pond, all observed island apple snails and associated egg clutches were removed every 2 wk for 1 year, and the population was not eradicated; however, sampling methods that included the removal of *P. maculata* in the middle of the pond may have been more successful. Bernatis and Warren (2014), for example, were more successful in their efforts. They waded in and collected *Pomacea* sp. from a relatively isolated pond in Florida for 3 y, with abundances decreasing each year (Bernatis & Warren 2014). Varying levels of eradication success (Raines 2009, Werner 2010, Martin et al. 2012) and the ability of *P. maculata* to potentially expand its range suggest that additional research is needed to determine effective strategies for containment and eradication.

CONCLUSIONS

These results provide valuable information regarding the status of island apple snails along coastal SC, most importantly demonstrating that established populations are reproducing throughout the year. Based on its broad diet range and high fecundity, it seems probable that *Pomacea maculata* will continue to spread. Consequently, it remains necessary to continue monitoring the populations and to consider implementing control techniques. Priorities for future research should include determining salinity tolerances of this species in SC and the application of genetic and molecular tools to investigate the population genetic structure of this species both within the state and among invaded states in the southeastern United States and along the Gulf of Mexico. Island apple snails have the potential to negatively affect natural habitats, so it is important to learn more about this species to manage the possible impacts it may have on native flora and fauna.

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