ENVIRONMENTAL LIMITS ON THE DISPERSAL OF INVASIVE *POMACEA MACULATA* IN LAKE SEMINOLE

by

NICHOLAS SCOTT MARZOLF

(Under the Direction of Stephen W. Golladay and Alan P. Covich)

ABSTRACT

The introduction of the Island apple snail, *Pomacea maculata* in the past 20 years is one of the largest threats to freshwater ecosystems in the tropics and subtropics. In the United States, *P. maculata* has direct impacts on native snail species, submerged aquatic vegetation, and its dispersal to novel habitats is of great concern. Environmental factors, particularly temperature and calcium, have shown to affect behavior and physiology. In Lake Seminole, a large shallow reservoir, *P. maculata* was introduced in 2003, and has since dispersed throughout large sections of the lake. Temperature variability in the lake may alter snail behavior, but localized refugia will allow *P. maculata* to persist. Calcium concentrations around the lake and in nearby lakes suit the elemental requirements of *P. maculata* growth and survival, except when combined with cooler conditions. This suggests *P. maculata* can disperse throughout Lake Seminole, and throughout the lower ACF basin in future.

INDEX WORDS: *Pomacea maculata*, reservoir, climate warming, invasive species, novel ecosystem, refugia, ACF basin, *Hydrilla verticillata*

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Introduction and Literature Review

Ecology of Invasive Apple Snails (*Pomacea* spp.)

Snails of the genus *Pomacea* are native to wetlands, rivers and lakes in North and South America and their introduction outside of their native range has shown that these gastropods are among the most invasive and ecologically destructive species to tropical and subtropical freshwater ecosystems (Hayes *et al.*, 2015; Hayes *et al.*, 2012). These snails have been intentionally introduced for aquaculture, as a food resource, as a biological control for disease vectors, and from unintentional introductions via the aquarium trade (Horgan, Stuart & Kudavidanage, 2014). Several *Pomacea* species have been widely introduced, including areas into Central America, the Caribbean, North America, Africa, Southeast Asia, New Zealand, and Asia.

Of the introduced *Pomacea* spp*.,* several are similar in shell morphology and in egg mass size and color, including *P. maculata, P. insularum,* and *P. canaliculata* (Howells *et al.*, 2006; Rawlings *et al.*, 2007; Thompson, 2004). In North America, genetic studies have shown the various introductions of this genus to be the same species: *P. maculata* (Hayes*, et al.*, 2012). The introduced range of *P. maculata* extends from the Everglades in South Florida, where it overlaps with the native apple snail *P. paludosa*, to Georgia, Alabama, Louisiana, Texas, Mississippi and South Carolina. Minimum water temperature and pH have been key in outlining areas susceptible to *P. maculata* range expansion in the southeast US, and will be used in the future to assess how climate change may increase the suitable habitat based on the current distribution of *P. maculata* (Byers *et al.*, 2013; Ramakhrishnan, 2007). Furthermore, other physicochemical properties may also affect the expansion of *Pomacea* spp., including salinity, dissolved oxygen and hydroperiod (Ramakhrishnan, 2007).

Apple snail activity and behavior has shown to be limited by low temperature. Several studies have shown that 13°-18° C is the range of low temperature thresholds for *P. canaliculata* juvenile growth and adult activity (Seuffert & Martin, 2010; Seuffert & Martin, 2013). Below the 13° C threshold, *Pomacea* spp. become inactive and retract into their shells and may attempt to bury themselves in sediment as an over-winter estivation behavior (Seuffert & Martin, 2008; Stevens *et al.*, 2002). Water temperature above 18° C has been reported to initiate reproduction (Albrecht, Carreno & Castro-Vazquez, 1999), though in more tropical species, hydroperiod influences reproductive timing more than temperature (Darby, Bennetts & Percival, 2008). Differences in temperature and hydroperiod outside of the native range may result in altered reproduction and seasonal snail behavior.

Female egg mass deposition has played a large role in the identification of *Pomacea spp.* (Barnes *et al.*, 2008; Bernatis & Warren, 2014; Burks, Kyle & Trawick, 2010; Darby *et al.*, 1999; Kyle *et al.*, 2013; Rawlings*, et al.*, 2007). Egg masses are deposited on emergent surfaces, and hatch after a 1-3 week incubation period, depending on species. Egg clutch size and number of eggs per egg mass also varies greatly by species: *P. paludosa* range from 3–141 eggs/egg mass (Cowie, 2002) and *P. maculata* range from 522–4751 eggs/clutch (Barnes*, et al.*, 2008).

In contrast to many smaller gastropods, the size of *Pomacea* spp. permits a macrophyte-dominanted diet (Wong *et al.*, 2010). Large radula size and mouthparts, high reproductive output and increased dietary demands result in these snails to consuming large amounts of submerged and emergent macrophytes, whereas smaller native and introduced snails generally consume periphyton (Carlsson, Bronmark & Hansson, 2004; Horgan*, et al.*, 2014; Sharfstein & Steinman, 2001). However, native and non-native macrophytes, periphyton, benthic detritus and amphibian eggs have all been documented in the diets of introduced *Pomacea*, suggesting omnivorous food habits (Baker, Zimmanck & Baker, 2010; Boland *et al.*, 2007; Burks, Hensley & Kyle, 2011; Carlsson*, et al.*, 2004; Fang *et al.*, 2010; Fellerhoff, 2002; Karraker & Dudgeon, 2014; Morrison & Hay, 2010).

Most gastropods require calcium to build their shell and consequently, freshwater habitats with sufficient calcium and a well-buffered pH are important to build and maintain shell strength (Brodersen & Madsen, 2003). Studies have indicated that gastropods are not observed in water with $[Ca^{2+}]$<5-mg/L (Lodge *et al.*, 1987). Calcium concentration is an important determinant in snail distribution and calcium limitation may affect the presence of both native and potentially invasive mollusks in freshwater habitats (Solomon *et al.*, 2009; Whittier *et al.*, 2008). Larger gastropods may similarly have greater calcium requirements due to larger shell size, and specific to *Pomacea*, the deposition of calcareous egg masses (Meier-Brook, 1978). However, gastropods have been observed in habitats with calcium concentration <5 mg/L (Jokinen, 1982), but the potential of calcium limitation is greater, and may be a deterrent in *P. maculata* distribution and expansion*.*

Environmental calcium, and its correlates including alkalinity, pH and conductivity, are known to influence freshwater mollusk species richness (Dillon, 2000).

Snails experimentally exposed to low calcium concentrations showed significant reductions in growth and in egg mass production (Thomas & Lough, 1974). In the case of the large ampullariid *Marisa cornuarietis*, closely related to *Pomacea,* calcium uptake in individuals increased as a function of the ambient calcium concentration, and individuals reared at lower concentrations exhibited lower survivorship and thinner shells (Meier-Brook, 1978). The concentrations used by Meier-Brook (1978) were greater than those used in previous studies (Thomas & Lough, 1974), suggesting that larger species have increased requirements and that their distribution will be influenced by their environmental calcium requirements. Calcium requirements of invasive mollusks may act as a filter for colonists, and serve as the mechanism to limit dispersal of non-native mollusks into habitats that have ill-suited water chemistry (Lodge*, et al.*, 1987; Spyra & Strzelec, 2014).

Study Site: Lake Seminole and the Lower Apalachicola-Chattahoochee-Flint Watershed

Lake Seminole is the 15,216-hectare impoundment in the Apalachicola-Chattahoochee-Flint (ACF) basin. The lake receives major surface water inflow from Spring Creek, the Chattahoochee and Flint Rivers, and serves as the headwaters for the Apalachicola River below Jim Woodruff Lock and Dam. The lake has a mean depth of 3m, and max depth of 10.7m. Much of the lake is dominated by submerged aquatic vegetation (SAV) during the growing season, and the expanse of SAV can influence nutrient storage and processing in the lower ACF (McEntire, 2009; Shivers, 2010).

The Silver Lake Wildlife Management Area (SLWMA) is located between the Spring Creek and Flint River arms of Lake Seminole, and is managed by the Georgia Department of Natural Resources. The property of SLWMA is bordered by channels connecting the Flint River to Spring Creek and receives substantial recreational boat traffic. Several of these channels are located near or adjacent to small ponds and lakes within SLWMA. These ponds were historically isolated depressional wetlands prior to the damming of the lake, which raised the groundwater potentiometric surface near Lake Seminole, creating the perennial lakes that are now present. Their origins as isolated wetlands results in different water chemistry compared to Lake Seminole, which receives substantial groundwater from the Upper Floridan Aquifer via Spring Creek and the Flint River (Torak, Crilley & Painter, 2005).

Located in the karst Dougherty Plain of southwest Georgia, Lake Seminole receives large amount of surface water and groundwater from unconfined fractures in the surrounding Ocala limestone. Limestone dissolution is responsible for the higher calcium concentrations and carbonate alkalinity observed in the Flint River and Spring Creek arms of the lake, both factors are of great importance to, not only *Pomacea* spp*.,* but to all gastropods (Hunter & Lull, 1977; Lodge*, et al.*, 1987; Rundle *et al.*, 2004).

The introduction of *P. maculata* in Lake Seminole was first observed by egg masses found in 2003 on the southern shoreline of the Flint River arm at Jack Wingate's Lodge, and the observation was confirmed in 2011 (Robertson, 2012; USACE, 2013). Robertson (2012) was able to determine the northern and southern extents of *P. maculata* egg masses along the southern shoreline of the Flint River. Among homeowners on Lake Seminole, little is known about *P. maculata* and what potential impacts dispersal and increases in abundance will have on the lake ecosystem. However, local visitors and homeowners near the introduction point in the Flint River have identified the pink egg masses on boat docks, seawall impoundments, and other artificial surfaces (pers. comm.). Although the pink egg masses are distinctive, little is known about the ecology of *P. maculata*, nor management actions that might be desirable on private property.

On the contrary, native *P. paludosa* was not recorded in USACE reports in Lake Seminole, but the presence of the Florida Apple snail in the lower Flint River basin has been recorded elsewhere (Howells *et al.*, 2006; Thompson, 2004). These snails' distributions were associated with locations of submerged springs in the lower stretches of the Flint River, suggesting the potential for historical presence in southern Georgia. *P. paludosa* are found in groundwater-fed rivers throughout the Florida panhandle, further suggesting the lower ACF as an extension of its native range (J. Van Dyke, P. Darby, pers. comm.). The spatial association with groundwater-fed springs suggests that these locations are refugia during the cold winter months. Within Lake Seminole, there are several submerged springs in the Flint River and Spring Creek arms of the lake that may be localized refugia for both *P. paludosa* and *P. maculata* during cold conditions (Torak*, et al.*, 2005).

Aims and Objectives

In this research, I aimed to track the dispersal of the invasive Island apple snail, *P. maculata,* in Lake Seminole, including historical and present and published observations to better understand the history of *P. maculata* in the lake. In comparing the historical distributions, the mechanisms and means of dispersal can be better understood, and the structural and physiochemical characteristics that may be important in future dispersal of the species can be identified. I also examined how seasonal variation in water temperature might affect the movement and temperature preference and tolerances of *P. maculata,* will help to understand how temperature may affect range expansion in the southeast US. In addition to temperature, the effect of variable calcium, an essential element for snail growth and survival, was assessed to examine how spatial calcium variability may limit the dispersal of *P. maculata* within Lake Seminole. The availability of dissolved and environmental calcium in certain freshwater habitats may not sufficiently meet the physiological needs of *P. maculata*, thereby precluding population dispersal and establishment of populations.

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CHAPTER 2

USING EGG MASSES TO TRACK AND QUANTIFY THE CHANGING DISTRIBUTIONS OF TWO APPLE SNAIL SPECIES IN A SOUTHEASTERN RESERVOIR[1]

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Abstract

The widespread dispersal of invasive apple snails (genus: *Pomacea*) has been described as one of the most consequential freshwater species introductions, but the ability to detect and estimate the adult abundance of this genus remains a challenge. Two species of apple snails, *P. paludosa* and *P. maculata,* were systematically surveyed by counting egg masses deposited on emergent surfaces during the peak reproductive months in 2013, 2014 and 2015 in Lake Seminole, Georgia. *P. maculata* egg masses were first recorded in Lake Seminole in 2003, and subsequent surveys showed expansion throughout the eastern part of the lake. In contrast, a reduction in native *P. paludosa* over the three-year survey was documented around the lake during the survey period. Physical and physicochemical characteristics were recorded to identify preferred habitats and used to predict areas of the lake that provide suitable habitat for future expansion of *P. maculata.* Survey results indicate that most of the lake provides suitable shoreline habitat and physicochemistry for both *Pomacea* species. Given time, *P. maculata*'s rapid expansion throughout the lake may have negative consequences for nutrient processing and nutrient storage in Lake Seminole by shifting dominant primary productivity from macrophyte-dominant to phytoplankton-dominant.

Keywords: Invasive species, *Pomacea, P. maculata, P. paludosa*, *Hydrilla,* habitat use, reservoir, Southeastern US

Introduction

The widespread dispersal of aquatic invasive species is one of the largest threats to freshwater bodies around the world (Howells*, et al.*, 2006; Pimentel, Zuniga & Morrison, 2005; Simberloff, 2014; Vitousek, 1990). The Island apple snail, *Pomacea maculata,* native to the Pantanal wetland in South America, has been introduced around the world through the aquarium trade and as a food source (Horgan*, et al.*, 2014). Introduction locations in North America include Florida, Georgia, South Carolina, and Texas (Chaichana & Sumpan, 2014; Hayes*, et al.*, 2012; Howells*, et al.*, 2006). There is wide interest in the consequences of introductions from an ecological perspective (Horgan*, et al.*, 2014), as wildlife disease vectors (Robertson, 2012) and a potential human health concern (Teem *et al.*, 2013).

Adult female apple snails of the genus *Pomacea* deposit calcareous, desiccation-resistant egg masses above the water line on emergent structures. The size and color these masses can be used to identify *Pomacea* species (Hayes*, et al.*, 2012; Rawlings*, et al.*, 2007) (Fig 2.1). In *P. paludosa,* there is some evidence for preference of emergent surfaces strong enough to support the weight of an adult female (Turner & Mikkelsen, 2004). In the native range of *P. paludosa* in the Florida Everglades, local egg mass abundance was weakly correlated with adult density in emergent vegetation stands (Darby*, et al.*, 1999).

In Lake Seminole, the first observation of non-native *Pomacea* egg masses (first reported as *P. canaliculata*) was made in 2003 (USACE, 2013) on the southern shoreline of the Flint River arm. However, *P. canaliculata* and *P. maculata* egg masses are similar in size and shape, and it is likely the initial identification in 2003 was incorrect (Rawlings*, et al.*, 2007). At the same location on the Flint River arm of the lake in 2010, Robertson (2012) reported the presence of non-native *P. insularum* which has recently undergone a name change to *P. maculata* (Hayes*, et al.*, 2012).

P. paludosa, the largest gastropod native to North American is predominantly found in the Everglades in South Florida. Its range also includes the Flint and Ocmulgee river basins (Howells*, et al.*, 2006; Thompson, 2004), and groundwater fed rivers throughout the panhandle of Florida, including Wakulla springs, Wacissa springs, Econfina creek and Wright's creek (Phil Darby, Jess Van Dyke, pers. comm.). *P. paludosa* has non-indigenous status along the Atlantic coast of Georgia, areas of Alabama and Puerto Rico (Benson, 2015; Horgan*, et al.*, 2014). The presence of *P. paludosa* throughout regions near Lake Seminole and in the Flint River suggest a history in Lake Seminole and native status, though no published observations are available for verification.

There is growing interest in examining the grazing effects of introduced *P. maculata* and *P. paludosa* on submerged aquatic vegetation (SAV), and the potential for grazing to alter the vegetation community of lakes, and over time, induce a shift from a SAV-dominated ecosystem to a phytoplankton-dominated ecosystem (Carlsson*, et al.*, 2004; Hilt *et al.*, 2011). *Pomacea* spp*.* are unusual among gastropods in in their ability to consume macrophytes in addition to periphyton and detritus. The role of an invasive macrophyte, *Hydrilla verticillata,* within Lake Seminole has been previously studied in regards to water quality and spatial abundance (McEntire, 2009; Shivers *et al.*, 2013; Shivers, 2010). The introduction and dispersal of *Pomacea* may reduce the *Hydrilla* distribution in the lake.

In this paper, the distribution of two apple snail species are reported based on egg mass presence and abundance. Also, an analysis of specific habitat and water quality parameters are used to predict the potential spread of *P. maculata* within a large, shallow, sub-tropical reservoir. The use of egg mass presence and abundance is hypothesized to be a tool to quickly monitor changes in the spatial distribution and relative abundance of these highly invasive gastropods, and may be related to the adult abundance of two potentially co-occurring *Pomacea*.

Materials and Methods

Site Description

Lake Seminole is a 15,216-ha impoundment located in southwestern Georgia, and is the most downstream reservoir in the Apalachicola-Chattahoochee-Flint (ACF) basin (Fig 2.2). The lake has a mean depth of 3m and a maximum depth of 10.7m at the Jim Woodruff Lock and Dam. The main surface water inflows to the lake are the Flint River, Chattahoochee River and Spring Creek, which drain a combined land area of 46,151 km^2 (Torak*, et al.*, 2005). The outflow of Lake Seminole is the Apalachicola River, which flows south into Apalachicola Bay and the Gulf of Mexico.

Smaller surface water inflows and connected water bodies include: Fish Pond Drain, located just east of the Chattahoochee River; Cypress Pond, oriented east-west between Fish Pond Drain and Spring Creek; and the Silver Lake Wildlife Management Area (SLWMA), located between the Flint River and Spring Creek. SLWMA is managed by the Georgia Department of Natural Resources, and has six small lakes that are areas of concern regarding the spread of invasive *P. maculata* (Fig 2.2).

The distributions of native and non-native macrophytes in Lake Seminole have been extensively studied, showing the distribution can vary greatly from year to year. Previous work has shown that *Hydrilla* beds alter the storage and transport of dissolved carbon and nutrients from the lake, and the year-to-year variability in macrophyte abundance may affect a variety of physicochemical properties in the lake (McEntire, 2009; Shivers, 2010). Waters *et al.* (2014) documented compositional differences in sediment cores between the Chattahoochee and Flint River arms of the lake, which reflect differences in water column carbon and nutrient dynamics over the history of the lake, which has partially been explained by the abundance and distribution of SAV post-impoundment of the lake. The expansion of *P. maculata* through grazing may negatively affect the distribution and size of SAV beds in Lake Seminole, and result in uncertain changes in primary productivity in the lake.

Egg Mass Surveys

To determine the presence of *Pomacea* spp. around Lake Seminole, survey locations were distributed at 1-km intervals along shoreline. At each survey location, a 5-minute, idle-speed, visual survey along the shoreline was completed by boat during July 2013, 2014 and 2015. Species were identified using egg mass color and size and the total number of egg masses was averaged for two observers (Fig 2.1). A nonparametric Kruskal-Wallis Rank Sum test and a post-hoc Nemenyi test for multiple comparisons were performed to determine if one species was observed in higher abundance in each year. Analyses were completed using the PMCMR package in R.

Dominant shoreline type was recorded at each location from one of five dominant types of surfaces found around the lake: beach, emergent vegetation, artifical, rock, and trees (including stumps and snags). Emergent vegetation was comprised of several species of grasses, including giant cutgrass (*Zizaniopsis miliacea*), *Typha spp.,* soft-stem bulrush

(*Scirpus validus*), water primrose (*Ludwigia spp.*) and horsetail (*Equisetum spp*)*.* Artificial habitat was predominantly boat launches, boat docks, houses, seawalls, or other structures. Outcrops of limestone were common rock habitat, and tree habitat was generally Bald Cypress (*Taxodium distichum*).

In 2014 and 2015, water temperature, pH, dissolved oxygen, and specific conductivity were recorded at each survey location using a Hydrolab Quanta (Hydrolab Corp., Austin, TX). Measurements were taken at the water surface and at the water-sediment interface to determine variation with depth. In 2015, the littoral vegetation community was characterized into six categories: open water, floating macrophytes, *Hydrilla*, *Hydrilla*-mixed SAV bed, *Lyngbya spp.*, and non-*Hydrilla* macrophyte species.

In 2013, 94 locations were surveyed in the main body of Lake Seminole. In 2014 and 2015, an additional 53 locations were surveyed in smaller drainages of the lake and in adjacent small water bodies to detect dispersal of *Pomacea* spp. on a larger scale for the lake. Egg mass counts were compared among the three survey years, between the three arms of the lake, and among shoreline categories. Trends were assessed to evaluate the change in spatial distribution and abundance of egg masses, and to identify areas of the lake susceptible to further invasion.

Shoreline Classification

Combining National Agricultural Imagery Program (NAIP) imagery for both Seminole and Decatur counties, Georgia and results from the shoreline survey, the entire shoreline of Lake Seminole was categorized into the same five-shoreline categories used during the egg mass surveys. The results of this classification were used to understand the distribution of available habitat, and to determine where the most preferred habitats were located based on egg mass abundance observed around the lake.

Results

Egg Mass Surveys

Two species of apple snails were identified during egg mass surveys in Lake Seminole: the Florida Apple Snail (*Pomacea paludosa*) and the Island Apple Snail (*Pomacea maculata*). *P. maculata* were abundant in the eastern portion of the Flint River arm of the lake, consistent with the locations documented previously (Robertson, 2012; USACE, 2013). *P. paludosa* were observed throughout the Chattahoochee River arm of the lake, western portion of the Flint River arm, and Spring Creek (Fig 2.3).

In 2013, *P. paludosa* were observed at 60% of survey locations, but observed at 58% of sites in 2014 and 53% in 2015. In contrast, *P. maculata* were observed at 22% of locations in 2013, 19% in 2014 and 25% in 2015. The decrease in *P. paludosa* and increase in *P. maculata* presence and abundance were also evident in the total and mean number of egg masses observed for each year (Fig 2.4B). The channel connecting Spring Creek and the Flint River was not surveyed in 2013, but showed an increase from 91 egg masses in 2014 to 244 in 2015. Based on independent observations, *P. maculata* was not found in this area in 2013, suggesting this area is part of the invasion front within the lake (S. Shivers, pers. obs.).

In 2014, *P. paludosa* were found at one less location than in 2013, and *P. maculata* were observed at one additional location. From 2014 to 2015, *P. paludosa* were absent at seven locations, and *P. maculata* were observed at five new locations. From 2014 to 2015, *P. paludosa* locations decreased from 58.2% to 52.7%, and *P. maculata* locations increased from 19.2% to 25.3% (Fig 2.3). Co-occurrence of both species was observed at one location in 2013. In 2014, both species egg masses were observed at 4 locations, and only 3 locations in 2015.

P. paludosa showed decreases in total number of egg masses observed in each arm of the lake (Flint River, Chattahoochee River, and Spring Creek) over the three annual surveys period. The greatest egg mass reductions were observed in Spring Creek, from 1118 total *P. paludosa* egg masses observed in 2013 to only 135 in 2015. *P. maculata* increased in abundance in the Flint River, Spring Creek and in the channel connecting these two rivers from 2013 to 2015, though the total observations decreased in 2014 (Fig 2.4). Results of Kruskal-Wallis Rank Sum and post-hoc Tukey-Nemenyi tests showed mean *P. maculata* egg mass abundance to be significantly greater than *P. paludosa* in both 2013 and 2015, but *P. paludosa* egg mass abundance was significantly greater than *P. maculata* in 2014 (Fig 2.4).

Habitat Distribution

The most widespread (n=98) and frequently observed shoreline type was emergent vegetation, which was used as a surface for egg mass deposition by both species in all arms of the lake (Fig 2.5). However, the greatest average egg mass density was observed on artificial shorelines. *Taxodium* knees (n=26) were also used frequently to deposit egg masses, and were the second most frequent habitat type surveyed on the lake (Fig 2.5).

In 2013, egg mass abundance for both species was greatest at emergent vegetation sites (2886 egg masses), followed by 740 egg masses at artificial sites and 510 egg masses at tree sites (Fig 2.5). The following year, 2287 egg masses were found at emergent vegetation sites, 586 at tree sites and 499 at artificial sites. In 2015, 2999 egg masses were found on emergent vegetation, 1268 egg masses at artificial sites and 576 egg masses at tree sites. The greatest number of egg masses observed in 2013 (count = 409) and 2015 (count = 368) were of *P. maculata* on artificial surfaces on the southern shoreline at the Flint River, near the reported point of introduction. For the remaining shoreline classifications, no egg masses were observed in any of the survey years at beach sites, and 171 total egg masses were observed at rock sites, 167 of which came from one location in 2013.

At survey locations where non-native *Hydrilla* was found either exclusively or with other macrophytes present, 3185 egg masses of both species were observed. *Pomacea* egg masses were observed in floating macrophyte beds as well (n = 1462 egg masses); areas of floating macrophytes included lotus (*Nelumbo lutea*), water lily (*Nymphaea aquatica*), water shield (*Brasenia schreberi*) and non-native water hyacinth (*Eichhornia crassipes*). Few egg masses were observed in open water (n=89) and in non-*Hydrilla* beds (n=108). No egg masses were observed on emergent surfaces where *Lyngbya* algal mats were observed in the water.

Water quality parameters varied among arms of the lake, by time of day, and by water depth (Table 2.1). Dissolved oxygen and pH varied greatly with depth, particularly when recorded near macrophyte or algal beds actively photosynthesizing. Specific conductivity from Spring Creek, which is predominantely groundwater fed, had a mean of 0.202 mS/cm, compared to the five SLWMA ponds, which range between 0.018 mS/cm to

0.086 mS/cm. Temperature was comparable between Lake Seminole (mean=31.4° C) and SLWMA (mean=31.6° C).

Shoreline Classification

Results of the shoreline classification from aerial imagery showed 64.4 km of emergent vegetation (68% of shoreline), 17.2 km of cypress tree (18%) and 10.4 km of artificial shoreline (11%). Rock habitat, in most cases outcrops of limestone bedrock, accounted for 0.9 km (0.9%) of the total shoreline, and was located near the dam on the western shoreline of the Chattahoochee River arm of the lake. Beach habitat comprised 1.0 km (1.1%) of the total shoreline, and found only in one area in the lake along the western shoreline of the Chattahoochee River.

Discussion

This study indicates that the use of egg mass surveys is a quick and efficient way to survey large areas of freshwater shoreline and emergent habitats for the presence of apple snails, particularly in documenting the presence, relative abundance and spread of invasive *Pomacea* species (e.g. *P. maculata,* (formerly *P. insularum*), *P. canaliculata, P. haustrum, P. bridgesii* and *P. diffusa*) and native *P. paludosa*. The distinctive coloration and size of the egg masses (Fig 2.1), particularly in distinguishing *P. maculata* and *P. paludosa* (Rawlings*, et al.*, 2007)*,* makes for a quick and reliable confirmation of the presence of an apple snail population. Continued egg mass surveys can document the spread of the population, as an alternative method to estimating abundance of adults which has shown to be challenging (Darby*, et al.*, 1999). Our surveys based on changes in egg-mass distributions show multidirectional movement of *P. maculata* both upstream and downstream from original reported locations, as well as movement northward to the opposite shoreline of the Flint River arm, where similar upstream and downstream dispersal has been observed.

Previous work on non-native *Pomacea* in Texas has reported that egg masses are deposited in greater abundance on non-native emergent vegetation (Burks*, et al.*, 2010). Egg mass surveys in Lake Seminole show greater egg mass abundance on artificial structures along the shoreline, although utilization of emergent vegetation and tree were also noted. Quantifying the relative use of artificial habitat versus the naturally occurring shoreline may help to identify a preferred surface, or even surfaces that result in a higher proportion of juvenile survival. Artificial structures are common in lakes and reservoirs and include boat ramps, boat docks, seawalls and homes. Artificial structures have been shown to aid the spread of invasive species, particularly in reservoirs (Johnson, Olden & Vander Zanden, 2008).

The prevalence of high egg mass abundance on artificial surfaces in all survey years suggests dense adult populations at these locations. Emergent artificial surfaces (e.g. boat-dock pilings, retainer walls) are generally more stable and able to sustain the weight of an egg-laying female compared to emergent vegetation which varies among species. However, observing and counting egg masses on artificial surfaces may be more efficient compared to emergent vegetation stands, where egg masses are hidden in dense aggregations of stalks and stems. Our observation that boat *Pomacea* spp. deposit egg masses on emergent vegetation is consistent with previous studies (Bernatis & Warren, 2014; Burks*, et al.*, 2010; Darby*, et al.*, 1999). The abundance of emergent vegetation throughout Lake Seminole suggests ample available habitat for egg mass deposition. Artificial habitat, while uncommon in most sections of the lake, resulted in the greatest abundance of egg mass counts. Artificial habitat also corresponded to the presumed first introduction sites. Previous studies have also shown significant association of introduced *Pomacea* spp. to artificial structures in canals, suggesting that these habitats are preferred by *Pomacea* (Hara *et al.*, 2014).

The northern shoreline of the Flint River arm has navigation channels that connect the Flint River to Spring Creek. In 2014, the presence of *P. maculata* was observed in the channel connecting the Flint River and Spring Creek. In 2015, the dispersal to Spring Creek (measured by survey locations where no egg masses were previously observed) was documented. There was a corresponding increase in total number of egg masses observed, indicating rapid dispersal to Spring Creek through channels connecting Spring Creek to the Flint River.

The dispersal and increase in *P. maculata* egg mass abundance can also be observed in a small cove on the western shoreline of Spring Creek, where the available habitat is mostly retainer walls, boat-dock pilings, boats and boat ramps. In this area, both *P. maculata* and *P. paludosa* were observed with low egg mass abundance in 2013. However, in 2014 and 2015, only *P. maculata* were observed and at much higher abundance. Survey locations at the mouth of this small cove have also shown a shift from either *P. paludosa* only or both species present in 2013, to both species present or *P. maculata* only, respectively, in 2014 and 2015.Both species co-occurred at two locations in 2013, four in 2014 and five in 2015 (Fig 3). Several of these co-occurrences were the result of observed spreading of *P. maculata* to areas where previously *P. paludosa* alone had been observed (Fig 2.3). These were often followed by a year where only *P. maculata* was observed. Co-occurrence has been observed in the Everglades, with juvenile invasive *P. maculata* inhibiting growth of juvenile *P. paludosa,* but the mechanism is yet undetermined (Posch, Garr & Reynolds, 2013). Our surveys indicate that in areas where both species are present in one year, in subsequent years, *P. maculata* remains the dominant species, potentially via the same mechanism hypothesized in previous studies, and further studies of these co-occurrence areas may help determine the interaction mechanism.

The timing in peak reproductive output for each species may have biased our survey results. Although the reproductive season for *P. maculata* is reported to last from May to September, newly deposited *P. maculata* egg masses were observed on Lake Seminole from April to November (N. Marzolf, pers. obs.) However, peak egg mass laying for *P. paludosa* may be in mid-spring (Darby*, et al.*, 1999). Bernatis & Warren (2014) showed peak *P. canaliculata* egg masses collected between May and August during their removal study from a small urban pond in Duval County, FL., suggesting the timing of our July surveys coincides with the observed peak output. For the purposes of these surveys, the number of egg masses for each species indicates substantial reproductive activity by both species, but more frequent surveys are needed to identify the peak in *Pomacea* spp. reproductive output for the lake.

The continued dispersal of *P. maculata* in Lake Seminole seems likely. Large areas of the lake provide suitable shoreline habitat, and during the growing season, there is ample vegetation to serve as a food source. Low winter temperatures and pH were found to be strong predictors of *P. insularum* dispersal in the southeast, and Lake Seminole falls near the limits of the low temperature tolerance (Byers*, et al.*, 2013; Ramakhrishnan, 2007). No pH recorded in the three years of surveys was <6.5. However, the potential of successive cold winters, altered thermal regimes in the lake through climate variation, and inability to access winter refugia may limit the population of both *P. paludosa* and *P. maculata*, or provide more suitable environmental conditions for one species or the other. The presence of submerged groundwater springs in the Flint River and Spring Creek arms of Lake Seminole, which remain thermally uniform throughout the year, may be used as winter refugia for each *P. paludosa* and *P. maculata* (Allums *et al.*, 2012; Thompson, 2004).

The ponds in SLWMA are not likely to be suitbale for *Pomacea* spp. establishment. Mean specific conductivity in these areas is much lower than the main body of the lake and these ponds are not hydrologically connected to the lake, but do receive high recreational use. These ponds also exhibit low calcium concentrations that may also make them unsuitable for apple snail dispersal (Marzolf *et al.*, 2015), although addition of lime (calcium oxides) has been used in some ponds to enhance fish populations for recreation, and could enhance the suitability of these ponds for *P. maculata* expansion.

During the course of the surveys, adults of each species were not routinely observed. Despite the large size of adults, direct observation by boat can be difficult except when in clear, shallow, non-vegetated areas of the lake. Using the presence and abundance of egg masses as a proxy for adult presence, relative abundance, the survey protocol represents an alternative to collecting adults, i.e., a repeatable and systematic method that can be effective in surveying large water bodies in a short period. Previous work using throw traps, vacuum dredges, seines, nets, SCUBA diving, and hand removal (Bernatis & Warren, 2014; Darby*, et al.*, 1999) have not been highly effective in capturing *Pomacea*. However, continuing to use egg mass surveys as a tool can help to develop correlations to adult density, biomass and abundance estimates as an effective alternative method.

The first occurrence of *P. paludosa* in Lake Seminole remains unknown, as there are records of *P. palduosa* in springs in the lower Flint River basin in 1974 (Howells*, et al.*, 2006; Thompson, 2004). There are populations of *P. paludosa* in several groundwater-fed rivers in the Florida panhandle, including the Chipola River, which discharges into the Apalachicola River (P. Darby, J. Van Dyke, pers. comm.). These observations suggest that *P. paludosa* have been historically present in the Florida panhandle, including areas now inundated in Lake Seminole. The presence of *P. paludosa* egg masses observed throughout the Chattahoochee River and Spring Creek arms of the lake, excluding beach and rock shoreline, suggest a history for this species in the lake.

It appears that the abundance and spatial distribution of *P. paludosa* across Lake Seminole is declining, though there may be issues with survey timing and peak reproductive output. Available habitat appears to meet the requirements of *P. paludosa*. As suggested in previous studies *P. paludosa* may be negatively affected through an as yet undefined interaction with introduced *Pomacea* spp. (Darby, Bennetts & Karunaratne, 2006; Darby, Mellow & Watford, 2007; Morrison & Hay, 2010; Posch*, et al.*, 2013). In areas where *P. maculata* has been established for >20 years, the ratio of non-native egg masses to *P. paludosa* egg masses can exceed of 10:1, regardless of habitat (T. Beck, pers. comm.). This suggests an underlying mechanism by which *P. paludosa* are being excluded by non-native apple snails, or that *P. maculata* are more fecund for longer periods of time (Barnes*, et al.*, 2008)

Continued long-term monitoring of *Pomacea* in Lake Seminole will provide an opportunity to observe: 1) the invasion rate of *P. maculata*, 2) the potential interaction between a native and introduced species for habitat and for resources (Posch*, et al.*, 2013),

3) the rate of dispersal among specific types of habitats with different food sources and 4) the effect of variable water quality in potentially limiting snail dispersal. The importance of SAV in Lake Seminole has been extensively studied (Shivers, 2010), and the interaction between *Pomacea* spp. and non-native and native SAV could have consequences from a water quality standpoint in Lake Seminole and other Southeastern US river basins. The specific ramifications for similar lakes in the southeastern US and other lakes are unknown (Baker*, et al.*, 2010; Burlakova *et al.*, 2009; Carlsson*, et al.*, 2004; Carlsson & Bronmark, 2006; Drizd, 2011; Fang*, et al.*, 2010). Climate models predict warming for the southeastern US, and under those circumstances, the potential range for *P. maculata* and other tropical and sub-tropical species could expand northward (Byers*, et al.*, 2013; Hellmann *et al.*, 2008; Hopkinson *et al.*, 2013; Parker *et al.*, 2013).

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FIGURES

Fig 2.1: Egg Masses of A) *P. paludosa* and B) *P. maculata* used to identify and quantify *Pomacea spp.* in Lake Seminole

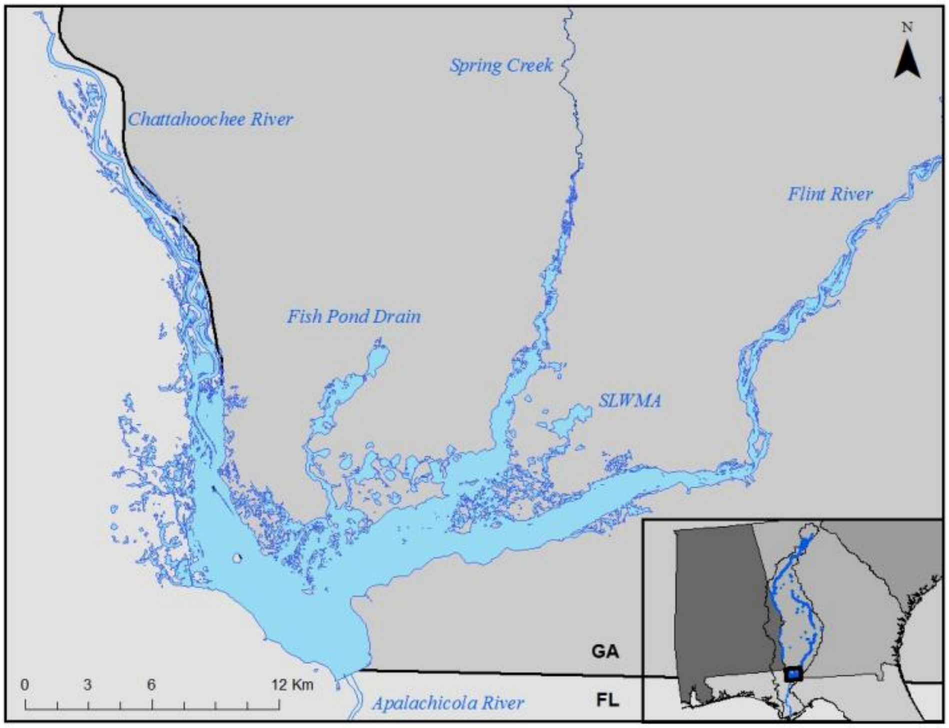

Fig 2.2: Location of Lake Seminole in ACF Basin, including major and minor surface water inflows and the Apalachicola River.

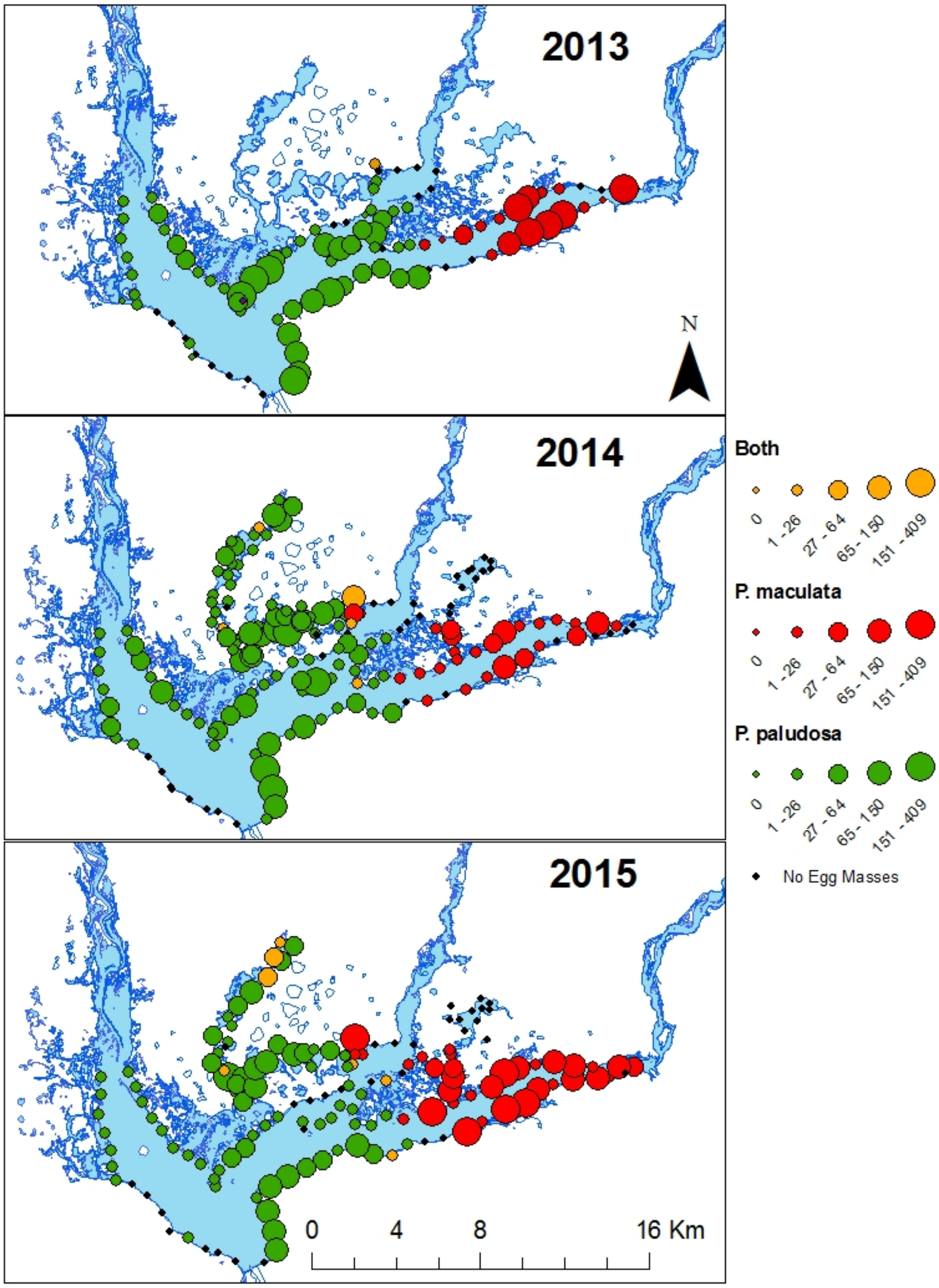

Fig 2.3: Egg mass survey results from 2013, 2014 and 2015. Circle size is proportional to the number of egg masses observed at each location and circle color related to the species observed at each location. Note the additional locations surveyed in 2014 and 2015 in Fish Pond Drain, Cypress Pond and SLWMA.

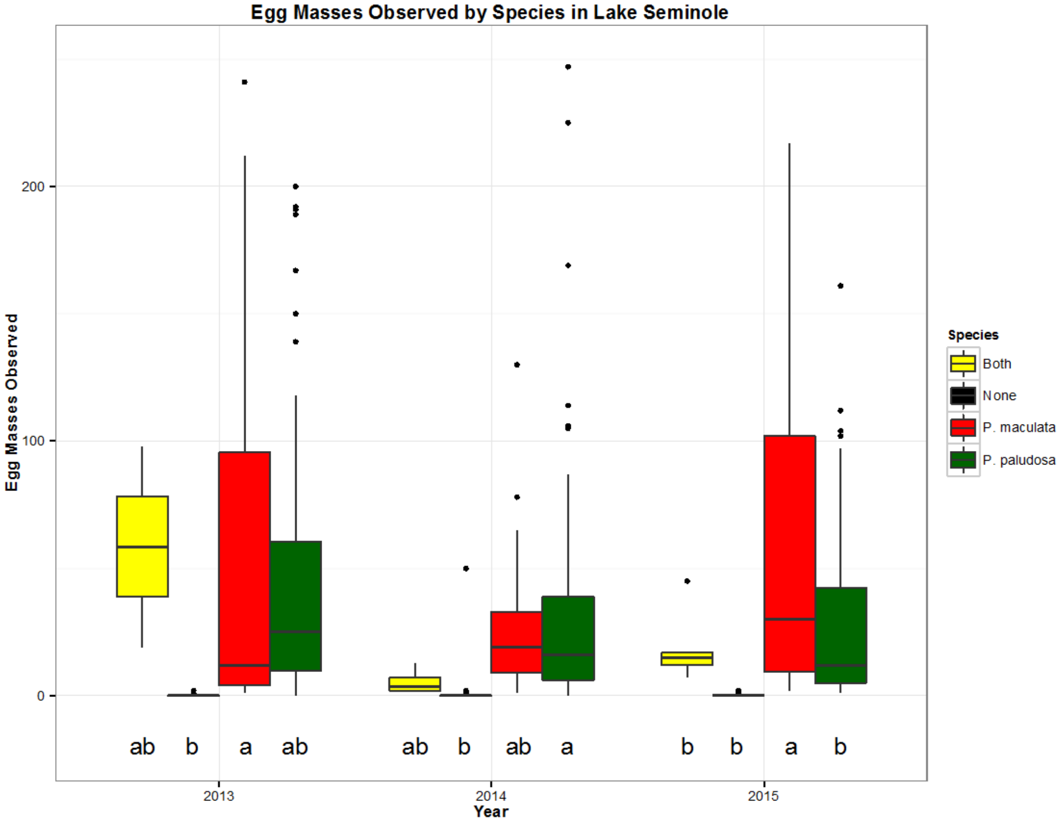

Fig 2.4 Summary statistics for egg mass surveys. A) Average number and total number of egg masses observed, by species, for all of Lake Seminole from 2013 – 2015 (± SE); B) Average number of egg masses observed from 2013 to 2015 of two apple snail species' in Lake Seminole. Error bars ± 1 SE.

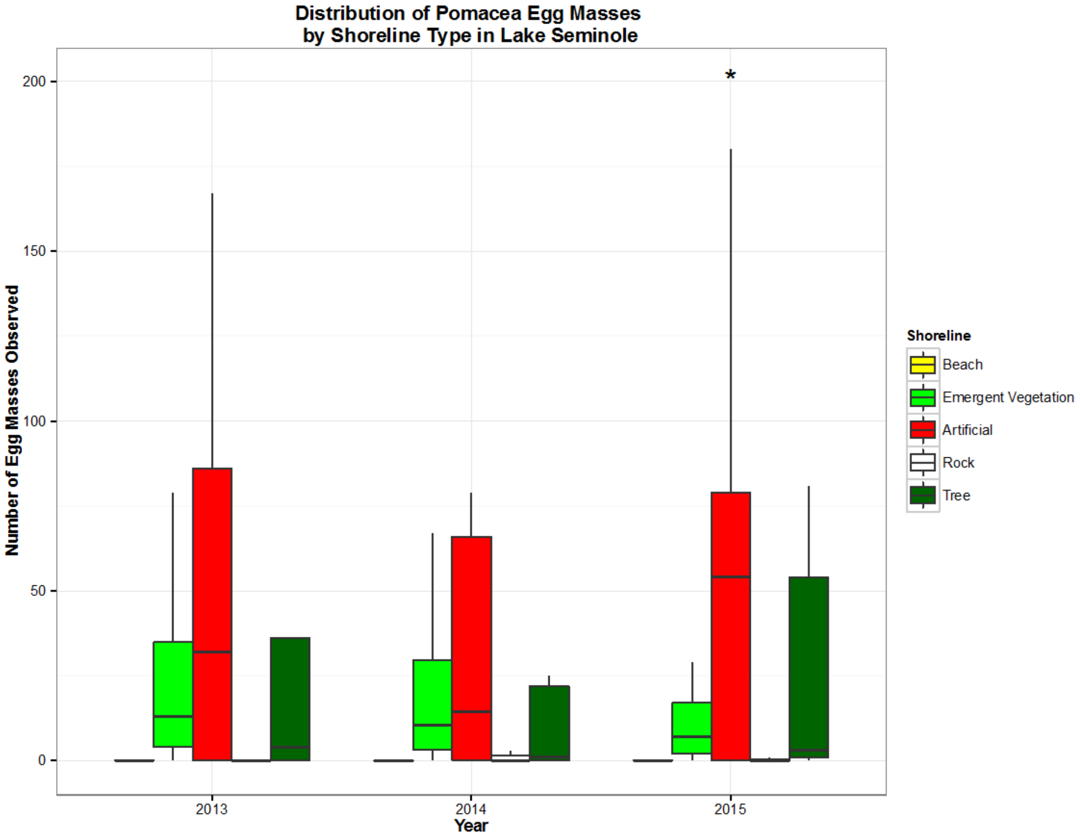

Fig 2.5: Number of egg masses observed at different shoreline habitats for 2013, 2014 and 2015.

Table 2.1: Water quality data averaged for 2014 and 2015 surveys in each arm of the lake and SLWMA ponds. Depths are reported as average of surface recordings, and the deepest location for the three arms of Lake Seminole.

2014	Depth (m)	Temperature (˚C)	Specific Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	pH
Flint River	0.0	31.76	0.179	9.18	8.39
	2.0	29.96	0.200	2.37	7.07
Spring Creek	0.0	31.54	0.202	7.39	8.05
	1.3	30.10	0.162	3.18	7.66
Chattahoochee River	0.0	32.10	0.112	7.63	7.86
	2.5	28.83	0.106	4.70	7.36
SLWMA Ponds*	0.0	31.60	0.047	7.49	7.90

2015	Depth (m)	Temperature (˚C)	Specific Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	pH
Flint River	0.0	30.89	0.136	6.59	8.17
	2.0	27.23	.0176	1.50	7.57
Spring Creek	0.0	31.32	0.146	6.05	7.91
	1.3	24.04	0.343	0.69	7.12
Chattahoochee River	0.0	32.03	0.129	7.53	8.56
	2.5	27.90	0.127	4.48	7.57
SLWMA Ponds*	0.0	31.60	0.054	4.15	7.79

*At sites in SLWMA, only surface water was analyzed

CHAPTER 3

ASSESSMENT OF THERMAL PREFERENCES OF INVASIVE *POMACEA MACULATA* IN A SOUTHEASTERN RESERVOIR[2]

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[2] Marzolf, N.S., Golladay, S.W., Covich, A.P., McCormick, P.V., and Wilde, S.W. To be Submitted to *Journal of Thermal Biology*

Abstract

The introduction and dispersal of non-native species is one of the largest threats to freshwater habitats. The Island Apple Snail, *Pomacea maculata*, has been widely introduced outside of its South American native range, and resulting in negative consequences to freshwater ecosystems. Temperature variation may affect their ability to disperse into and successfully establish in different freshwater ecosystems. Related *Pomacea* snails have shown to associate with microhabitats in order to avoid extreme temperatures, and similar behavior may be observed in *P. maculata*. To understand how temperature maybe affecting *P. maculata* dispersal in Lake Seminole, GA, USA, seasonal temperatures were measured monthly from 2010 to 2015 and in upstream surface water inflows to the lake. Laboratory experiments assessed the temperature preference and selection of *P. maculata* when offered a range of temperatures (27-34°C). Results demonstrate large seasonal variation in lake temperature, ranging from 31.7° C in the summer to 9.2° C in the winter. In temperature preference trials, snails significantly selected cooler (26-28° C) temperatures compared to warmer (32-34°C) conditions. Snail aversion to seasonal temperature extremes suggests a capacity to select thermal microhabitats and localized thermal refugia. If present, locating thermal refugia may facilitate survival in introduced ecosystems, promoting the dispersal of *P. maculata* into a wider range of freshwater habitats.

Keywords: Invasive species, *Pomacea maculata,* Island Apple snails, temperature, refugia, southeastern US, climate warming, reservoir

Introduction

The global spread of invasive species is one of the largest threats to freshwater ecosystems (Havel *et al.*, 2015; Strayer & Dudgeon, 2010). The success of invasive species can be predicted in part based on similarity of habitats between native and introduced ranges (Parker*, et al.*, 2013). However, physical and chemical variation can direct the dispersal of invasive species to microhabitats, or localized refugia, which may not be available in native ranges (Fei, Phillips & Shouse, 2014). Understanding the various physicochemical factors contributing to the survival and persistence of invasive species can help controlling their spread (Moran & Alexander, 2014).

The Island Apple Snail, *Pomacea maculata*, along with several congeners (Rawlings*, et al.*, 2007), is a widely introduced species native to rivers, wetlands and lakes of the Pantanal region in South America (Hayes*, et al.*, 2012). In Southeast Asia, invasive *P. canaliculata* is an agricultural pest in rice paddies, and has altered vegetation communities in wetlands (Carlsson*, et al.*, 2004). In North America, the non-native *P. maculata* occur across much of the southeastern US (Byers*, et al.*, 2013; Hayes*, et al.*, 2015), and has measurable effects on food webs, native gastropod species, and as vectors for wildlife and human disease (Darby*, et al.*, 1999; Darby*, et al.*, 2007; Posch*, et al.*, 2013; Teem*, et al.*, 2013).

Projections of climate variation have been used to predict future distributions of invasive species, and may be of particular use in *P. maculata* expansion (McDowell, Benson & Byers, 2014; Rahel & Olden, 2008). *P. maculata* behavior is largely regulated by physicochemical factors, such as seasonal temperature variation, which may affect the establishment and persistence of this species in new habitats (Seuffert & Martin, 2010;

Seuffert & Martin, 2013; Tiecher, Seuffert & Martín, 2015). Activity of *P. canaliculata,* a closely related congener from South America, is greatest at temperatures >15˚ C, but under conditions <15˚ C, several *Pomacea* spp. become dormant, and estivate during cold and dry periods by burrowing into sediments (Seuffert & Martin, 2010). Behavioral responses to temperature by non-native *Pomacea* spp. may be useful in predicting expansion in introduced ranges.

Reservoirs are especially susceptible to establishment of invasive species, and often contain multiple non-native species that affect ecosystem function (Johnson*, et al.*, 2008; Waters*, et al.*, 2014). *P. maculata* was first observed in 2003 in Lake Seminole, GA, US (Fig 3.1), the most downstream impoundment in the Apalachicola-Chattahoochee-Flint (ACF) basin and has since expanded across significant portions of the lake (Shivers *et al.*, Submitted; USACE, 2013). The spread of *P. maculata* in Lake Seminole provides an opportunity to study how the distribution of this invasive species expands relative to variable physicochemical conditions. Lake Seminole lies near the boundary of the range expansion predicted in Byers*, et al.* (2013). The continued range expansion of *P. maculata* within Lake Seminole and increased abundance is expected to affect submerged aquatic vegetation (SAV) abundance through herbivory, which may in turn affect nutrient transport and storage in Lake Seminole (Shivers, 2010; Waters*, et al.*, 2014).

Large sections of the lower ACF basin, including portions of Lake Seminole, overlie the unconfined karst of the Upper Floridan Aquifer (UFA) system, primarily in lower sections of the Flint River (Torak*, et al.*, 2005). In this physiographic district, there is high connectivity of surface waters and groundwater, which have distinct thermal characteristics. Surface water is affected largely by atmospheric temperature, and varies seasonally. In contrast, groundwater from the UFA discharged into surface water bodies is, in general, thermally uniform (Rugel *et al.*, 2012; Torak*, et al.*, 2005). The occurrence of groundwater springs and seeps in the lower Flint River basin provides thermally uniform microhabitats, which can result in biologically diverse habitats (Walsh, 2001).

In this study, seasonal temperature variation was assessed across Lake Seminole to determine the range of temperatures to which *P. maculata* may be exposed. Laboratory experiments were designed to quantify the preference of *P. maculata* to a range of summertime temperatures. These thermal preferences were then used to predict snail movement relative to observed temperatures and to availability of thermally uniform microhabitats in Lake Seminole.

Materials and Methods

Site Description

Lake Seminole is a 15,216 ha run-of-the-river reservoir in the Apalachicola-Chattahoochee-Flint (ACF) watershed in southwestern Georgia. The lake was formed in 1957, and has a mean depth of 3 m and maximum depth of 10.7 m. The major surface water inflows to the lake are the Chattahoochee River, the Flint River, and Spring Creek, which drain a combined area of 46,141 km^2 before discharging into headwaters of the Apalachicola River (Fig 3.1, inset). From 2010-2015, the lake had a mean annual temperature of 22.0 ˚C, and ranged in extreme temperatures from 30.8 ˚C in the summer to 9.1 ˚C in the winter.

Temperature Measurements

Surface water temperatures in Lake Seminole were measured monthly from September 2010-August 2015 at locations upstream in each arm of the lake, within the body of the lake, and below the dam in the Apalachicola River (Fig 3.1). Measurements were performed using a Hydrolab Quanta (Hydrolab Corp., Austin, TX).

Monthly temperature data were compared to temperature records for two USGS gauges located in the Lower ACF: 02357150, located in Spring Creek near Reynoldsville, GA; and 02356000, located in the Flint River near Bainbridge, GA. Data at these gages collected as daily mean temperature for the period of record from 9/16/2012–9/16/2015. These data were used examine seasonality of water temperatures in two of the major surface water inflows upstream of the lake.

Lab Experiment: Temperature Gradient

Temperature preferences of juvenile and adult *P. maculata* were assessed in an artificial temperature gradient, using a previously designed laboratory channel to examine the effect of temperature on invasive species in the ACF (Sargent *et al.*, 2010). The gradient was created in a 224 cm-long, 25.4 cm-diameter PVC pipe, divided into eight equally sized zones (zone length=28 cm), with polycarbonate sheets to separate adjacent zones (Fig 3.2). A 12.7 cm x 20.32 cm section was removed from the bottom of the polycarbonate sheets to allow snail movement between zones. Submersible aquarium heaters (n = 8) and an aquarium chiller were used to create the desired temperature gradient, ranging from 34.0° C to 27.0° C in ~1°C increments between adjacent zones. Water temperatures in each zone were monitored throughout the experiment using an Extech model 39240 waterproof thermometer. The temperature gradient was representative of water temperature variation observed in Lake Seminole during the period from spring (27˚) through summer (34˚) and fall (27˚).

Twenty-four hours prior to experimental trials the channel was filled with aged, dechlorinated tap water and the aquarium heaters and chillers were turned on to create the temperature gradient. The channel was emptied weekly between trial runs and cleaned to remove accumulated organic matter and any snail-produced slime trails from the channel. Adult snails used in this experiment were collected from the Flint River arm of Lake Seminole (30.7749, -84.7326) during the summer and fall of 2014. Juveniles were reared from egg masses collected in Lake Seminole, but grown in Athens, GA. Snails were classified as adult when shell length (whorl apex to bottom of shell) ≥40mm (Youens & Burks, 2007). All snails were held in aquaria set to 27˚C using similar heaters in the experiment, and fed a combination of *Hydrilla* collected from Lake Seminole and spinach. Experiments took place from December 2014 to June 2015.

Temperature preference and activity of *P. maculata* were examined using two different experimental procedures. The first procedure involved direct observation and recording of the movements of two snails at 5-minutes intervals for 1-hour. Trials took place in a darkened room, and were observed using a red light-emitting headlamp to minimize effects of lighting on snail behavior. Both snails were placed in the middle of the temperature gradient (between zones 4 and 5), allowing movement to warmer or cooler temperature zones. At each 5-minute interval, the zone where each snail was located was recorded, and the associated water temperature was measured. The time to first movement for each snail was recorded when an open operculum or antennae outside the aperture of the operculum was observed. A time-series was created reflecting the movement of each snail through time and temperature, and the overall movement at the end of the 1-hr trial was compared to the starting temperature conditions.

The second procedure relied on time-lapse observation over a 3-hour period to determine the longer-term movement in relation to temperature of two snails. Hobo Pendant Temperature Data Loggers (UA-001-64, Onset Computer Company, Bourne, MA) were placed in each zone of the gradient, and recorded water temperature at five-minute intervals. A GoPro Hero 2 (GoPro, San Mateo, CA, USA) camera was mounted above the experimental channel so that all temperature zones were visible and programmed to take a photograph of the channel every 60-seconds. The temperature gradient was established 24hr prior to experiments and snails were placed between zones 4 and 5 at the start of each trial, with the only difference being that low-intensity lighting was required to allow photography. Time-lapse photos were then compressed into a single movie using GoPro Studio software (GoPro, San Mateo, CA, USA). Total number of temperature zones occupied, net movement, starting and final temperature, temperature difference over time, and the final zone occupied were recorded for each snail.

Data collected using the time-lapse method were analyzed by comparing the frequency with which different temperatures were used between adults and juveniles. Snail movement was assessed based on the number of zones occupied and the temperature zone occupied at the end of each respective trial. Temperature difference from the start to the end of each trial was examined to better identify snail movement towards warmer or cooler conditions. Final temperature zone for snails that showed any movement was compared to the initial zone (between zones 4 and 5) using nonparametric Wilcoxon Rank sum test with $\mu = 4.5$. 95% confidence interval from this test indicates direction movement in the channel, either towards cooler zones or towards warmer zones.

Results

Lake Seminole Temperature Seasonality

Monthly temperatures in Lake Seminole varied seasonally in a similar manner across all arms of lake. Mean maximum monthly temperature over the 5-year period was 29.5° C and occurred in July or August. Mean minimum temperature was 12.0° C, and occurred between December and March. Mean temperature for the lake over the 5-year period was 22.0° C (Fig 3.3).

Data from USGS 0235600 in the Flint River show similar temperature patterns, with a mean annual temperature of 20.4° C (± 6.1 SD), maximum recorded temperature of 30.0° C in July 2015, and minimum temperature of 8.2° C in January and February of 2014 over the three-year period. At USGS 02357150 in Spring Creek, the average temperature was 19.7° C (± 3.9 SD), and a maximum temperature of 26.8° C was recorded in July 2015, and a minimum temperature of 8.8° C was recorded in January 2014. Mean annual temperatures along with maximum and minimum annual temperatures for 2012-2015 are reported in Table 3.1. Data from monthly measurements and USGS daily mean temperatures clearly show that Lake Seminole and upstream reaches of the major surface water inflows perennially fall below 15° C, which has been shown to be the low temperature threshold for non-native *Pomacea* spp. activity.

Temperature Gradient Experiment- Direct Observation

In the direct observation trials, 49 snails were assayed (32 adults, 17 juveniles). Of the 49 total trials, 16 showed movement (32.7%), defined as movement to a different zone; of these, 10 snails (62.5%) moved >2 zones, 5 snails (31.2%) moved >3 zones, and one snail (6.25%) moved seven zones. Average gradient temperature at the start of all trials was 28.5 ± 0.09 ˚C and in trials where snails moved, the average ending temperature where snails were recorded was 28.3 ± 0.17 ˚C (Fig 3.4, x-axis ranges warmest zones to coolest, left to right).

Juvenile snails showed signs of movement at 17.1 ± 5.8 minutes after starting the trial, while adult snail showed movement at 19.4 ± 4.5 minutes. Overall, snails occupied an average of 2.3 ± 0.3 zones per one-hour trial, and had an average net movement of 0.3 ± 0.1 zones toward the cooler temperature zones of the gradient. Mean temperature where snails were present at the end of each trial was 0.2 ± 0.1˚ C lower than at the start of each trial.

Temperature Gradient Experiment- Time-Lapse

Using indirect observation, a total of 69 snails were assayed (60 adults, 9 juveniles). Of the 69 snails, 50 (72.4%) moved >1 zone, 32 (46.3%) moved >2 zones, 22 (31.9%) moved >3 zones, 10 (14.6%) moved >4 zones, one snail (1.5%) moved 7 zones, and two snails (2.9%) moved 8 zones Snails that moved through 8 zones moved to one end of the channel, and then reversed to the opposite end of the channel. Average starting temperature for each assay was 29.3 ± 0.1˚ C and the average water temperature zone at the end of each trial was 29.1 ± 0.2˚ C.

Snails moved toward the cooler end of the gradient by an average of 1.0 ± 0.2 zones per trial. Mean temperature where snails were present at the end of the trial was 0.2 ± 0.2 ˚C lower than at the start of each trial.

Combining all trials, 7.6% of snails were found in the warmest three gradient zones, 26.4% were found in the coolest three zones, and the remaining 57.6% were found in the two middle temperature zones (Fig 3.4). Snails that moved to the extreme warm (n = 3) and cool (n = 11) zones showed higher overall movement, moving an average of 4.0 ± 1.2 total zones over the course of each trial, while the average number of zones occupied for all snails was 2.3 ± 1.7.

Snails that showed any movement in the channel showed significant movement (p=0.0008) away from the middle of the channel. The 95% confidence interval shows snails significantly favored cooler temperature zones between zones 5.0 and 6.0.

Discussion

Seasonal low temperature extremes have significant effects the activity of invasive *P. maculata*, and the change in temperature regimes between native and introduced ranges may result in novel behavioral responses. In the southeastern US, the establishment and dispersal of *P. maculata* in more temperate habitats provides an opportunity to observe movement patterns and the utilization of microhabitats specific to introduced ranges. Microhabitat availability such as localized refugia may enable *P. maculata* to persist in extreme seasonal temperatures in temperate habitats.

Large sections of Lake Seminole, located in the lower Flint watershed, are incised in the Ocala Limestone of the Upper Floridan aquifer, where groundwater springs and seepages are common (Torak*, et al.*, 2005). Groundwater inflows in these areas remain thermally uniform year-round, which provide cool refugia in the summer and warm refugia in the winter for a variety of fauna, including *P. paludosa* where it occurs in Florida (Walsh, 2001). This study suggests that these areas could also serve as thermal refugia for *P. maculata*, facilitating expansion in more temperate conditions. Several springs are submerged within Lake Seminole in both the Flint River and Spring Creek arms of the lake (Torak*, et al.*, 2005). Submerged springs and the thermal stability in these areas may provide localized microhabitats within Lake Seminole, particularly in Spring Creek and the Flint River.

Both monthly measured temperatures and hourly temperatures from USGS gages show Lake Seminole and its surface water inflows fall below the 15˚ C temperature threshold documented to induce *P. maculata* inactivity (Seuffert & Martin, 2010; Tiecher*, et al.*, 2015). Lake Seminole and the lower ACF basin were predicted to be suitable habitat based on minimum temperature (Byers*, et al.*, 2013). This study suggests that certain areas of the lower ACF are below this temperature threshold. However, predicting the dispersal range of *P. maculata* in the lower ACF based solely on temperature will be difficult due to the presence of thermal refugia. The duration of temperatures <15˚C will also affect *P. maculata* activity, and monthly collected data and USGS daily mean tempeartures show that much of the Lower ACF falls below this 15˚C mark. However, cold temperatures may not persist for long enough time to affect either *P. maculata* movement, activity, or physiology.

Movement of *P. maculata* in an experimental temperature channel indicates movement away from warmer temperatures towards cooler temperatures. Preference of cooler temperatures in a range of measured summer temperatures from Lake Seminole provides evidence for utilization of cooler temperature refugia in the presence of warm and cool temperatures. Similar studies using lower temperatures and temperature preference will be needed to develop movement patterns and how movements under experimental conditions relate to *in situ* conditions. It may be that certain individuals will take advantage of nearby microhabitat springs and persisting during extreme temperature conditions whereas other individuals may die off as a result of low temperatures.

Temperatures in the lower ACF are similar to those in *P. maculata* native range in the Pantanal wetland in South America, though there is greater seasonal variation in precipitation in native ranges (Keddy *et al.*, 2009). In the Pantanal, summer air temperatures range from 25–40˚ C, and winters range from 18-30˚ C, though air temperatures ~0˚ C are not uncommon (Heckman, 1998; Oliveira, Hamilton & Jacobi, 2010). In this region, seasonal flooding and drought control *P. maculata* behavior, by dictating reproductive onset and estivation during the dry season (Seuffert & Martin, 2010). Under more stable hydrologic conditions in the Lower ACF, temperature regulation may exert larger control on snail behavior, and more study will be needed to better understand important habitat differences between native and introduced ranges.

Much of the concern in the lower ACF and the southeastern US is the extent to which *P. maculata* will be able to successfully expand its range and affect a wider range of aquatic ecosystems (Carlsson*, et al.*, 2004; Robertson, 2012; Zhao *et al.*, 2012). The historical and present distributions of this species within the ACF are well understood (Benson, 2015; Robertson, 2012), but with projections of warming temperatures (Catano *et al.*, 2015; Hellmann*, et al.*, 2008), predicting future range expansion becomes more challenging. Climate projections for the southeastern US suggest warmer year round mean temperatures, increasing in range from 0.5° C to 3.0° C (Hopkinson*, et al.*, 2013; IPCC, 2014). Corresponding increases in water temperatures in Lake Seminole may warm the lake so that the low temperature inactivation threshold of *P. maculata* is rarely reached, allowing snails to actively graze and reproduce for a longer period each year. Warming temperatures will also expand the available habitat upstream throughout the lower ACF, and expanding the northern extent of *P. maculata* dispersal across all freshwater habitats in the southeast.

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Figures

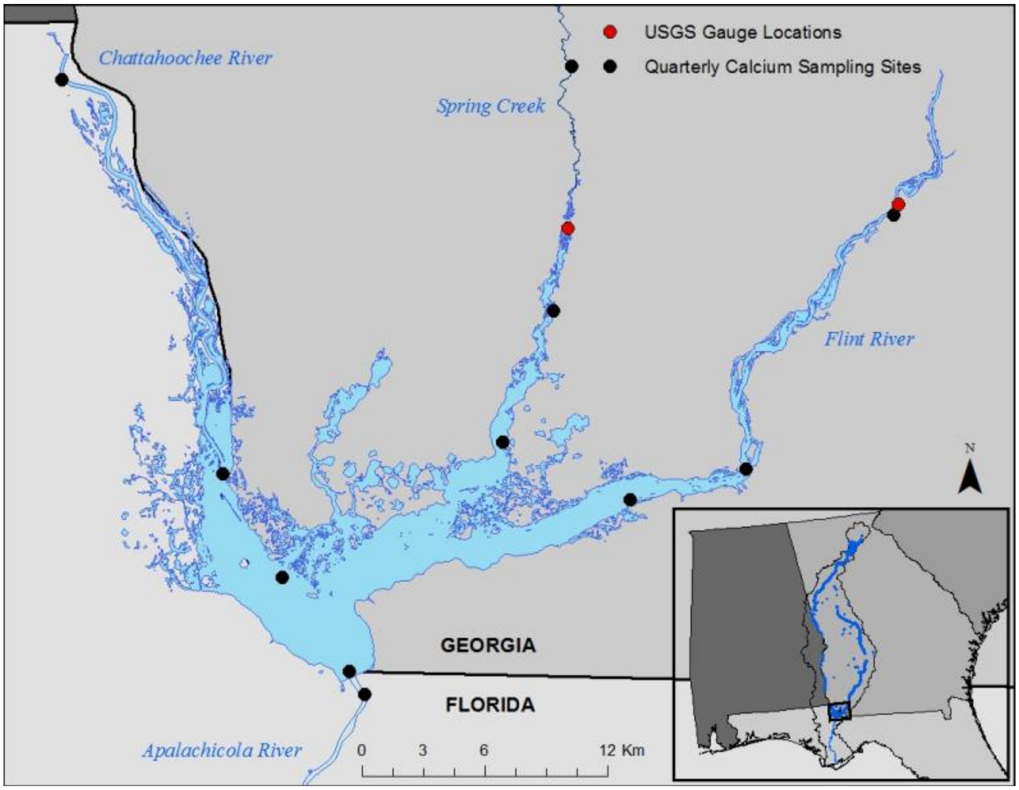

Fig 3.1: Location of Lake Seminole in the ACF watershed, and locations of monthly temperature locations (black circles) and USGS gauges (red circles).

Fig 3.2: Experimental temperature gradient channel from above, as was used in time-lapse observations. Total channel length is 228 cm, with each zone 28 cm in length. In this view, Zone 1, the warmest zone, is the farthest left zone, and Zone 8, the coldest zone is farthest to the right. Aquarium warmers and air stones were mounted in each zone to warm and oxygenate all zones. Hobo temperature recorded temperature every 5-minutes. In this trial, two snails were placed in the channel: one can be seen in Zone 5 (on left), and the other in Zone 6 (on right). The apparent curvature of the channel results from distortion from the wide camera lens.

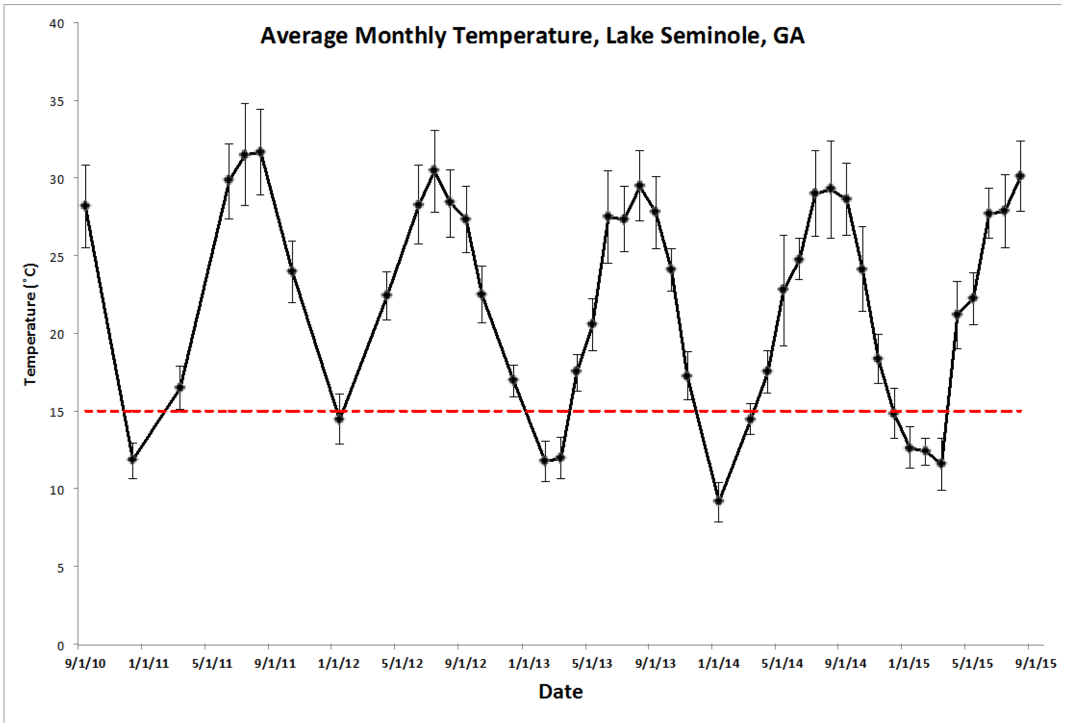

Fig 3.3: Average monthly temperatures recorded in Lake Seminole from 9/2010 to 9/2015. Monthly temperatures are reported as mean temperature across all sampling locations (Fig 1, B), ± SD. *Pomacea spp.* have reported reduced activity in the native ranges ~15˚ C, of which the lake can reach on an annual basis.

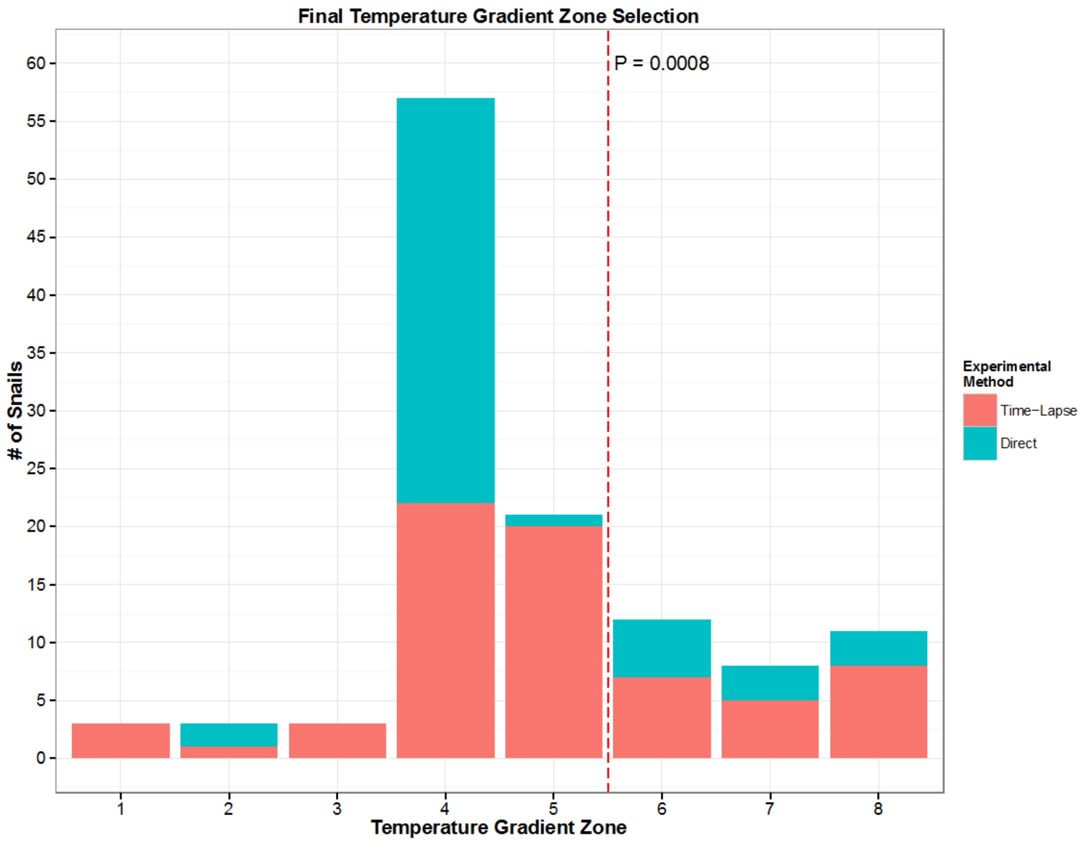

Fig 3.4: Final temperature gradient zone selection for all snail trials, separated by indirect and direct observation. Values on the x-axis indicate zone number, from warmest zone on the left (Zone 1) to coolest zone on the right (Zone 8). Indirect trials occurred over a 3-hour time period, while direct observations occur over 1-hour. Vertical red-dashed line indicates mean zone occupied, which was distributed significantly different (P = 0.0008) than the starting zone at zone 4.5

Table 3.1: Summary statistics for temperatures from two USGS Gauges in the Lower ACF basin form 2012 to 2015. Locations for each gauge relative are found in Fig 1.

USGS Gauge	2012			2013			2014			2015		
	Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**
02356000 (Flint River)	19.4 ± 4.4	10.4	27.1	20.4 ± 5.6	10.0	29.3	19.8 ± 6.3	8.2	28.8	21.7 ± 6.6	9.6	30.0
02357150 (Spring Creek)	19.4 ± 2.2	13.9	23.9	19.8 ± 3.8	10.0	25.7	19.2 ± 4.1	8.8	25.1	20.5 ± 4.2	10.2	26.8

CHAPTER 4

INFLUENCE OF TEMPERATURE AND CALCIUM CONCENTRATIONS ON THE GROWTH AND SURVIVAL OF JUVENILE *P. MACULATA*: PHYSICOCHEMICAL TOLERANCE OF AN INVASIVE SNAIL IN THE SOUTHEAST US[3]

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[3] Marzolf, N.S, Golladay, S.W., Covich, A.P., McCormick, P.V., and Wilde, S.W. To be submitted to *Journal of Molluscan Studies*

Abstract

The introduction and dispersal of invasive species is one of the largest threats to freshwater habitats. The Island Apple Snail, *Pomacea maculata*, a native of wetlands, rivers and lakes of South America, has been widely introduced in the tropics and subtropics. These fast-growing herbivores are affecting freshwater ecosystems, biodiversity and human health. Snail behavior and growth are influenced by temperature, which can affect their successful establishment in freshwater ecosystems. In addition, snail growth may be limited by ambient concentrations of calcium (Ca), which is a key component of gastropod shells. In Lake Seminole, a large, shallow reservoir in the Apalachicola-Chattahoochee-Flint (ACF) basin, temperature and Ca concentrations were sampled quarterly to quantify ambient concentrations. Highest concentrations occurred in the Spring Creek arm of the lake (44.3 mg/L), followed by the Flint River arm (16.2 mg/L) and the Chattahoochee River arm (9.9 mg/L). Small ponds near Lake Seminole were also sampled, and had mean concentrations of 5.2 mg/L. Observed differences in Ca and temperature among locations were used to design treatments for a two-week 3x3 factorial laboratory assay to quantify the effects of different levels of these factors on the growth and survival of juvenile *P. maculata.* Ca and temperature significantly affected juvenile snails growth and survival. Snail growth was significantly greatest at the warmest temperature treatment and at the highest two Ca treatments. This indicates that low Ca and temperature may affect the establishment and persistence of *P. maculata* in the lower ACF basin.

Key words: Calcium, Invasive species, *Pomacea maculata*, reservoir, southeastern US, climate warming, Apple snails, environmental tolerance, *Hydrilla*

Introduction

The global spread of invasive species is one of the largest threats to freshwater habitats (Havel*, et al.*, 2015; Strayer & Dudgeon, 2010). The success of invasive species can be traced to similarities between native ranges and introduced habitats, but differences may exist that promote the dispersal of the species in non-native ranges compared to native ranges (Parker*, et al.*, 2013). Understanding factors contributing to the establishment and persistence of invasive species in new environments may help in predicting and controlling their spread (Moran & Alexander, 2014).

The Island Apple Snail, *Pomacea maculata*, is a widely introduced species native to wetlands, rivers and lakes in South America, along with several congeners (Hayes*, et al.*, 2012). In SE Asia, introduced *Pomacea* is a significant pest in rice paddies, and is altering vegetation communities in wetlands (Carlsson*, et al.*, 2004). In North America, *P. maculata* has been introduced in Florida, Georgia, Alabama, South Carolina,, North Carolina, Louisiana and Texas (Byers*, et al.*, 2013). *P. maculata* can have large effects on food webs, native gastropod species, wildlife, and to human health (Darby*, et al.*, 1999; Darby*, et al.*, 2007; Posch*, et al.*, 2013; Teem*, et al.*, 2013).

Determining the environmental limits on the dispersal and expansion of *P. maculata* will help identify the specific environmental factors that could limit future dispersal of *P. maculata* in introduced areas. Temperature and hydroperiod have been found to control the activity and behavior of related species, such as *P. canaliculata*, a similarly invasive congener native to the same region in South America (Seuffert & Martin, 2010; Seuffert & Martin, 2013; Tiecher*, et al.*, 2015). Specifically, at temperatures <15° C, movement and physiology of *P. canaliculata* is significantly reduced. Identifying *P.*

maculata physiological responses to variable temperatures will help to assess the potential for establishment and persistence outside its native range under current and future climate conditions (Havel*, et al.*, 2015; McDowell*, et al.*, 2014).

As a gastropod species with a calcium (Ca) carbonate shell, water bodies with a well-buffered pH and source of abiotic Ca are important to maintaining shell size and strength, and pH has been used to predict spatial limits of dispersal (Byers*, et al.*, 2013; Glass & Darby, 2009). Previous studies also concluded that gastropods are less likely to occur in water with environmental Ca<5-mg/L (Lodge*, et al.*, 1987) as Ca concentration is essential in shell formation (Brodersen & Madsen, 2003). Gastropods have been observed in habitats with Ca concentration less than 5 mg/L (Jokinen, 1982), but the potential of Ca limitation is greater, and may be a deterrent for *P. maculata,* which likely has a high Ca requirement (shell growth and maintenance, egg mass production). The effect of differences in environmental Ca has been studied in several freshwater macroinvertebrates (Edwards, Jackson & Somers, 2015; Greenaway, 1971), and is an important driver of mollusk distribution, as Ca limitation may affect the presence of both native and potentially invasive mollusks in freshwater habitats (Solomon*, et al.*, 2009; Whittier*, et al.*, 2008).

Environmental Ca, and its inter-correlations with alkalinity, pH and conductivity, has been shown to drive freshwater mollusk species richness in general (Dillon, 2000), and more specifically, the distributions of non-native gastropods (Spyra & Strzelec, 2014). For example, snails experimentally exposed to low Ca concentrations showed significant reductions in growth and egg mass production (Thomas & Lough, 1974). In the case of *Marisa cornuarietis*, an organism closely related to *Pomacea,* Ca uptake in individuals increased as a function of the ambient Ca concentration, and individuals reared at lower concentrations exhibited lower survivorship and thinner shells (Meier-Brook, 1978). The concentrations used in Meier-Brook (1978) were greater than used in previous studies (Thomas & Lough, 1974) suggested that larger species have higher Ca requirements and their distribution is limited by these requirements. Specific environmental Ca requirements for introduced species may act as a filter for colonists and limit dispersal into habitats that have ill-suited physiochemistry (Lodge*, et al.*, 1987).

Populations of *P. maculata* were first observed in Lake Seminole and the lower Apalachicola-Chattahoochee-Flint (ACF) basin (Fig 4.1) in 2003 and have since dispersed widely throughout the Flint River arm of the lake (Shivers*, et al.*, Submitted; USACE, 2013). Lake Seminole potentially can have a significant role in seasonal nutrient recycling through uptake by macrophyte beds, predominately invasive *Hydrilla verticillata.* Grazing by *P. maculata* may result in removal of native species of submerged and floating macrophytes and reduced nutrient retention by macrophytes. The lake is near the northern limit of the predicted range for *P. maculata* and has a large volume of potentially Ca-rich groundwater and surface water inflows. The wide range in seasonal temperature and spatial Ca availability make it ideal to examine the effects of variable temperature and Ca concentration on *P. maculata* growth, survival and prospects for continued expansion in the lake and throughout nearby habitats in the lower ACF.

In this study, temperature and Ca concentrations were measured across Lake Seminole to characterize spatial variability among the major surface water inflows. Additionally, laboratory experiments were used to assess the effects of the observed ranges of Ca and temperature on the growth and survival of *P. maculata.* The goal was to identify the range of ambient conditions under which *P. maculata* growth and survival was most successful in order to predict how much of Lake Seminole may be susceptible to invasion.

Materials and Methods

Site Description

Lake Seminole is a 15,216 ha impoundment located in the Apalachicola-Chattahoochee-Flint (ACF) watershed in southwestern Georgia (Fig 4.1, A). The lake has a mean depth of 3-m and maximum depth of 10.7 m (Shivers, 2010). The major surface water inflows to the lake are the Chattahoochee River, the Flint River, and Spring Creek, which drain a combined 46,141 km^2. The lake discharges into the Apalachicola River on the Florida-Georgia border. Lake Seminole has a mean annual temperature of 22.0° C, and from 2010-2015, a mean annual low temperature of 12.0° C and mean annual high temperature of 29.5° C (Chapter 3, this volume).

Several freshwater ponds (n=6) occur near Lake Seminole between the Flint River and Spring Creek in the Silver Lake Wildlife Management Area (SLWMA), managed by Georgia Department of Natural Resources (Fig 4.1, B). During the impoundment of Lake Seminole, the resulting rise in the groundwater potentiometric surface in the lower ACF caused the conversion of several geographically isolated wetlands in SLWMA into perennial ponds. These small ponds, although not hydrologically connected to Lake Seminole, are located near populations of *P. maculata,* and could be occupied by future *P. maculata* dispersal (Chapter 2, this volume). These ponds have public access boat launches and are managed for recreational fishing by Georgia DNR.

Calcium and Alkalinity Sampling

Triplicate 1-L grab samples were obtained every three months across Lake Seminole from September 2014–June 2015. Samples were taken upstream of the lake, within the body of the lake, and at the outflow of the lake (Fig 4.1). Samples were also taken at each of the six ponds in SLWMA. At each of these locations, a Quanta Hydrolab (Hach Environmental, Loveland, CO, USA) was used to measure temperature (˚C), specific conductivity (mS/cm), and dissolved oxygen (DO) (mg/L and %). Grab samples were stored at 4˚ C, and returned to the lab, where unfiltered subsamples were analyzed for alkalinity (mg/L) using a Mettler Toledo DL15 titrator (Mettler Toledo Inc., Toledo, OH, USA). Subsamples were filtered through 0.7μm GF/F filters (EMD Millipore, Billerica, MA, USA) and analyzed for Ca using a PerkinElmer AAnalyst 400 (Perkin Elmer Corporation, Norwalk, CT, USA).

Laboratory Experiments: Calcium and Temperature Effects

The effect of Ca and temperature on the growth of hatchling *P. maculata* was assessed using a 3x3 factorial growth experiment that bracketed ranges of each parameter observed across Lake Seminole. Experimental levels of Ca concentrations were based on samples taken from Lake Seminole and surrounding ponds in SLWMA. Water used for experimentation was taken from three locations with different Ca concentrations: Spring Creek (Spring253 mean $[Ca^{2+}]=42.16$ mg/L), the Flint River (FFace mean $[Ca^{2+}]=24.93$ mg/L), and Silver Lake, the largest SLWMA pond (SilverN average $[Ca^{2+}]=0.50$ mg/L). Temperature treatments were selected based on the seasonal gradient measured in the lake, including a 34˚ C (summer), 26˚ C (fall and spring), and 18˚ C (winter) treatments.

Temperature conditions in the laboratory experiment were maintained in a Thermo Fisher Revco BOD Incubator under a 14-h:10-h light:dark regime.

Five one-L water samples were collected from each of the three sites 24-hr prior to the beginning of each experiment and placed in the incubator to acclimate to temperature. Trials were run for each temperature treatments separately. After 24-hr, 750mL of each sample was poured into a 1-L plastic chamber replicate. For each temperature setting, five replicate Ca chambers were added for the three Ca treatments, for a total of 15 chambers per temperature setting and 45 chambers across the three temperature settings.

Juvenile *P. maculata* used in each temperature treatment were collected 1-day following hatching from the same egg mass cohort, ensuring all snails were the same age. Juveniles from different egg masses were used in each of the three trials, but egg masses were collected from the same location in Lake Seminole. Newly hatched snails were acclimated to their designated experiment temperature for 24-hr in a 1L plastic chamber in tap water. After the acclimation period, 10 randomly selected snails were blotted dry, weighed, and randomly placed into one of five replicates of each Ca treatment chambers. Additionally, 10 snails were randomly selected from each cohort to determine wet mass, dry mass and ash weight to compare to snails used in the experiment. Snails were fed with three single-hole punched discs from spinach leaves *ad libitum*. Spinach leaves had a Ca content of 0.133 mg Ca/g leaf, and hole-punched discs had a mean weight of 7.3-mg dry mass and 0.3-mg AFDM per disc.

Snails were removed from the treatment chambers at Day = 0, 1, 3, 7, 10 and 14, and measured for cohort wet mass. Water in each chamber was replaced out at Day 7 with new water collected from the appropriate sites as previously mentioned. Two of the five replicates per treatment were randomly selected for Ca and alkalinity determination (see methods above) to track changes in these parameters over the course of the experiment. On day 14, all snails were weighed for cohort wet mass, then placed in pre-weighed pans and dried at 50° C for 24-hr for dry mass determination, and then placed in a muffle furnace at 500° C for 1-hr to determine ash mass.

Statistical Analysis

Linear mixed-effect models were used to model the change in wet mass and snail survival, with the inclusion of treatment effects for Ca concentration and temperature. Tukey's post-hoc multiple comparisons with Bonferroni adjustments identified significant experimental treatments for change in wet mass and in snail mortality.

Following Hunter & Lull (1977) and Glass & Darby (2009), who concluded that 95% of gastropod ash mass is $CaCO_3$ and 40% of the molecular weight of $CaCO_3$ is Ca, ash mass was multiplied by 0.4 to determine Ca content of each snail cohort. Snail data were analyzed using factorial ANOVA (Ca*Temperature) for Day 14 wet mass, dry mass, ash mass, and Ca content for each replicate, given the additive model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the response variable on interest (wet mass, dry mass, ash mass, Ca, Ca/g), μ represents the grand mean of the response variable of interest, α_i is the treatment effect of Ca concentration, β_j is the treatment effect of temperature, $\alpha\beta_{ij}$ is the interaction of Ca and temperature, and ε_{ijk} is the residual error. Tukey's post-hoc multiple comparisons were performed to identify significant temperature and Ca effects.

Results

Calcium and Alkalinity Variation Across Lower ACF

Ca concentrations varied spatially among the arms of the lake, but were less variable at the lake outflow (Fig 4.2). Spring Creek had greatest Ca concentration of the major lake inflows (average Ca^{2+}=44.3 mg/L), followed by the Flint River (Ca^{2+}=16.2 mg/L) and the Chattahoochee River (Ca^{2+}=9.9 mg/L). Sites within Lake Seminole (Fig 1, A) showed slight mixing and dilution of dissolved Ca (mean Ca^{2+}=22.1 mg/L), and the outflow values of the lake (Apalachicola River), was relatively uniform during each sampling period (Ca^{2+}=19.6 mg/L). Mean Ca across all lake sampling sites ranged from lowest concentration (13.2 mg/L) in March to greatest concentration (18.2 mg/L) in June.

Ca concentrations in SLWMA ponds ranged from near detection limits (detection limit = 0.001 mg/L) to equivalent to the lowest concentrations observed in the lake. Overall, the ponds had a mean Ca of 5.2 mg/L Ca^{2+}, and ranged from 1.6 mg/L in Silver Lake to 9.1 mg/L in Panic Pond. Specific conductivity in these ponds (mean=0.048 mS/cm, range=0.014-0.100 mS/cm) was greatly reduced when compared to the main body of the lake (mean=0.152 mS/cm, range=0.080-0.326 mS/cm).

Patterns for alkalinity were similar to those for Ca. Of the surface water inflows, greatest alkalinity was measured in Spring Creek (mean = 116.8 ppm as $CaCO_3$), followed by the Flint River (55.7 ppm) and the Chattahoochee River (39.2 ppm). In the lake, these water sources mixed (74.4 ppm) before being discharged into the Apalachicola River (alk = 58.4 ppm). SLWMA ponds had low alkalinity, ranging from 6.1 ppm to 30.8 ppm. Panic Pond, the pond nearest to Spring Creek (Fig 4.1, B) measured highest in Ca (mean = 9.1) and specific conductivity (0.091) for all sampling times, but was not available for sampling in September due to management restrictions for access.

Laboratory Experiment- Physicochemistry

Water samples collected for the laboratory experiments occurred twice per 14-day assay. At the start of each assay, Spring Creek Ca concentration averaged 41.3 mg/L, Flint River averaged 25.6 mg/L and Silver Lake averaged 0.50 mg/L. Over the course of the experiment, Ca concentrations in the respective treatment chambers increased to an average of 51.7 mg/L in Spring Creek treatments, 35.7 in Flint River treatments, and 1.2 mg/L in Silver Lake treatments (Table 4.1). Alkalinity had similar patterns of concentration at the start of the experiment and increased in all treatments over the two-week experiment. Alkalinity measured from final set of samples taken in Spring Creek measured 9.1 ppm, well below previously recorded values from Spring Creek

Laboratory experiments- Pomacea Daily Growth

At Day 0 for each experiment, average cohort wet mass was 15.3 ± 2.5 mg across all experiments. In the 18˚ C treatment, mean snail growth in water from the Flint River (23.4 mg) and Spring Creek (22.0 mg) were greater than Silver Lake snails (15.7 mg) (Fig 4.3). At 26˚ C, Spring Creek average snail wet mass (24.0 mg) was less compared to both Flint River (26.9 mg) and Silver Lake (26.8 mg). At 34˚ C, Spring Creek cohort wet mass (22.1 mg) was also less than both Flint River (26.4 mg) and Silver Lake (25.2 mg).

Survivorship in Silver Lake treatments at 18˚ C was greatest, with an average of 64% of snails surviving through the experiment, compared to 86% survival in Spring Creek treatments and 98% survival in Flint River treatments (Fig 4.3). At 26˚ C, snail survival averaged 86% in Spring Creek, 96% survival in the Flint River and 98% survival in Silver Lake. At 34˚ C, survival was 90% in Spring Creek and 98% for each Flint River and Silver Lake treatments.

The linear mixed effect model showed a significant effect of Ca ($F_{2,212}$, $p = 0.001$), time ($F_{5,212}$, $p < 0.001$), and temperature ($F_{2,212}$, $p < 0.001$) on snail growth. The statistical interaction of temperature and Ca was significant ($F_{4,212}$, $p < 0.001$), and the interaction of time with temperature ($F_{10,212}$, $p < 0.001$), Ca ($F_{10,212}$, $p < 0.01$) and the interaction of time, Ca and temperature ($F_{20,212}$, $p = 0.001$) were all significant (Fig 4.3).

The linear mixed effect model showed significant effect of Ca ($F_{2,212}$, $p < 0.001$), time ($F_{5,212}$, $p < 0.001$), and some evidence of an effect of temperature ($F_{2,212}$, $p = 0.089$) for snail survival. The interaction of temperature and Ca was significant ($F_{4,212}$, $p < 0.001$), and the interaction of time with temperature ($F_{10,212}$, $p < 0.0001$), Ca ($F_{10,212}$, $p < 0.0001$) and the interaction of time, Ca and temperature ($F_{20,212}$, $p < 0.0001$) were all significant (Fig 4.4).

Snail wet mass at Day 14 was significantly affected by Ca concentration ($F_{2,36}$, $p = 0.001$), temperature ($F_{2,36}$, $p < 0.001$), and temperature*Ca interaction ($F_{4,36}$, $p = 0.003$). Results of Tukey's post-hoc comparison showed that warmer temperatures (26˚ C and 34˚ C) resulted in greater wet mass compared to the lowest temperature (18˚ C), and moderate Ca concentration (Flint River) resulted in greater dry masses compared to the extreme Ca concentration treatments (Spring Creek and Silver Lake).

Dry mass was significantly affected by Ca concentration ($F_{2,36}$, $p < 0.001$) and temperature ($F_{2,36}$, $p < 0.001$), and there was no interaction between these factors ($F_{4,36}$, p

= 0.129). Tukey's post-hoc multiple comparisons showed that dry mass declined significantly with decreasing temperature across all three temperature treatments. Spring Creek and Flint River Ca-treatment dry masses were significantly greater than the lowest-Ca concentration treatment, Silver Lake.

Snail ash mass was significantly affected by temperature ($F_{2,36}$, $p < 0.001$) and Ca concentration ($F_{2,36}$, $p < 0.001$), and there was a marginal interaction between these two factors ($F_{4,36}$, $p = 0.066$). Results of Tukey's post-hoc multiple comparisons showed that snail ash weight declined significantly with decreasing temperature across all three temperature treatments. Spring Creek and Flint River Ca concentration treatments were significantly greater than Silver Lake concentration treatment (Fig 4.5).

Discussion

Variable dissolved calcium concentrations and temperatures were shown to affect the growth and survival of invasive *P. maculata* in Lake Seminole and its dispersal throughout the lower ACF basin. The combined effects of Ca spatial variation and seasonal temperature show that there are areas in the lower ACF basin that are unsuitable for *P. maculata* and may limit their establishment and persistence. However, within Lake Seminole, the measured Ca concentrations and temperatures do not have a negative effect on snail growth or survival, which indicates that *P. maculata* will likely not be limited by Ca in their dispersal throughout the lake.

Calcium Variation in the Lower ACF and Lake Seminole

In the surface water inflows to Lake Seminole, dissolved Ca varied greatly, and showed strong correlation with carbonate alkalinity ($R^2 = 0.81$) across all sites. Ca concentration was greatest in Spring Creek, which receives a substantial portion of its flow from groundwater from the Upper Floridan Aquifer (UFA). Large sections of the Spring Creek and lower Flint basin are incised in the Ocala limestone, the water bearing strata of the UFA, where there is high connectivity of groundwater to surface water (Torak*, et al.*, 2005). Dissolution Ocala limestone bedrock contributes to increased Ca and alkalinity concentrations in the Spring Creek and Flint River arms of the lake relative to the Chattahoochee River and the isolated ponds in SLWMA, which do not have the same connectivity to the UFA. In the Lake Seminole basin, mixing of waters with high and low groundwater connectivity resulted in relatively uniform Ca concentrations (mean in-lake concentration = 22.4-mg/L), and discharge into the Apalachicola River (19.6-mg/L).

Ponds in SLWMA had low Ca concentrations, although several of the ponds were similar in concentration to the lowest concentrations measured in the Lake Seminole basin (e.g. Chattahoochee River) (Fig 4.2). The ponds are morphologically very shallow, filled with bald cypress (*Taxodium distichum*) stumps just below the water surface, and are similar in the water chemistry to isolated depressional wetlands and cypress domes found throughout the lower ACF and southeastern USA (Battle, Golladay & Clayton, 2001). Additionally, these stumps have shown to be used by *P. maculata* to deposit egg masses in Lake Seminole, but egg masses were not observed in any of these ponds, which further suggests unsuitable conditions in these ponds (Chp 2, this volume). These wetlands are topographic low points that fill with surface water from precipitation according to the regional climate patterns of the area, and are important refugia and habitat for aquatic and terrestrial fauna in the southeastern US (Batzer, Day & Golladay, 2012). Due to its proximity to Lake Seminole, it is likely that these ponds were isolated depressional wetlands before impoundment, and have since become perennially inundated ponds as a result of the local elevation of groundwater potentiometric surface in the adjacent water bodies around Lake Seminole following lake formation (Torak*, et al.*, 2005).

At present, the ponds are occupied several species of submerged, emergent and floating macrophytes also found Lake Seminole (e.g. *Brasenia spp. Ceratophyllum spp., Equisetum spp., Typha spp.*), along with filamentous cyanobacteria, *Lyngbya wollei*, in several of the ponds. Limited research has been undertaken in the SLWMA ponds, and little is known on historical hydrologic conditions, or the present biological communities. Management practices to promote recreational fishing in several of these ponds include addition of pond fertilizers and lime (calcium oxide). Continued addition of lime may increase the suitability of these ponds to sustain gastropod populations, particularly *P. maculata.* Introduction of apple snails to these ponds could occur through human recreational traffic. Migration from the main body of Lake Seminole is less likely, as the ponds are not hydrologically connected to the lake. The introduction of non-native species by humans is well documented, including in the wide dispersal of *P. maculata* (Benson, 2015; Drake & Mandrak, 2014).

***Pomacea* Experiments**

Experimental results from the Ca and temperature experiments indicate that significant growth and survival of juvenile *P. maculata* can occur under a wide range of environmental conditions found in Lake Seminole, with the exception being in the lowest Ca concentration and coolest temperature conditions (Figs 4.3 and 4.4). Linear mixed-effect model indicate that Ca concentration and temperature were each significant factors related to the growth of juvenile snails, along with the statistical interaction of those treatment factors. Although no Ca concentration or temperature treatment level significantly influenced day-to-day snail mass, snail production as measured by dry mass and ash mass indicate that warmer temperatures and Ca-rich experimental conditions resulted in greater organic and inorganic snail mass compared to cooler conditions at low Ca concentrations (Fig 4.5). Previous work on *P. canaliculata* behavior noted increased activity in warmer water (>15˚ C), suggesting that lower experimental temperature conditions in this study may have induced inactivity and slowed growth rates (Seuffert & Martin, 2010; Seuffert & Martin, 2013). Although the reported thermal inactivity threshold is below the lowest experimental temperature, temperatures in Lake Seminole annually fall below 15˚ C (average minimum annual temperature = 12.0˚ C) (Chapter 3, this volume). Experimental results from this study suggest that *P. maculata* growth and survival will be inhibited by cooler conditions, which occur in Lake Seminole, and especially when combined with low Ca concentrations. Conditions unfavorable to *P. maculata* growth and survival occur in Lake Seminole and in the SLWMA.

Historically, gastropods were thought to be limited to freshwater with Ca concentrations greater than 5 mg/L. However, recent studies have shown survival and growth in lower Ca concentrations (Lodge*, et al.*, 1987; Thomas & Lough, 1974). Surveys of *P. maculata* in Lake Seminole support the 5 mg/L limit, using conductivity as a proxy of Ca concentration (Chapter 2, this volume). The experimental results from this study suggest that under warmer conditions, snails will be able to survive and grow when Ca is less than 5 mg/L. However, induced stress in low Ca environments accumulates over time and may partially explain reduced growth and increased mortality observed in the lowest calcium treatment. At the lowest Ca concentration treatment in all temperature conditions, increased snail mortality was observed. In several of the experimental units, the remaining shells of dead snails were collected and were less rigid than shells collected from dead snails in higher Ca treatments (N. Marzolf, personal observation). Prolonged exposure to low Ca concentrations, especially in large ampullariids, may have large effects on growth, shell thickness and size, and reproduction (Meier-Brook, 1978; Thomas & Lough, 1974). This suggests that it is unlikely that *P. maculata* could establish and maintain a reproducing population in habitats of the lower ACF and Lake Seminole at the low-end of observed environmental Ca concentration.

P. paludosa is known to have greater shell size and strength under high pH and Ca conditions (Glass & Darby, 2009). Gastropod shell strength and morphology are known to be important in predator defense (Covich, 2010; Rasser & Covich, 2014). Under lower Ca environmental conditions, the ability to adequately defend against predators could be limited (Alexander & Covich, 1991; Bukowski & Auld, 2014; Crowl & Covich, 1990; Hoverman, Cothran & Relyea, 2014). Low Ca concentrations may result in juvenile snails being more susceptible to macroinvertebrate, fish, and reptile predators, and limit snail recruitment in non-native ranges (Valentine-Darby, 2015).

Over the course of the experiment, Ca and alkalinity concentrations in each experimental unit slightly increased, likely as a result of leaching from of spinach discs and other particulate material in each sample. The largest increases in concentrations were in the higher Ca treatments (Flint River and Spring Creek), and the difference in concentration within the Ca levels remained constant. The lowest Ca concentration treatment (Silver Lake) showed smallest increase (0.66-mg/L to 1.32-mg/L), which remained sufficiently low to ensure no confounding of Ca in the lowest treatment. During Day 14 of the warmest temperature assay, the chambers nearest the light source in the incubator began producing algae on the walls of the chamber. Because the water samples used in the assay came directly from the field, algal biomass and the growth of algae in the experimental units may have provided the snails with a small amount of additional food resource in addition to the spinach provided, and could have altered the growth rates observed in the warmest (34° C) temperature treatment at all Ca concentration treatments. Samples taken from Spring Creek prior to the final week of the assay was deficient in carbonate alkalinity, which may have altered the desired experimental conditions. The timing of these samples coincided with abundant *Hydrilla* at the sampling location, which likely depleted inorganic carbon at the water surface at the time of sampling.

Implications for *P. maculata* Dispersal in Lake Seminole and the Lower ACF

Calcium concentrations in Lake Seminole are greater than what has previously shown to inhibit gastropod growth and survival. Furthermore, this study shows that invasive *P. maculata* can potentially survive and grow even near the detection limit of dissolved Ca concentration at high temperature. This result suggests that Ca availability does not limt the dispersal of *P. maculata* in Lake Seminole and the lower ACF (Fig 4.2). This is reflected in the current distribution of *P. maculata* primarily in the Flint River and Spring Creek where growth and survival of juveniles is not Ca limited (Shivers*, et al.*, Submitted). Future monitoring of *P. maculata* distribution will help to determine the extent to which *P. maculata* can sustain populations at lower Ca concentrations, because their distribution has expanded since 2013 to areas adjacent to SLWMA, where the lowest environmental Ca concentrations were observed.

Given low physicochemical inhibition to the growth and survival of *P. maculata* in Lake Seminole, continued expansion throughout the lake seems likely. The widespread introduction of this snail has altered subtropical and tropical wetland, river, and lake habitats, communities and ecological function, and the same consequences seem likely in Lake Seminole (Carlsson*, et al.*, 2004; Karraker & Dudgeon, 2014; Posch*, et al.*, 2013). The abundance of submerged aquatic vegetation (SAV), *Hydrilla* in particular, and the carbon and nutrient processing attributed to SAV in Lake Seminole has greatly affected the functioning of the lake, and its role as a site of high biogeochemical transformations (McEntire, 2009; Shivers*, et al.*, 2013; Waters*, et al.*, 2014). As fast-growing macrophytophagous snails graze on SAV (including *Hydrilla*), significant reductions in SAV may occur in Lake Seminole. Grazing could induce a shift from a macrophyte-dominant to a phytoplankton-dominant lake ecosystem as has been observed elsewhere (Burks*, et al.*, 2011; Meza-Lopez & Siemann, 2015; Morrison & Hay, 2010; Scheffer *et al.*, 2001)

Climate projections suggest the southeastern US will experience warmer temperatures, and more variable precipitation (Hopkinson*, et al.*, 2013; IPCC, 2014). Continued warming of water bodies in the lower ACF will provide more suitable thermal habitat for *P. maculata*, and potentially expand the available habitat into upstream reaches of the ACF basin (Byers*, et al.*, 2013). During the wintertime, *Pomacea spp.* snails can survive some degree of cold and dry periods by estivating until favorable conditions return

(Tiecher, *et al.*, 2015). In the lower ACF, *P. maculata* can perhaps survive the relatively colder winters by burrowing into lake sediment, and/or using thermal refugia such as groundwater springs, which maintain a constant temperature of 22.2° C. It seems unlikely that once established in southeastern rivers, that *P. maculata* will be eliminated.

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Figures

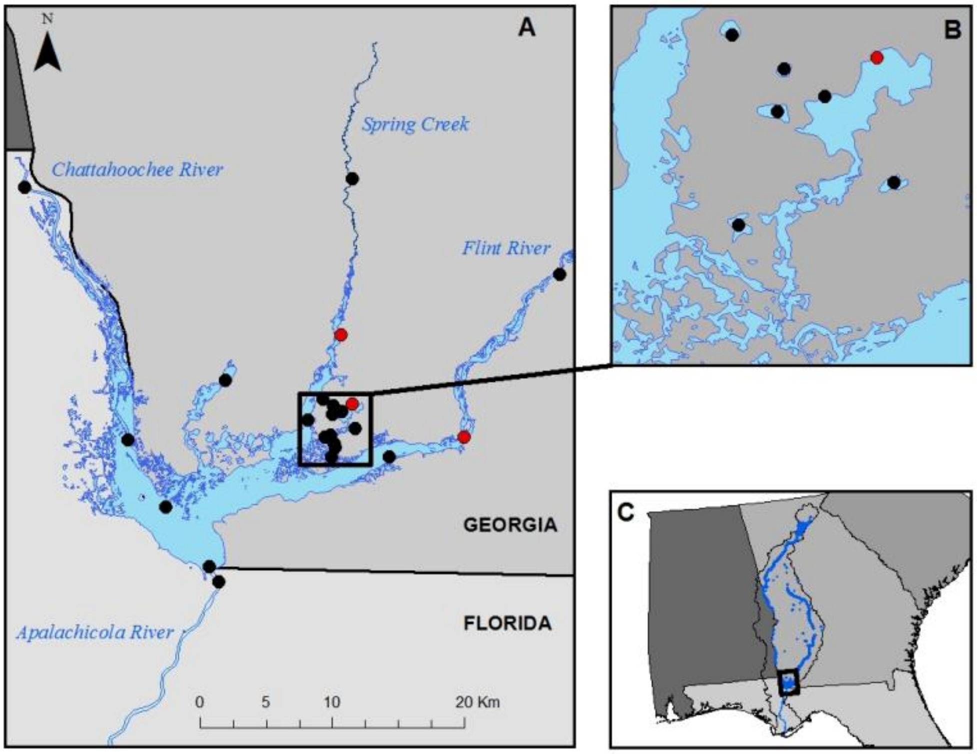

Fig 4.1: Study sites in the lower ACF. A) Lake Seminole, and sampling locations (black circles) across the three arms of the lake and below Jim Woodruff Lock and Dam. Red circles indicate sampling locations that doubled as sampling locations for juvenile *P. maculata* laboratory experiments. B) Location of Silver Lake WMA (SLWMA) between Spring Creek and the Flint River arms of Lake Seminole, and sampling locations in the various ponds found in SLWMA. C) ACF watershed in southeastern US, and location of Lake Seminole in the basin.

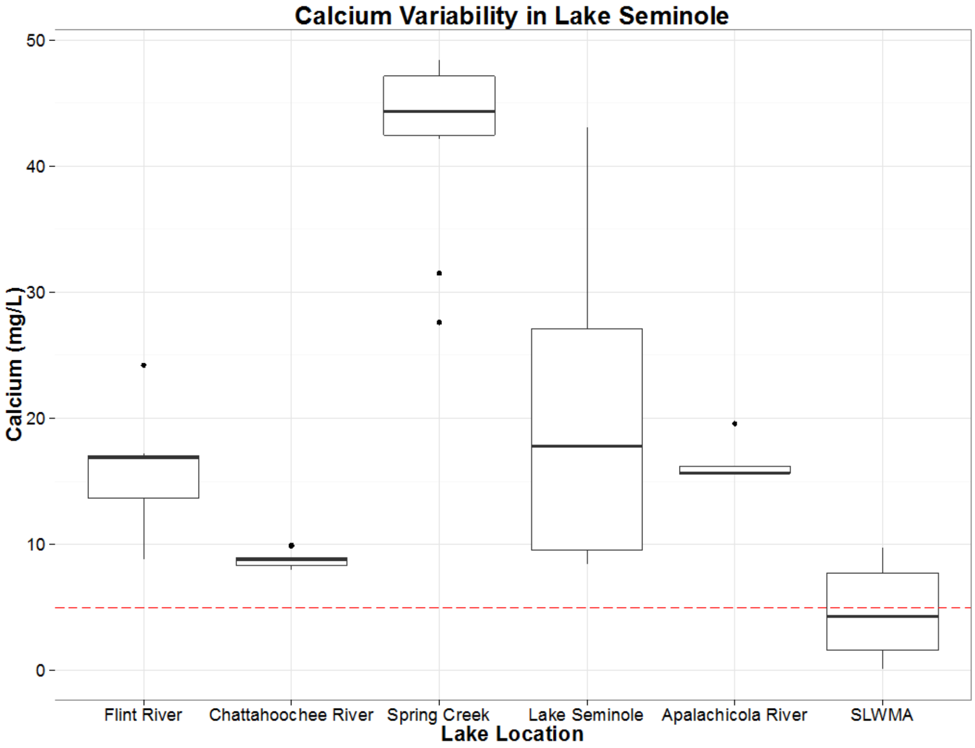

Fig 4.2: Calcium variability across quarterly sampled locations across the lower ACF basin. The plot reads left-to-right upstream of Lake Seminole to the outflow of the Lake, excluding SLWMA. The red dashed line at $[Ca^{2+}]$ = 5 mg/L represents the concentration where gastropods are presumed not to be found below (Lodge 1987).

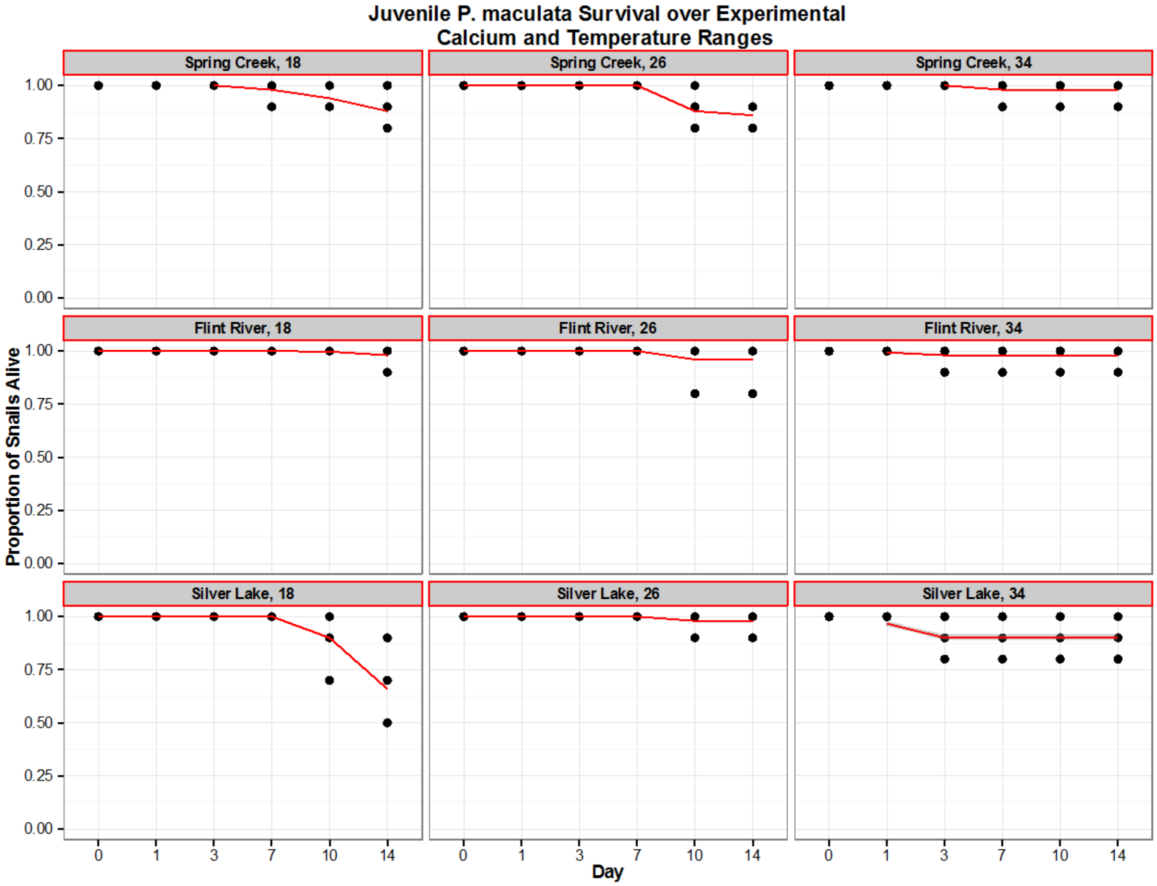

Fig 4.3: Proportion of surviving *P. maculata* over the two-week experiment. Calcium concentrations are ordered from top to bottom, Spring Creek, Flint River and Silver Lake. Temperature treatments are ordered left to right, 18˚ C, 26˚ and 34˚. Circles represent the proportion of surviving snails in each 10-snail cohort, and the line represents the predicted values from the linear mixed-effect model.

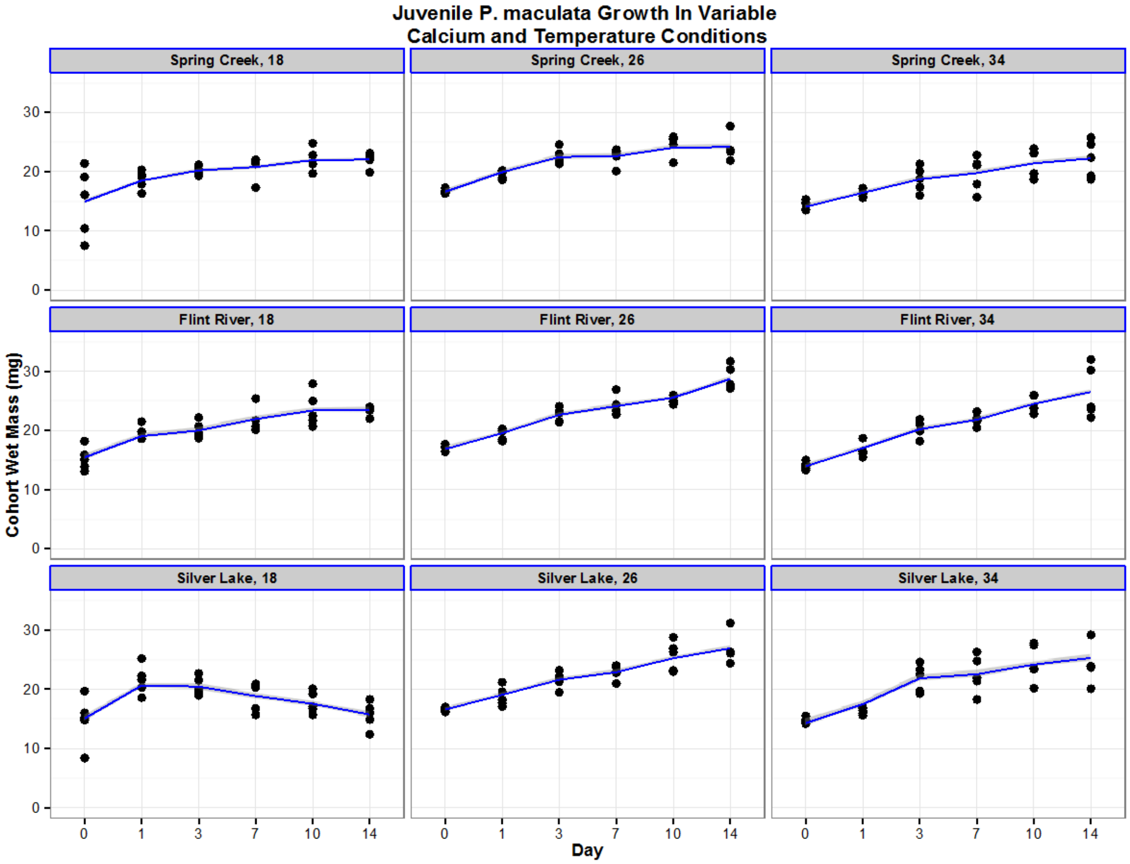

Fig 4.4: *P. maculata* growth over the course two-week experiment. Calcium concentrations are ordered from top to bottom, Spring Creek, Flint River and Silver Lake. Temperature treatments are ordered left to right, 18˚ C, 26˚ and 34˚. Circles represent the wet mass of each cohort from the respective treatment in each of the possible experimental combinations. The line represents the predicted values from linear mixed-effect model.

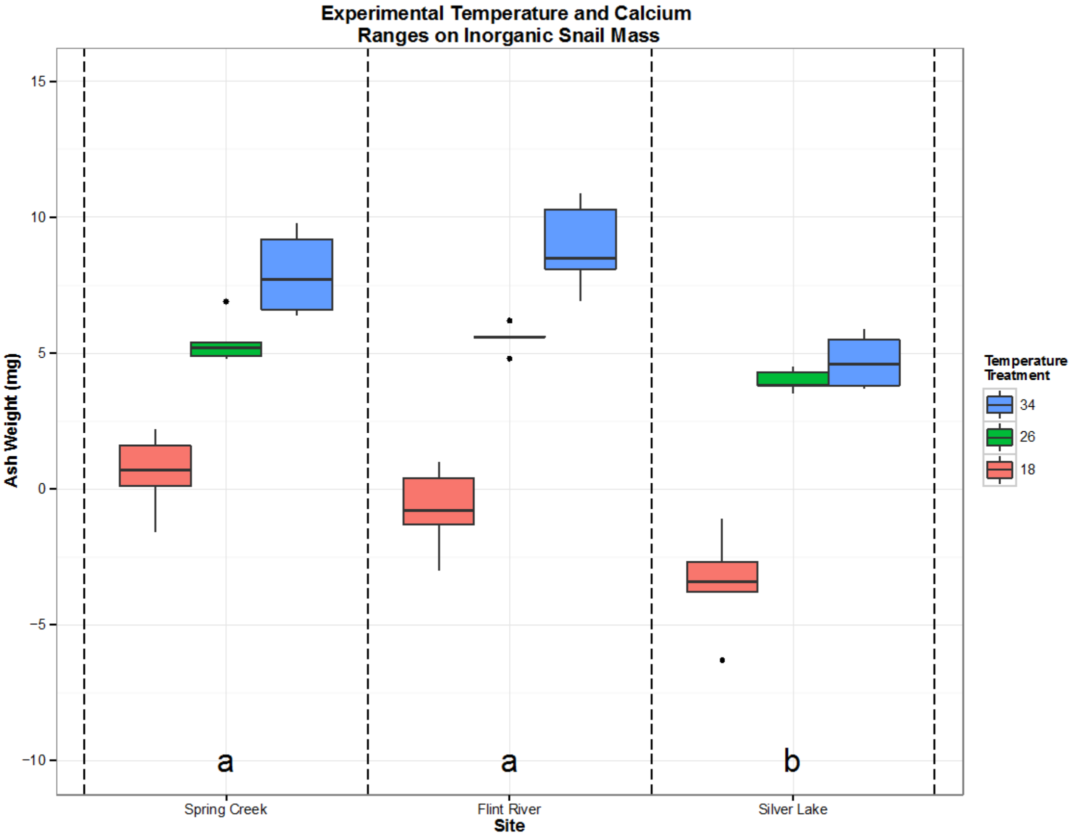

Fig 4.5: Ash mass of snails assayed over variable temperature and calcium conditions. Significant treatment levels are indicated for site using letters above each significant grouping, and significant temperature treatments are grouped by similar color, as shown in the legend.

Table 4.1: Mean physicochemical field data from sampling for three sites used in *P. maculata* experiments along with calcium and alkalinity measurements from Days 0 and 14 used in the experiments.

					Day 0		Day 14	
Site	**Temperature (˚C)**	**Specific Conductivity (mS/cm)**	**DO (mg/L)**	**pH**	**Calcium (mg/L)**	**Alkalinity (ppm)**	**Calcium (mg/L)**	**Alkalinity (ppm)**
Spring Creek	**27.7**	**0.235**	**6.15**	**7.47**	**41.3**	**117.7**	**51.7**	**107.9**
Flint River	**29.3**	**0.173**	**7.20**	**7.57**	**25.6**	**93.0**	**35.7**	**129.2**
Silver Lake	**30.9**	**0.018**	**4.35**	**7.28**	**0.50**	**4.4**	**1.2**	**42.9**

CHAPTER 5

Conclusions and Implications

In North America, *Pomacea maculata* is a widely introduced invasive species and is likely to expand from the current distribution (Byers*, et al.*, 2013). This species has been documented to affect a variety of ecosystem processes and native species where it has been introduced (Carlsson*, et al.*, 2004; Darby*, et al.*, 2007; Hayes*, et al.*, 2015; Horgan*, et al.*, 2014). Similar to some other invasive species, *P. maculata* can grow better in some of its introduced locations relative to its native range in South America. Understanding the reasons for this increased growth requires more information on the ecology of this invasive snail (Parker*, et al.*, 2013).

Lake Seminole is a large shallow reservoir with a history of invasive species introductions that have altered ecological processes (Shivers, 2010; Waters*, et al.*, 2014). *P. maculata* was first observed in the lake in 2003. The dispersal of *P. maculata* throughout Lake Seminole may significantly affect the availability of submerged aquatic vegetation (SAV), which can cover up to 55% of the lake surface area in certain years (Shivers*, et al.*, 2013). Widespread grazing by *Pomacea* may cause a shift from a macrophyte-dominant state to a phytoplankton-dominant state (Hilt*, et al.*, 2011; Scheffer*, et al.*, 2001). This decline in SAV could negatively impact water quality, productivity, and biodiversity in the lower Apalachicola-Chattahoochee-Flint watershed. For these reasons, determining the environmental factors affecting the distribution and dispersal of *P. maculata* in Lake

Seminole is crucial to understanding the extent to which this species might affect lake processes.

Egg masses around the shoreline of the lake were quantified to map the distribution of two *Pomacea* spp. over a three-year period (2013-2015). Results from these surveys indicate that *P. maculata* is present in two of the three arms of the lake (Flint River and Spring Creek), and the number of survey locations in which *P. maculata* was observed increased over the three-year period. The native apple snail, *P. paludosa*, was observed in all three surface water inflows: Flint River, Chattahoochee River and Spring Creek. However, total number of *P. paludosa* egg masses decreased over the survey period and declined or disappeared over three years at some survey locations where both species were present initially. These changes in the two species' distributions may reflect indirect competition for preferred food resources between the two species, which has been previously documented, though the mechanism is unknown (Darby*, et al.*, 2007; Posch*, et al.*, 2013). Continued *P. maculata* dispersal throughout Lake Seminole could result in the local extirpation of *P. paludosa* from parts of Lake Seminole if the current trend of reduced *P. paludosa* egg mass production continues.

Assessing the environmental limits to *P. maculata* dispersal in Lake Seminole showed that the temperature regime and range of water calcium concentrations are both suitable to *P. maculata*, except when both parameters are at the lower limits of their ambient range. Movements of *P. maculata* across an experimental temperature channel gradient showed that snails preferred cooler temperatures when offered a range of observed summer temperatures. The abundance of groundwater-fed springs that are thermally uniform may provide refugia during extreme warm and cold periods. These refugia occur both within Lake Seminole and in the Flint River and Spring Creek, and may increase the suitability of these rivers to the dispersal for *P. maculata*, offering corridors for future dispersal.

Calcium concentrations varied slightly in Lake Seminole, but were considerably higher compared to small ponds near the main body of the lake. Juvenile *P. maculata* exhibited growth in calcium concentrations found throughout Lake Seminole, and were inhibited only under low calcium and low temperature conditions. This suggests that *P. maculata* will not be limited by spatial calcium variation within the lake, but their dispersal into small, calcium-limited ponds may be inhibited, particularly during cold periods.

Implications

Given the expanding distribution of *P. maculata* in Lake Seminole, observed from 2003 to 2010 it is apparent that the lake provides appropriate habitat and environmental conditions for this species. Although Lake Seminole is near the predicted northern limit for *P. maculata* dispersal based on minimum annual temperature, the presence of springs and thermal refugia throughout the lower ACF provide seasonal habitats that could allow *P. maculata* to survive seasonal temperature extremes. Calcium concentrations, significant in the distribution of all gastropods, vary across the lake, but the effect of low calcium and temperature significantly affect snail growth and survival.

Continued expansion northward in the ACF will expose *P. maculata* to more frequent extreme low temperatures. However, warming climate conditions and water temperatures could increase the suitability of more temperate water bodies in the ACF, and nearby watersheds (IPCC, 2014). Increased abundance of *P. maculata* in Lake Seminole may begin to affect the availability and distribution of SAV. Reductions in SAV could result in changes in the productivity of the lake, and have impacts on water quality, recreation and fishing, which are of great ecological, economic, and political concern in the ACF watershed.

The environmental factors in this study indicate a strong likelihood for continued expansion. However, there may yet be factors to limit the growth and dispersal of *P. maculata* in the ACF. Predation, particularly of the smaller juveniles, by fishes, turtles, macroinvertebrates, reptiles and mammals could potentially reduce snail recruitment over time (Valentine-Darby, 2015). No eradication or removal practices have been attempted in Lake Seminole, but concentrated management efforts in areas where dense egg masses or adults are observed to reduce the population may prevent further dispersal and reduce the effect of localized snail grazing.

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