

**Interactive effects of environmental stressors and the invasive apple snail, *Pomacea maculata*, on tapegrass, *Vallisneria americana***

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Presented to

The Faculty of the College of Arts and Sciences

Florida Gulf Coast University

In Partial Fulfillment

Of the Requirements the Degree of

Masters of Science

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By

Shannan C. McAskill

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## Abstract

The island apple snail, *Pomacea maculata*, family Ampullariidae, is a large freshwater gastropod native to South America. *P. maculata* is known as a heavy consumer of aquatic plants. *P. maculata* was introduced to Florida in the 1990s and has rapidly spread throughout natural and man-made wetlands and waterways in the southeastern United States. Negative ecosystem impacts associated with *P. maculata* invasion include destruction of macrophyte communities via overgrazing, competitive exclusion of the native Florida apple snail *Pomacea paludosa*, and the potential transmission of toxins and parasites to predators.

Populations of *P. maculata* have been documented in freshwater tributaries of estuaries such as Mobile Bay, Alabama and the Caloosahatchee Estuary, Florida, and the snails may be moving into the estuaries themselves. The objectives of this study were: 1) to evaluate *P. maculata*'s potential to harm macrophytes like tapegrass, *Vallisneria americana*, in low-salinity estuarine environments, 2) to determine how the grazer's destructiveness might be moderated by environmental context (salinity, temperature, and the presence of other macrophyte species), and 3) to identify management and restoration strategies for *V. americana* to minimize the harm done by *P. maculata*. We accomplished these objectives with feeding trials and mesocosm experiments conducted under varying conditions of salinity, temperature, and macrophyte community composition.

We found that increasing salinity lowered *P. maculata* grazing pressure on *V. americana* but increasing temperature increased grazing pressure. Herbivory on *V. americana* was not reduced and was sometimes intensified when other aquatic plant species were present. The results of two mesocosm experiments suggested that salinity and snail presence have a nonadditive, antagonistic, effect on *V. americana*. I.e., in the absence of snails the plant

performed best at 0 psu, whereas when snails were present the plant did best at 5-10 psu due to reduced snail grazing. Due to the significant sub-lethal impacts of salinity on *P. maculata*'s grazing and health it is unlikely that the snail's invasion will proceed beyond the lowest salinity portions of estuaries. These estuarine regions can therefore serve as a valuable refuge for *V. americana* populations, providing that effective water management keeps salinity below the approximately 10 psu threshold where significant direct harm occurs to the plants.

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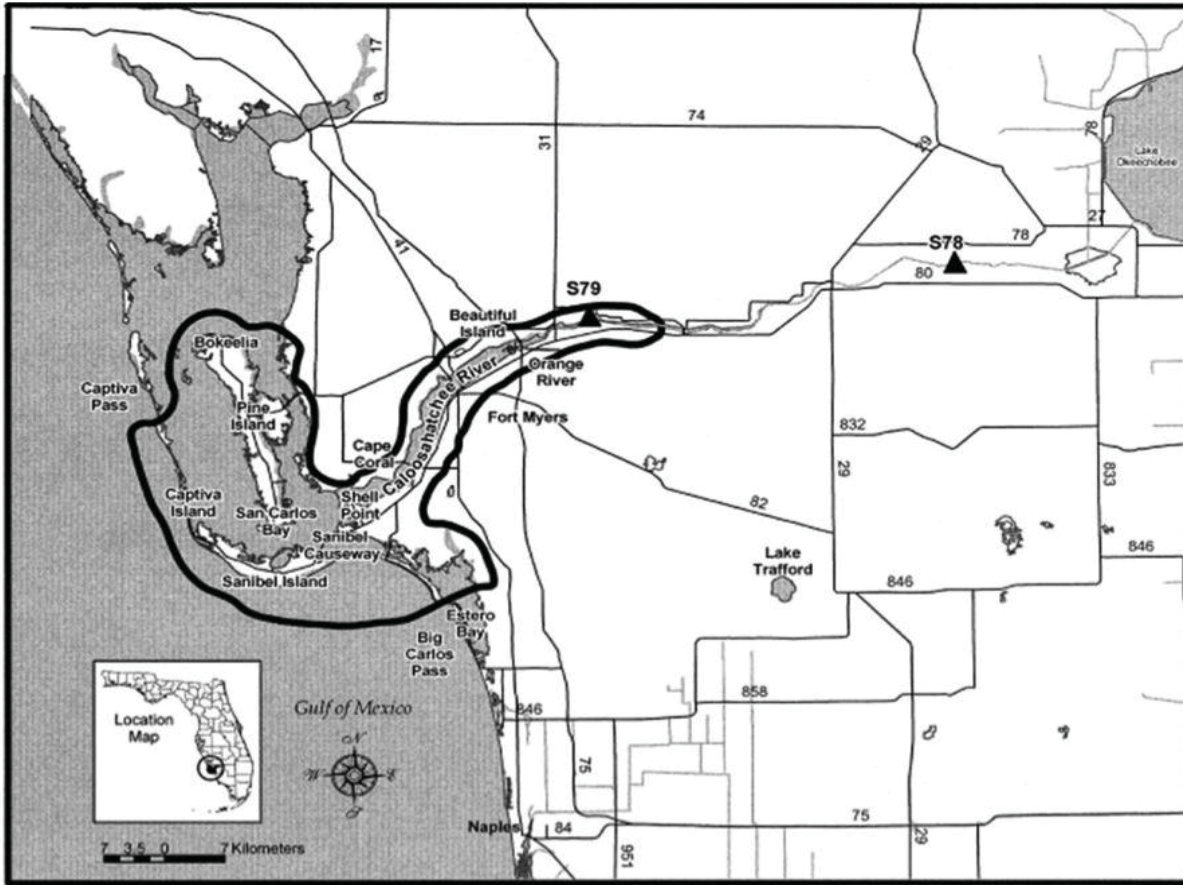
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## Chapter 1: Introduction

Estuaries, recesses in the coastline where fresh and marine waters mingle, are hotspots of biological productivity and biogeochemical cycling (Barbier et al. 2011). In addition to serving as a vital nexus of aquatic, terrestrial, and marine habitats, estuaries provide economically important resources and ecosystem services to humans (Barnes 2005, Barbier et al. 2011).

The Caloosahatchee Estuary (CE), located at 26.63° N 81.85° W along the southwest coast of Florida, USA (Fig. 1.1), is a drowned river valley estuary formed during the post-glacial rise in sea level (Antonini et al. 2002). The CE has a 42 km linear extent from the S-79 lock and dam to the Gulf of Mexico, and its width of the ranges from 160 m in the upper portion to 2,500 m in near the Gulf (Scarlatos 1988, Xia et al. 2010). Depths within the CE range from 0.3 to 6 m depending on location. The average depth of the estuary west of Beautiful Island is 1.5m. In the past, the typical annual range in water temperature was reported to be between 17°C to 28°C (Scarlatos 1988). The mean temperature of the upper Caloosahatchee Estuary (CE) from November 2012 – November 2014 was 27°C, with a winter minimum of 17°C and a summer maximum of 34°C (Sanibel Captiva Conservation Foundation 2015).



**Figure 1.1:** Map of the Caloosahatchee Estuary (Barnes 2005).

The CE supports many recreationally and commercially important fisheries species such as blue crab *Callinectes sapidus*, snook *Centropomus undecimalis*, spotted sea trout *Cynoscion nebulosus*, and red drum *Sciaenops ocellatus*. In addition it supports charismatic megafauna which add value to the area's tourist economy; organisms such as the smalltooth sawfish *Pristis pectinata*, the Florida manatee *Trichechus manatus*, and numerous species of wading birds. These valued species are in part supported by submerged aquatic vegetation (SAV), which plays a key role in the ecosystem function of the CE (Ward 1984, Doering et al. 2002, Barnes 2005).

SAV, a functional grouping of species that includes both seagrasses and freshwater macrophytes, plays numerous beneficial roles in the environment. Beds of SAV reduce erosion

and maintain water clarity by trapping fine sediments (Doering et al. 2002, Wigand et al. 2000, Barnes 2005). Additionally, they can improve overall water quality by taking up excess nutrients that could otherwise accelerate eutrophication within the system (Barnes 2005, Camp et al. 2014). SAV can also provide important habitat and contribute to secondary production by acting as foraging grounds for fish, as well as invertebrates, waterfowl, wading birds, and the endangered Florida manatee (Doering et al. 2002, Barnes 2005, Camp et al. 2014). The CE historically supported four species of bed-forming SAV: the seagrasses *Thalassia testudinum* and *Halodule wrightii*, the euryhaline SAV *Ruppia maritima*, and the moderately salt tolerant freshwater plant *Vallisneria americana* (Barnes 2005, Stevens et al. 2010).

*Vallisneria americana*, known commonly as tapegrass, is a deep-rooted macrophyte that is a particularly valuable SAV species in freshwater and low-salinity estuarine habitats (Hogan et al. 2014). Tapegrass populations within the CE can help improve water clarity, reduce erosion, and stabilize shoreline by trapping sediments, provide shelter for aquatic fauna, and serving as an important food source for many species including *T. manatus* (Robbins 2004, Barnes 2005, Moore et al. 2010). Robbins (2004) collected 44 species of fish, along with numerous invertebrate taxa, from *V. americana* and *Ruppia maritima* beds in the CE. Dominant invertebrates were amphipods and mysid shrimps but commercially important blue crabs, *Callinectes sapidus*, and pink shrimp, *Penaeus duorarum*, were also abundant.

While all estuaries exhibit some temporal variation in salinity, anthropogenic changes in the hydrography of the Caloosahatchee watershed have substantially increased salinity variability (Barnes 2005, Douglass 2014). Historically, the estuary graded into the shallow, meandering Caloosahatchee River, with its headwaters located near Lake Hicpochee. However, for purposes of flood control, land reclamation, and other human needs, the Caloosahatchee River was heavily

modified (Barnes 2005). Modifications included the dredging of a canal to connect the headwaters of the river to Lake Okeechobee, and deepening and straightening of the river channel to accommodate navigation and freshwater releases from Lake Okeechobee. Lock and dam structures were also put in place in order to improve navigation and regulate water flow (Scarlatos 1988, Barnes 2005). Today, the freshwater portion of the Caloosahatchee has been engineered into a canal called C-43. There are three main lock and dam structures that control water flow from C-43 into the estuary (Barnes 2005). In order from upstream to downstream these are S-77, S-78, and S-79. The S-79 lock and dam, also known as the W.P. Franklin Lock and Dam, is located furthest downstream (Scarlatos 1988, Barnes 2005). This lock and dam structure marks the beginning of the Caloosahatchee Estuary and acts as an impediment to tidal exchanges and saltwater intrusion farther up the river (Barnes 2005). However, as long as flows through S-79 are maintained above 450 cubic feet per second (cfs), the upper CE just below S-79 has low salinities consistent with a tidal freshwater / oligohaline system (Douglass 2014).

Tapegrass, *Vallisneria americana*, has historically been the dominant SAV species in the upper portion of the estuary (Barnes 2005), faring well where salinities remain below 10 psu (Doering et al. 1999, Wigand et al. 2000). Unfortunately, *V. americana* beds have been largely absent from the Caloosahatchee Estuary since the early 2000s due unnaturally extreme salinity fluctuations (Stevens et al. 2010, Douglass 2014).

Variations in salinity within the CE are strongly influenced by freshwater discharge into the head of estuary, now mediated by managed flows through S-79. Salinity near the mouth of the estuary can drop below 5 psu during periods of high freshwater discharge, with dire consequences for the seagrasses *Thalassia testudinum* and *Halodule wrightii* in that region. Conversely, during periods of low freshwater discharge, saltwater intrusion occurs as far



upstream as the S-79 dam where salinities often exceed 10 psu (Doering et al. 2002). Saltwater intrusion near Beautiful Island, the center of *V. americana*'s historical distribution in the CE, now averages between 13-14 psu at the winter dry season maximum. This is near or above *V. americana*'s upper salinity tolerance. Although there is evidence that *V. americana* can recover from short term saltwater intrusion events, in recent years *V. americana* has been continuing to decline even when conditions are favorable for growth (Doering et al. 2001, Douglass 2014).

Herbivory is another major limiting factor for the establishment and growth of *V. americana* populations, as has been demonstrated in previous studies in freshwater portions of the Caloosahatchee (Ceilley et al. 2003, 2009, 2013), Crystal River (Hauxwell et al. 2004) and Chesapeake Bay (Moore et al. 2010). While herbivory may be important in both Virginia and Florida, the herbivore species involved are quite different in Florida. An herbivore of particular concern in Florida is the recent invader *Pomacea maculata* (Perry 1810; formerly described as *P. insularum*, D'Orbigny 1839), commonly known as the island apple snail (Baker et al. 2010, Ceilley et al. 2013). *P. maculata* is an aquatic gastropod in the Ampullariidae family. It is native to warm temperate and tropical South America (Conner et al. 2008). Like other members of its family, *P. maculata* possesses both gills and an air-breathing siphon, allowing it to survive in hypoxic waters and shallow wetlands. The island apple snail was initially introduced to Florida in the 1990s, and since then populations have rapidly spread throughout Florida as well as seven other states in the southeastern USA (Byers et al. 2013). In contrast with its Florida-native congener *P. paludosa*, which feeds mainly on periphyton, aquatic algae, and detritus, *P. maculata* is infamous for feeding predominately on macrophytes (Sharfstein and Steinman 2001, Horgan et al. 2014). The feeding proclivities of this snail, in conjunction with its high reproductive and consumption rates, gives it the ability to cause rapid changes in macrophyte

community structure, in some cases eliminating nearly all plants (Monette 2014). This can alter nutrient cycling and turbidity, shifting healthy, macrophyte-dominated ecosystems to eutrophic, phytoplankton-dominated systems (Burks et al. 2010, Hogan et al. 2014). The snail has been particularly damaging in manmade wetlands such as the stormwater treatment areas of the Florida Everglades (Monette 2014), and as an agricultural pest in Asian rice paddies where it has been invasive for decades (Byers et al. 2013).

In addition to devastating plant communities, *P. maculata* has strong harmful impacts on native fauna (Byers et al. 2013, Horgan et al. 2014). In addition to indirect impacts on native wildlife due to destruction of macrophyte habitat, direct harmful impacts of *P. maculata* in Florida include: competitive exclusion of the native Florida apple snail (*Pomacea paludosa*), transmitting parasites such as the rat lungworm, and bioaccumulating algal toxins that cause Avian Vascular Myelopathy (Conner et al. 2008, Byers 2013).

Much of the concern about *P. maculata*'s effects on native fauna is focused on its potential displacement of *P. paludosa*, the only apple snail native to the southeastern United States (Conner et al. 2008). *P. maculata* grows three to four times larger than *P. paludosa*, lives longer (>3 years as opposed to 1-1.5 years), and has higher consumption, growth, and reproductive rates (Conner et al. 2008, Burks et al. 2010, Byers et al. 2013). For example, an average *P. maculata* lays 2000 eggs per clutch and can spawn 4-5 clutches per month during warm months (Barnes et al. 2008, Bernatis 2014), whereas *P. paludosa* lays only 20-30 eggs per clutch and spawns 1-3 clutches per month (Garr et al. 2012, Byers et al. 2013). The combination of all of these factors makes *P. maculata* an ideal candidate for invasive expansion. In addition to harming native apple snails via exploitation competition, invasive *P. maculata* can feed directly on benthic fauna including microcrustaceans, worms, and other snails, likely including juvenile

*P. paludosa* (Horgan et al. 2014). Conner et al. (2008) found that juvenile *P. paludosa* had a higher mortality rate when adult *P. maculata* were present.

Although there is strong evidence of direct negative impacts of *P. maculata* on *P. paludosa*, the most severe impacts of *P. maculata* on other aquatic fauna appear to be those mediated indirectly, via decimation of aquatic vegetation. This implies that overgrazing and exploitation competition are the primary mechanisms underlying loss of native biodiversity and ecosystem functions following an apple snail invasion (Horgan et al. 2014). Better understanding the impacts of *P. maculata* on native vegetation, and determining what environmental factors might mitigate those impacts, could help reduce *P. maculata* damage in the Caloosahatchee Estuary and in other aquatic ecosystems in the snails' introduced range.

A laboratory study of the feeding rates of *P. maculata* on Florida native and nonnative vegetation found that *Vallisneria americana* is among the preferred species consumed by the snail (Baker et al. 2010). Overgrazing of *V. americana* beds has also been documented in the field by Ceilley et al. (2013), who found that *V. americana* planted in freshwater portions of the Caloosahatchee River did well when snails were removed, but was decimated by the snails otherwise. Loss of *V. americana* due to *P. maculata* in the freshwater Caloosahatchee may also impact tapegrass in the brackish estuary below S-79. I.e., it may remove a seed source critical for reestablishing estuarine tapegrass beds after dieoffs due to high salinity events.

Our current understanding of *P. maculata* herbivory on *V. americana* is based on a small number of laboratory feeding trials (Gettys et al. 2008, Baker et al. 2010, Bernatis 2014) and a few observations and anecdotes from the field (e.g., Ceilley et al. 2013). Few if any studies having rigorously tested the snails' effects on live, rooted *V. americana*. Likewise, there have been a number of studies of the environmental tolerances of the snail with regards to salinity

(Ramakrishnan 2007, Bernatis 2014, Martin and Valentine 2014), pH (Ramakrishnan 2007, Byers 2013, Bernatis 2014), temperature (Ramakrishnan 2007, Gettys et al. 2008), and emersion stress (Ramakrishnan 2007, Bernatis 2014). However there is limited knowledge of how sub-lethal effects of these environmental factors might influence the demographic rates and grazing impacts *P. maculata* populations. Further, aquatic vegetation species such as *V. americana* have their own sets of environmental tolerances, which might affect their responses to grazing. In table 1.1, and in the sections below, we summarize what is known about the environmental tolerances and optima of *P. maculata* and *V. americana*. However, to make useful predictions about the extent and impacts of the apple snail invasion, and to improve management strategies for protecting macrophyte communities, we must build on the existing information with realistic ecological studies of the interactions between environmental conditions, snails, and macrophytes.

**Table 1.1:** Reported environmental tolerances of *Pomacea maculata*

| Environmental Factor | Range of Tolerance | Optimal Range                            | Study                     |
|----------------------|--------------------|--|---------------------------|
| Temperature          | > 0°C              | -  | Yoshida 2014              |
|                      | > 6°C              | -  | Byers et al. 2013         |
|                      | 15-35°C            | Consumption: 20-35°C;<br>Growth: 20-30°C | Gettys et al. 2008        |
|                      | 15.2-36.6°C        | 20-30°C                                  | Ramakrishnan 2007         |
| Salinity             | 0-8.0 psu          | 0- 8.0 psu                               | Bernatis 2014             |
|                      | 0-15.0 psu         | 0-5 psu                                  | Martin and Valentine 2014 |
|                      | 0-13.6 psu         | 0-6.8 psu                                | Ramakrishnan 2007         |
| pH                   | 5.5-9.5            | 7.5-9.5                                  | Bernatis 2014             |
|                      | 4.0-10.5           | 7.0-9.0                                  | Ramakrishnan 2007         |

**Table 1.2:** Reported environmental tolerances of *Vallisneria americana*

| Environmental Factor | Range of Tolerance | Optimal Range | Study                 |
|----------------------|--------------------|---------------|-----------------------|
| Temperature          | 13-38°C            | 28°C          | Bartleson et al. 2014 |
| Salinity             | 0-15 psu           | 0-3 psu       | Doering et al. 1999   |

## Temperature impacts:

*Pomacea maculata* – Reports on the temperature tolerance range for *P. maculata* have typically suggested 15 – 36 °C (Ramakrishnan 2007, Gettys et al. 2008). However, Byers et al. (2013) report that *P. maculata* populations can persist in areas with average monthly temperatures as low as 6°C, and *P. maculata* populations in Asia can even tolerate 0°C for short periods of time (Yoshida 2014). Based on the latter two studies, it would seem likely that *P. maculata* will continue to spread into temperate regions of the US, barring other limiting factors. However, a species' range is not necessarily dictated by its maximum physiological tolerances. The spread of *P. maculata* into temperate regions of the US will also depend upon the region falling within the snail's optimal temperature range during the summer months. A study done by Gettys et al. (2008) looked at the effects of temperature on the consumption of *Hydrilla verticillata* and growth rate of *P. maculata* and suggested that the maximum consumption rate of the snail occurred between 20-35°C, and that maximum growth occurred within the slightly narrower range of 20-30°C (Gettys et al. 2008).

*Vallisneria americana* – *V. americana* has a broad geographic range from subtropical climates to cool temperate areas, and there appear to be considerable differences in the environmental tolerances and optima of populations from different regions (Kimber 1994, Bartleson et al. 2014). Therefore, we focus on tolerances reported from southwest Florida. Bartleson et al. (2014) examined the temperature tolerance of *V. americana* in southwest Florida and reported that growth occurred between 13-38°C. The optimal temperature for growth of *V. americana* in this study was 28°C, which falls towards the upper range of *P. maculata*'s optimal temperature range for growth (Gettys et al. 2008, Bartleson et al. 2014).

### **Salinity Impacts:**

*Pomacea maculata* - It is also known that *P. maculata* has the ability to tolerate low levels of salinity. However, there are some varying reports on what the salinity tolerances of *P. maculata* actually are. Discrepancies among these reports likely owe to differences in the time of exposure to elevated salinities, the size and age of the snails exposed, and genetic differences among the populations studied. A study on the survival of *P. maculata* embryos and hatchlings (Martin and Valentine 2014) reported 100% survival at 0 psu, 80% survival at 5 psu, and 50% survival at 10 and 15 psu after a period of two weeks. In contrast, Ramakrishnan (2007) reported the maximum salinity tolerance of juvenile and adult *P. maculata* as 6.8 psu, with 10-20% mortality after 28 days at 6.8 psu and 100% mortality after just 7 days at 13.6 psu. Therefore, Ramakrishnan (2007) classified the snail as a 2<sup>nd</sup> degree euryhaline limnobiont, following the classification system outlined by Remane and Schlieper (1971). In this system, stenohaline limnobionts are species restricted to salinities between 0-0.5 psu. Euryhaline limobionts can tolerate salinities ranging from 0.5-8+ psu. Euryhaline limobionts can be divided into three groups, 1<sup>st</sup> degree (0.5-3 psu), 2<sup>nd</sup> degree (3-8 psu), and 3<sup>rd</sup> degree (>8 psu) (Ramakrishnan 2007, Remane and Schlieper 1971). The optimal salinity range for *P. maculata* reported by Bernatis (2014) was <8.0 psu. This agrees with Ramakrishnan (2007), and suggests that the survivorship at 10-15 psu reported by Martin and Valentine (2014) may not be typical for the species in all of its introduced range. Because the salinity tolerance of *P. maculata* by region and by life history phase, we recommend additional, region-specific studies of the species' tolerance.

*Vallisneria americana* - The salinity tolerance of *V. americana* populations within the Caloosahatchee Estuary has been extensively researched. Doering et al. (1999) exposed *V. americana* to salinity levels ranging from 0-15 psu for a 43 consecutive days. Although *V.*

*americana* was able to tolerate 15 psu for the full 43 days, net production of blades was zero at this salinity. Blade production was similarly low at 10 psu. The study concluded that the salinity tolerance of *V. americana* in the Caloosahatchee estuary ranges from 0-15 psu, with 0-3 psu being optimal conditions for growth (Doering et al. 1999). Further study of *V. americana* salinity tolerance within the Caloosahatchee estuary revealed that acute mortality of the tapegrass occurred at 18 psu (Doering et al. 2001), with 50% mortality after 31 days of exposure. If salinity is reduced to 3 psu or lower no more than a month after high salinity exposure, *V. americana* populations can recover roughly 50% of lost blades and shoots within one month. This would suggest that the plants have the ability to survive and recover from saltwater intrusion events within the Caloosahatchee Estuary (Doering et al. 2001), providing that the intrusions last no more than 30 days and the recovery periods are long.

#### **Feeding Preferences:**

Several studies have examined *P. maculata* herbivory on native and nonnative plants in Florida by quantifying the rates of consumption of different plant species (Gettys et al. 2008, Burlakova 2009, Baker et al. 2010, Bernatis 2014). In these studies, *Vallisneria americana* was one of the species most readily consumed by *P. maculata*. For example, Baker et al. 2010 quantified the feeding rate of *P. maculata* on twenty-two different SAV species found in Florida. Plant species including *Vallisneria americana*, *Hydrilla verticillata*, and *Chara* sp. were among the most heavily damaged plants from *P. maculata* herbivory. However, caution should be applied when interpreting feeding preferences from studies where only one plant species at a time was presented to consumers. Indeed, feeding preferences tend to be relative, and consumption rates on one or another plant species can be drastically altered by the “community context” of what other plant species are available to consumers.

Multiple macrophyte species occur along with *V. americana* in the Caloosahatchee watershed. This is especially true in the heavily *P. maculata*-invaded urban canals of Cape Coral (26°36'25.55"N 81°58'23.11"W), which are separated from the Caloosahatchee Estuary by a series of weirs. There is little quantitative information on the extent of *V. americana* in the Cape Coral canals, but based on the destructive overgrazing of *V. americana* by *P. maculata* observed in the nearby Caloosahatchee River by Ceilley et al. (2013), *V. americana* populations in the canals are in serious peril. However, it is unclear whether or not the other macrophytes occurring in Cape Coral canals will alleviate or exacerbate grazing pressure on *V. americana*. Determining *P. maculata*'s preference for alternative food sources relative to *V. americana* in the Cape Coral canals may have important implications for *V. americana* populations there and elsewhere. It is possible that alternative food sources reduce herbivory on *V. americana*, and that management to enhance aquatic macrophyte diversity could reduce the harmful impacts of *P. maculata* in southwest Florida.

### **Summary:**

Based on the feeding preferences, negative ecosystem impacts, and known salinity tolerances of the island apple snail, the struggling *V. americana* populations in the upper Caloosahatchee Estuary, and in other estuarine habitats in the southeastern USA, may be vulnerable to this invader. However, moderate levels of salinity could also afford this SAV species some protection. I.e., if *V. americana* can be restored in the Caloosahatchee Estuary below the S-79 lock and dam, it may be moderately protected from the freshwater-based invader. Of course, this is contingent upon salinity in the estuary remaining below levels directly harmful to the *V. americana* itself. To manage threatened populations of *V. americana* we need to achieve a better



understanding of its responses to the combined, interactive effects of *P. maculata* and environmental stressors. Towards this end, our research has three main objectives:

1. Determine how salinity and temperature directly impact the grazing rate of *P. maculata* on *V. americana*.
2. Compare *P. maculata* grazing rate on *V. americana* in monoculture versus grazing rate on *V. americana* in polyculture with other SAV species that may be more or less preferred food sources.
3. Assess the interactive effects of salinity and *P. maculata* herbivory on the growth and survivorship of *V. americana*.

In addition to addressing conservation and management issues specific to *P. maculata* and *V. americana* in southwest Florida, this research addresses a broader scientific goal. That goal is to determine how environmental context (e.g., temperature, salinity, and the presence of alternate prey species) affects the outcome of interactions between an invasive herbivore and a habitat-forming foundation species. We hope that the knowledge gained will enable natural resources managers around the world to incorporate environmental context in efforts to mitigate the impacts of invasive herbivores.

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## Chapter 2: Factors mediating *Pomacea maculata* herbivory rates on *Vallisneria americana*: salinity, temperature, and macrophyte community context

### Abstract

The island apple snail, *Pomacea maculata*, is native to South America but has become an invasive pest in freshwater ecosystems in Asia and North America. It tolerates oligohaline conditions and may also invade estuaries. By overgrazing submerged aquatic vegetation (SAV) *P. maculata* have impaired ecosystem functions and threatened key species such as Tapegrass (*Vallisneria americana*) in the Caloosahatchee Estuary in Southwest Florida. Abiotic stressors such as salinity and temperature appear to strongly influence the grazing rate of *P. maculata* and offer potential means to mitigate its impacts. We found that increases in salinity reduced *P. maculata* grazing rate, whereas elevated temperature increased grazing. In addition, we examined the role macrophyte community context plays in alleviating or exacerbating grazing pressure on *V. americana*. In feeding trials where the snails were offered a choice between *V. americana* and another SAV species, they generally consumed a proportion of *V. americana* equal or greater to the proportion *V. americana* consumed when no alternative SAV was presented. The results of this experiment indicate that low levels of salinity could potentially alleviate *P. maculata* herbivory on *V. americana*, but that *V. americana* might be especially threatened by *P. maculata* in warm summer months and when in competition with other aquatic macrophytes.

## Introduction

Environmental context can dramatically alter the nature, strength and outcome of interspecies interactions (Chamberlain et al. 2014). For example, a population of herbivores might severely overgraze a population of plants under one set of abiotic and biotic conditions, yet the same herbivores could be harmless or even beneficial to the plants under another set of conditions (Elton 1958, Levine and D'Antonio 1999, Martin and Valentine 2011). Understanding how environmental context affects interspecies interactions is particularly important for addressing the harmful impacts of invasive species, because it can give environmental managers tools to mitigate the harm done by the invaders.

The Island Apple Snail, *Pomacea maculata*, is an aquatic gastropod native to warm temperate and tropical South America (Conner et al. 2008). The snail was introduced in Florida and Texas during the early 1990s and quickly became invasive, spreading throughout the southeastern USA (Byers et al. 2013). Like other members of the Ampullariidae family, *P. maculata* possesses both gills and an air-breathing siphon, allowing it to survive in hypoxic waters and shallow wetlands. In contrast with its Florida-native congener *P. paludosa*, which feeds mainly on periphyton, aquatic algae, and detritus, *P. maculata* feeds predominately on macrophytes (Sharfstein and Steinman 2001, Horgan et al. 2014). *P. maculata*'s diet, in conjunction with its high growth and reproductive rates, can lead to rapid changes in the structure and function of invaded ecosystems. By removing macrophytes and altering nutrient cycling and sediment processes, the snails can shift clear-water, macrophyte-dominated ecosystems to turbid, phytoplankton-dominated ecosystem (Burks et al. 2010, Horgan et al. 2014). Overgrazing by invasive *Pomacea* spp. on a variety of emergent and submerged aquatic vegetation species has been documented in natural lakes and wetlands (Carlsson et al. 2004,

Martin et al. 2012, Horgan et al. 2014), in engineered wetlands and waterways (Teem et al. 2013, Horgan et al. 2014), and in agricultural ecosystems (Cowie 2002, Joshi and Sebastian 2006). The goal of our research is to determine how aspects of environmental context (salinity, temperature, and the presence of alternate prey species) affect the outcome of interactions between the invasive herbivore *P. maculata* and the habitat-forming foundation species *V. americana*. We are particularly concerned with the impact of *P. maculata* herbivory on beds of tapegrass, *Vallisneria americana*, in the freshwater and oligohaline portions of the Caloosahatchee Estuary.

The Caloosahatchee Estuary (hereafter CE), located at 26.63° N 81.85° W along the southwest coast of Florida, USA, is a drowned river valley estuary extending 42 km NE from the Gulf of Mexico to the S-79 lock and dam (Antonini et al. 2002, Xia et al. 2010, Douglass 2014). The CE historically supported four species of bed-forming SAV: the seagrasses *Thalassia testudinum* and *Halodule wrightii*, the euryhaline SAV *Ruppia maritima*, and the moderately salt tolerant freshwater plant *Vallisneria americana* (Barnes 2005, Stevens et al. 2010). *V. americana* was once abundant throughout fresh and oligohaline regions of the Caloosahatchee, where salinities generally remain below 10 psu (Doering et al. 1999, Wigand et al. 2000). The salinity tolerance of *V. americana* ranges from 0-15 psu with an optimal range from 0-3 psu (Doering et al. 1999). *V. americana* populations within the CE improved water clarity by trapping nutrients and sediments, increased diversity and secondary production of benthic fauna, and fed native grazers such as the Florida Manatee, *Trichechus manatus latirostris* (Barnes 2005, Moore et al. 2010, Horgan et al. 2014). Unfortunately, hydrologic changes in the CE watershed, including ditching and urban development, channelization and damming of the river, and the establishment of a synthetic link to the large Lake Okeechobee watershed, have increased temporal variability

in salinity levels within the CE and largely eliminated *V. americana* (Scarlatos 1988, Doering et al. 1999, Barnes 2005, Douglass 2014).

We suspect that *P. maculata* herbivory is another major limiting factor for the reestablishment and growth of *V. americana* populations within the CE, based on documentation of overgrazing and depletion of *V. americana* in the freshwater portion of the Caloosahatchee River where *P. maculata* is also present (Ceilley et al. 2013). It is known that *V. americana* is readily consumed by *P. maculata* under freshwater conditions (Gettys et al. 2008, Baker et al. 2010, Bernatis 2014), and that *P. maculata* can tolerate some degree of estuarine salinity. However, our current understanding of *P. maculata* herbivory on *V. americana* is mostly based on laboratory feeding trials where the consumption rate of the snail was examined under freshwater conditions. There is limited understanding of how estuarine salinity conditions influence the grazing impacts *P. maculata*.

The lethal limit of salinity for *P. maculata* has been variously reported as 6.8 psu (Ramakrishnan 2007), 8.0 psu (Bernatis 2014), and 15+ psu (Martin and Valentine 2014). For the purposes of this study, we examined the effects of salinity in the range from 0-8 psu, which was the “optimal salinity range” for the snails as reported by Bernatis (2014). Note that this range is below the 10-18 psu lethal limit of salinity established for *V. americana* in the CE by Doering et al. (2001).

High salinities in the CE typically occur during the winter dry season, when Caloosahatchee River flow is diverted for municipal and agricultural users. In contrast, high temperatures occur in the summer rainy season when salinity is generally low. Summer water temperatures in the CE can exceed 30°C and may exacerbate grazing impacts by increasing *P. maculata* metabolic rates. A study done by Gettys et al. (2008) tested the effects of temperature



between 15-35°C on the grazing rate of *P. maculata* on *Hydrilla verticillata*. This study was based on research by Ramakrishnan (2007) which concluded the physical temperature tolerance of *P. maculata* is between 15.2-36.6°C. However, more recent studies have shown *P. maculata* can tolerate temperatures lower than 15°C for short periods of time (Yoshida 2014, Byers et al. 2013).

Based on the research done by Gettys et al. (2008), the optimal temperature range for *P. maculata* grazing is between 20-35°C. The optimal temperature range for *P. maculata* growth is between 20-30°C (Gettys et al. 2008). In addition, Gettys et al. (2008) found that when kept at 15°C *P. maculata* ate less food compared to snails kept at higher temperatures and lost an average of 1.8g of body weight during the 10 day experiment. Although the impacts of temperature on *P. maculata* grazing rate have been assessed it is still necessary to quantify the impacts of temperature on *P. maculata* grazing rate on *V. americana* due to its importance as a valued ecosystem component (Ceilley et al. 2013). This is the first time a study will be done using *V. americana* to assess the impact of temperature on *P. maculata* grazing rate.

In addition to variation in temperature and salinity, freshwater and estuarine *V. americana* habitats in southwest Florida may vary in terms of the presence and diversity of other aquatic macrophytes. The presence of plant prey more or less favored by *P. maculata* could either exacerbate or alleviate grazing impacts on *V. americana*. Therefore, our goals for this study are to:

1. Determine how sub-lethal levels of salinity affect the grazing rate of *P. maculata* on *V. americana*.
2. Determine how changes in water temperature, within the range experienced seasonally in the CE, influence *P. maculata* grazing rate.

3. Compare *P. maculata* grazing rates on *V. americana* to grazing rates on other SAV species in the CE watershed to determine how the biotic context of plant diversity affects the vulnerability of *V. americana*.

We hope that the knowledge gained will enable natural resources managers to incorporate environmental context in efforts to restore *V. americana* and mitigate harmful impacts of *P. maculata*.

## Methods

### *Collection and Maintenance of Organisms*

Adult *P. maculata* were collected between April and August 2014 from three sites in Lee County, FL: urban canals in Cape Coral (26.6069°N 81.9730°W and 26.6088°N 81.9725°W), a wet detention pond in Estero (26.4911°N 81.8069°W), and the freshwater Caloosahatchee River at the W.P. Franklin Campground (26.7236 °N 81.6927 °W). Snails were maintained in outdoor holding tanks covered with a mesh screen to prevent any snails from escaping. They were fed daily from cultured stocks of southern naiad (*Najas guadalupensis*), chara (*Chara* sp.), pondweed (*Potamogeton illinoensis*), and water fern (*Salvinia minima*) until the start of the experiment. The *V. americana* used for the experiment was collected from Lake Trafford (26.4219°N 81.4963°W) and an urban canal in Cape Coral (26.6088°N 81.9725°W) and was cultured in outdoor tanks on the Florida Gulf Coast University campus until needed.

### *Snail and Vegetation Measurements*

To account for possible effects of snail size on consumption rate, the operculum width of each snail was measured to the nearest mm prior to starting a feeding trial. Although there are

multiple techniques for quantifying apple snail size, operculum width is thought to be the most reliable form of measurement for live specimens (Fig. 2.1, Youens and Burks 2008).



**Figure 2.1:** *P. maculata* operculum measurement (Youens and Burks 2008).

Although food freshness is not thought to affect the survival, growth, and reproduction of *Pomacea spp.*, it can significantly alter feeding behavior (Qiu et al. 2011). Therefore, the *V. americana* used for the feeding trials was collected from culture tanks within 24 hours of the start of the feeding period. After collection, live blades were separated from roots and rhizomes, and were carefully cleaned of epiphytes, debris, and dead foliage as in Baker et al. (2010). Blade bundles of standardized wet weight were assembled and bound at one end with a binder clip to consolidate the SAV and sink it to the bottom of aquaria where snails could access it. To better standardize wet weight, blades were wetted and spun for ten seconds in a Zyliss® Easy Spin™ salad spinner before being added to bundles, and at the end of each trial. Each bundle was within  $\pm 0.01$  g of the desired wet weight at the beginning of each trial.

### *Feeding Trials*

Feeding trials were conducted inside a temperature controlled laboratory kept at 24°C, which is within *P. maculata*'s optimal temperature range for growth and consumption (Gettys et al. 2008). For each feeding trial, snails were placed in 30 x 20 x 20 cm acrylic tanks with ventilated lids. Each tank was filled with 6 L of tap water, and 2 ml of Tetra® AquaSafe Plus

Aquarium Dechlorinator Water Conditioner was added to dechlorinate and chelate any heavy metals present in the water. Tanks were allowed to sit for 24 hours before adding snails. Once the snails were added to the tanks they were starved for 48 hours to standardize gut fullness, then presented food bundles and allowed to feed for 24 hours (Baker et al. 2010). After the 24 hour feeding period all remaining plant material was removed from the tanks and reweighed in order to calculate consumption rate (Baker et al. 2010).

*Salinity and Temperature Trials-* Salinity in tanks was manipulated before snails were added, using Instant Ocean™ artificial seawater mix. Salinity trials were conducted at 0, 2, 4, 6, 8, and 10 ( $\pm 0.02$ ) psu. Each replicate in these trials tested an individual adult snail (3.2 – 6.7 cm operculum width) with 5 ( $\pm 0.01$ ) g of *V. americana*. Due to space constraints only two salinity treatments (twenty total tanks) could be tested concurrently. Therefore, each trial always included 10 replicates of a 0 psu control treatment, and 10 replicates of an elevated salinity treatment. Because there were no significant differences in snail consumption rate among matched salinity treatments in repeated trials, replicates were pooled for each salinity level (total  $n = 30$  for 2, 4, 6, 8, and 10 psu and  $n = 150$  for 0 psu) for analysis. For temperature trials, individual Hydor 50W water heaters were placed in each tank. Two different temperature ranges were tested: ambient 23-24°C, which served as the experimental control, and elevated 28-29°C, which is near *P. maculata*'s upper temperature limit for optimal growth and consumption (Gettys et al. 2008). Water temperatures were checked twice daily during feeding trials. Water heaters were adjusted accordingly if water temperatures fluctuated from their intended range. However, most water heaters were able to maintain consistent temperatures with their intended range and rarely needed to be adjusted. Because there were no significant differences among matched temperature treatments from repeated rounds of the experiments, replicates from three

rounds, each with 10 ambient and 10 elevated temperature treatments, were pooled, for a total n of 30 for each temperature treatment. Snail size and food weights used in varied-temperature feedings trials were the same as in the varied-salinity trials.

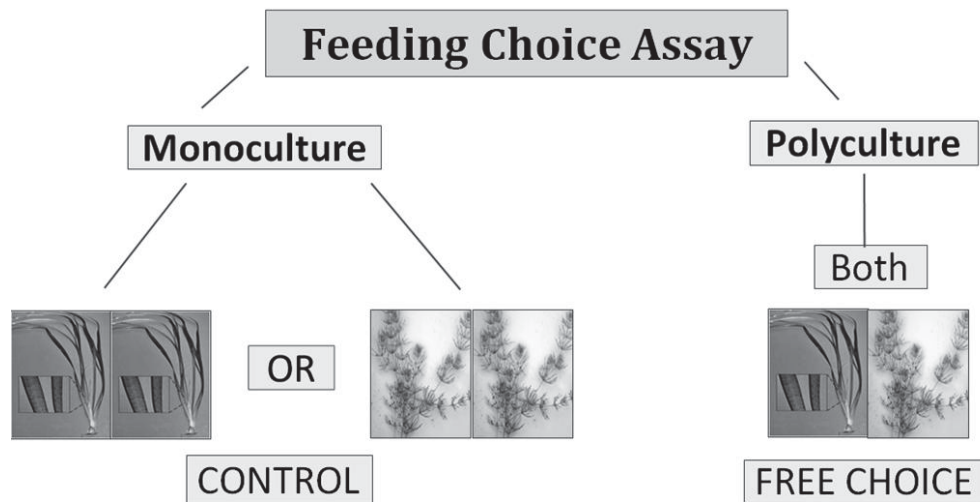
*Feeding Choice Assay*- This experiment compared *P. maculata*'s feeding rate on *V. americana* to its feeding rate on other aquatic macrophytes commonly observed at *P. maculata* and *V. americana* collection sites. The macrophyte taxa selected for comparison were the native calcareous green alga *Chara* sp., the exotic leafy SAV *Hydrilla verticillata*, and the exotic floating water hyacinth *Eichhornia crassipes*. To determine the snails' preference for each species relative to *V. americana*, feeding rate on each macrophyte was tested for that macrophyte in monoculture, and in polyculture with *V. americana* (Table 2.1).

**Table 2.1:** Macrophyte taxa and wet weights used for feeding choice experiment.

| Trial | Treatments                |                                    |  |
|-------|---------------------------|------------------------------------|--|
|       | Monoculture 1             | Monoculture 2                      | Polyculture  |
| 1     | <i>V. americana</i> (2 g) | <i>Chara</i> sp. (2 g)             | <i>V. americana</i> (1 g) and <i>Chara</i> sp. (1 g)             |
| 2     | <i>V. americana</i> (2 g) | <i>Hydrilla verticillata</i> (2 g) | <i>V. americana</i> (1 g) and <i>Hydrilla verticillata</i> (1 g) |
| 3     | <i>V. americana</i> (2 g) | <i>Eichhornia crassipes</i> (2 g)  | <i>V. americana</i> (1 g) and <i>Eichhornia crassipes</i> (1 g)  |

Because of the smaller food amounts used in these trials, we used juvenile rather than adult apple snails. Four juvenile snails between 0.4-2.0 cm operculum widths were placed in each tank for the feeding choice experiments. *P. maculata* is reported to reach sexual maturity at 2.5 cm which is why we chose 2.0 cm to be our upper size limit for juvenile snails used in the experiment (Ostrom and Chesnes 2014). Each trial consisted of three treatments; a monoculture of *V. americana*, a monoculture of another macrophyte species, and a polyculture treatment containing both macrophyte species, giving snails a choice between food sources (Fig. 2.2,

Bernatis 2014). For monoculture trials snails were given  $2 \pm 0.01$  g of food. For polyculture trials snails were given  $1 \pm 0.01$  g of each macrophyte species for a total food mass equal to that of monocultures. Each unique treatment was replicated five times, with the exception of the *V. americana* monoculture treatment, which was included in each trial and therefore replicated a total of 15 times.



**Figure 2.2:** Feeding choice assay experimental design.

### *Data Analysis*

For the salinity and temperature trials grazing rate was normalized to snail size by dividing the wet mass of *V. americana* consumed over 24 hours by the operculum diameter of the snail, resulting in units of g vegetation consumed \* snail operculum width  $\text{cm}^{-1}$  \* day $^{-1}$  (Youens and Burks 2008, Baker et al. 2010, Bernatis 2014). For both temperature and salinity trials, treatment differences in grazing rate were assessed with One-Way ANOVA. Prior to the ANOVA, a Goodness-of-Fit test was used to test for normal distribution. Homogeneity of variance was also tested by running O'Brien[.5], Brown-Forsythe, Levene, and Bartlett tests. The

data was square-root transformed appropriately if needed. ANOVA was followed with a post-hoc Tukey HSD pairwise comparison (Zar 1996).

For the feeding choice assay the response variable was based on the total proportion of each macrophyte taxa consumed, determined by dividing the amount consumed during the 24 hour trial by the initial wet weight. Using this type of response variable allowed statistical comparison between polyculture and monoculture treatments, which had the same total macrophyte weight but different weights for each individual species. The response variable was also normalized to account for snail size. We calculated the total normalized rate of consumption of each macrophyte taxa (NRC, Bernatis 2014) using a modified formula from Bracken et al. (2014).

$$\text{NRC} = [(M_f - M_i) * M_i^{-1} * S_o^{-1} * d^{-1}]$$

$M_f$  was the final SAV mass,  $M_i$  was the initial SAV mass,  $S_o$  was summed operculum size of the four snails, and  $d$  was number of days. The NRC was therefore expressed in units of  $\text{g} \cdot \text{cm}^{-1} \cdot \text{day}^{-1}$ . For each trial, a Two-Way ANOVA was performed to assess how NRC varied by macrophyte species (*V. americana* vs. other) and by plant diversity treatment (monoculture vs. polyculture). As with the One-Way ANOVA for the salinity and temperature experiments, Goodness-of-Fit tests, O'Brien[.5], Brown-Forsythe, Levene, Bartlett tests, and Tukey HSD pairwise comparisons were performed for the feeding choice analyses (Zar 1996).

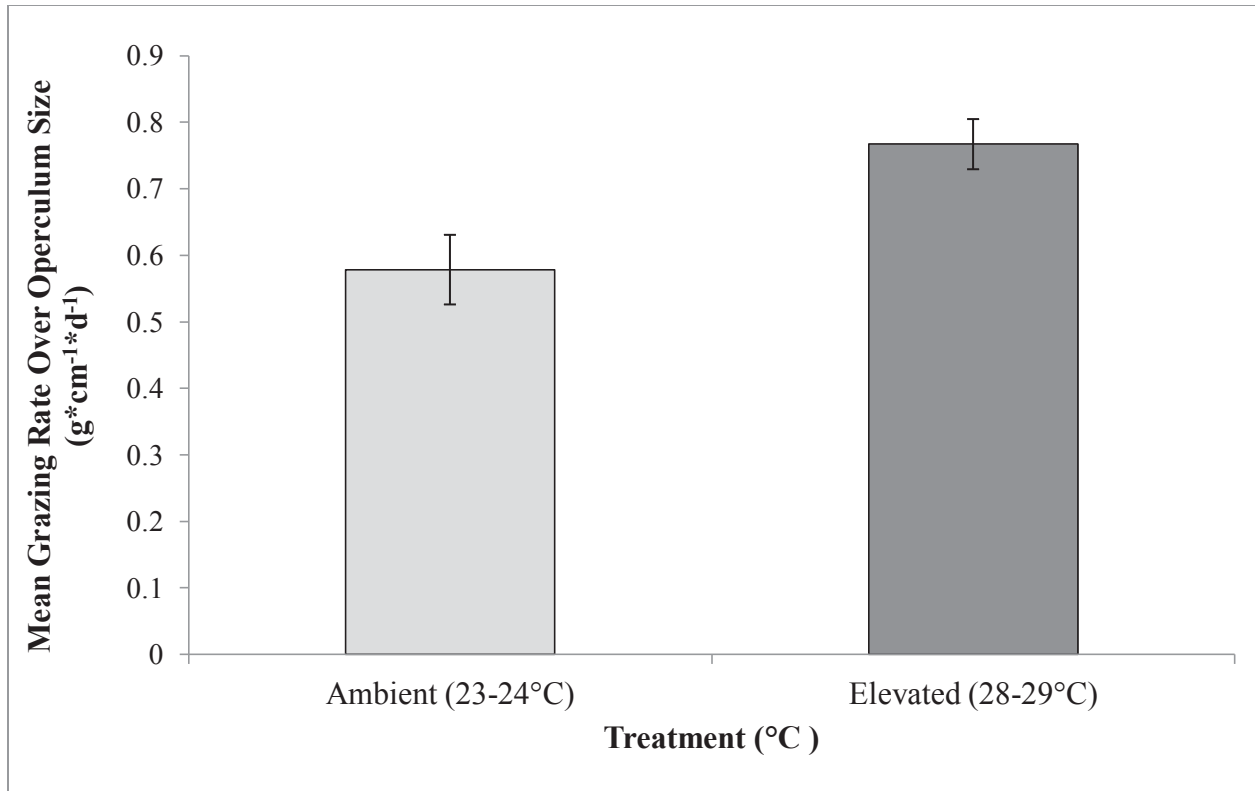
## Results

*Effects of Salinity and Temperature on Grazing-* The mean grazing rate of *Pomacea maculata* at 0 psu and ambient temperature (23-24°C) in the salinity trials was 0.56 g wet weight *Vallisneria americana* consumed \* cm<sup>-1</sup> snail operculum width \* day<sup>-1</sup>. As expected, this was virtually identical to the grazing rate of the ambient temperature control group in the temperature trials (0.57 g\*cm<sup>-1</sup>\*day<sup>-1</sup>). Elevated temperatures (28-29°C) increased grazing rate by 32%, on average, to 0.76 g\*cm<sup>-1</sup>\*day<sup>-1</sup> (ANOVA, p = 0.0049, Fig. 2.3). Increases in salinity decreased *P. maculata* grazing rate (ANOVA, p < 0.0001). At 10 psu the grazing rate of *P. maculata* was negligible, < 0.01 g\*cm<sup>-1</sup>\*day<sup>-1</sup>. Based on the Tukey HSD letters report, both 0 psu and 10 psu were statistically different from all other treatments. There was no significant difference between 2 and 4 psu or between 6 and 8 psu, but a general trend of decreasing grazing rate with increasing salinity was clear (Fig. 2.4).

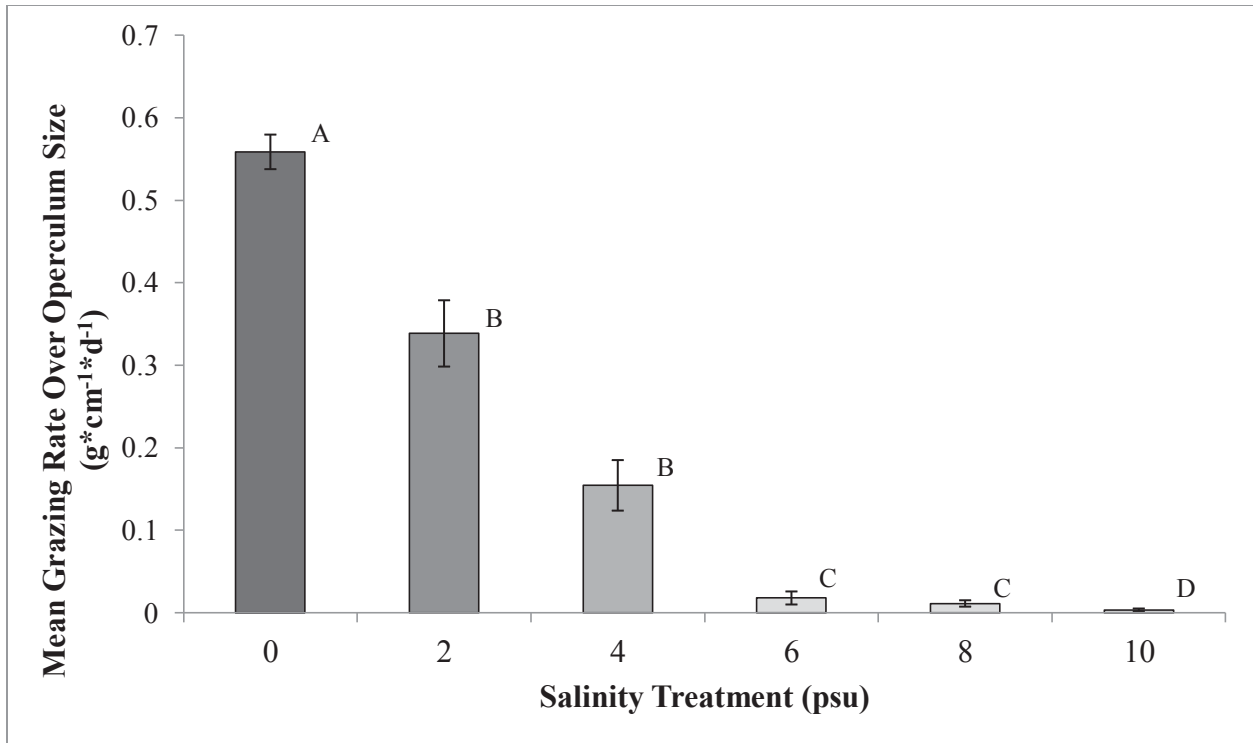
*Feeding Choice Assay-* In all polyculture treatments, snails consumed more *V. americana* than the other macrophyte presented, indicating that *V. americana* was the most preferred food source of those presented in this experiment (Table 2.2). However, differences in proportion consumed between *V. americana* monocultures and monocultures of the other macrophyte species were significant only for *Eichornia crassipes*, which the snails generally avoided even when it was the only food choice. In the *V. americana* vs. *E. crassipes* trial there was a significant interaction between plant diversity (monoculture vs. polyculture treatments) and plant species (Two-Way ANOVA square root transform, p < 0.0001), because *P. maculata* consumed a much greater proportion of *V. americana* under polyculture conditions than when *V. americana* was presented without *E. crassipes* (Fig. 2.5). There was also an interactive effect of plant diversity and plant species in the *V. americana* vs. *Chara* sp. trial (Two-Way ANOVA , p =



0.0185). Under polyculture conditions *P. maculata* ate more *V. americana* than *Chara* sp., whereas the proportions consumed in monocultures did not differ (Fig 2.6). In the *V. americana* vs. *H. verticillata* trial there was no interactive effect of plant diversity and plant species (Fig. 2.7) but the plant diversity effect itself was significant (Two-Way ANOVA ,  $p = 0.0067$ ), because *P. maculata* consumed more *H. verticillata* when it was the only species present.



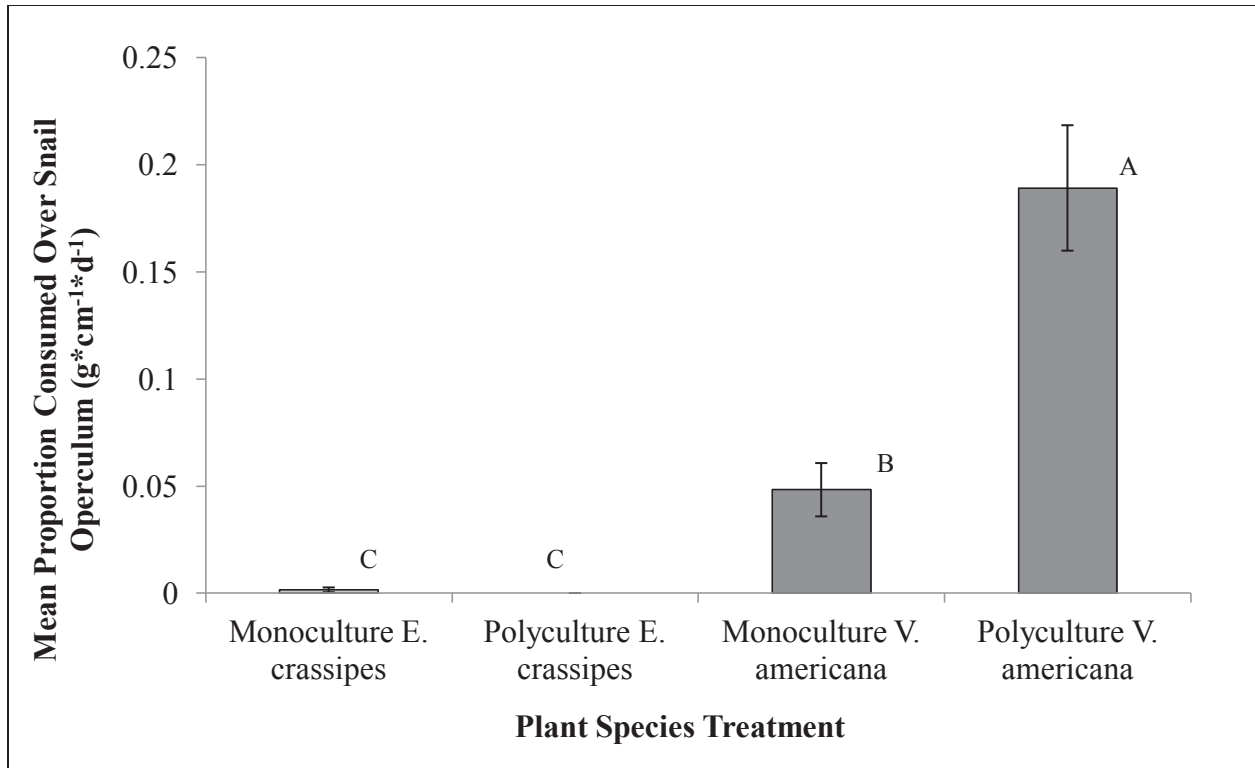
**Figure 2.3:** *Pomacea maculata* grazing rate on *Vallisneria americana* at ambient vs elevated temperature. Grazing rate is expressed as g *V. americana* wet mass consumed over 24 hours, normalized to the operculum size of the snail in the feeding trial (see text). Temperature increased grazing rate (ANOVA,  $p = 0.0049$ ). Error bars depict standard error of the mean (SEM).



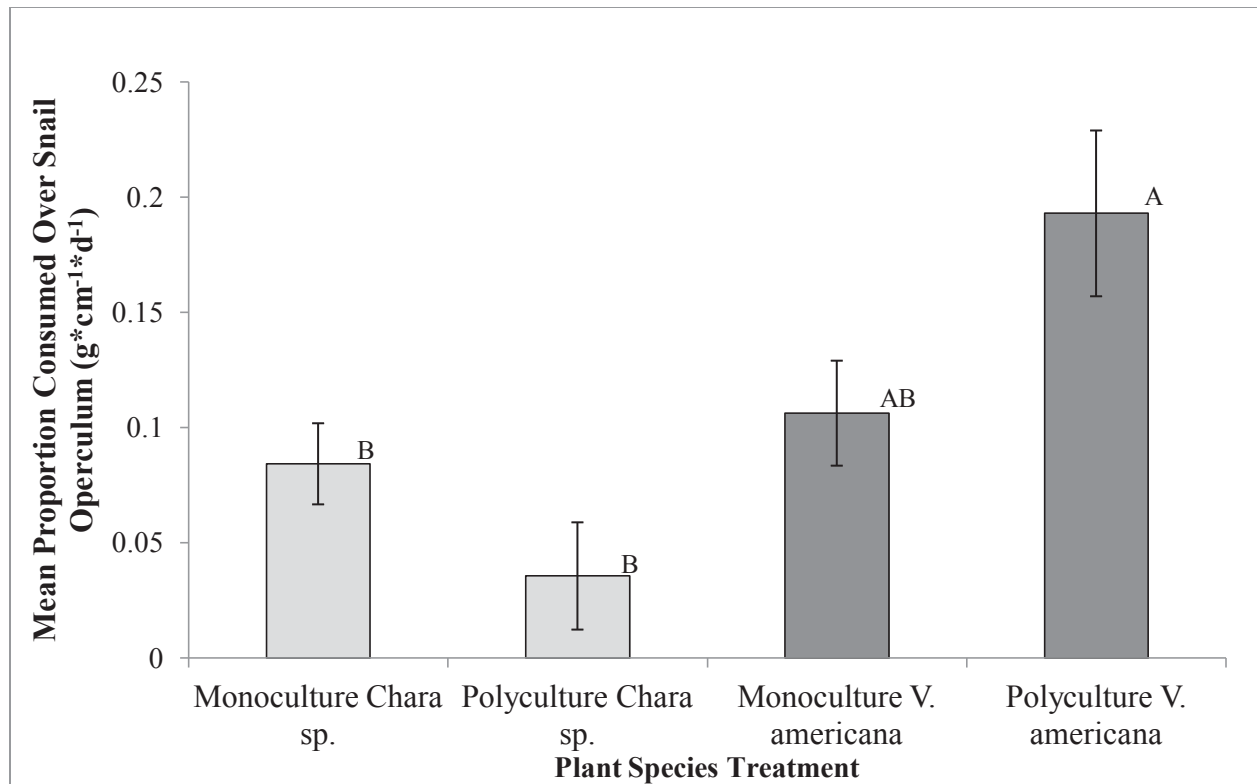
**Figure 2.4:** *Pomacea maculata* grazing rate on *Vallisneria americana* at different salinities. Grazing rate is expressed as g *V. americana* wet mass consumed over 24 hours, normalized to the operculum size of the snail in the feeding trial (see text). Salinity has a significant effect on grazing rate (ANOVA log10 transform,  $p = <0.0001$ ). Letters indicate significantly different groups. Error bars depict standard error of the mean (SEM).

**Table 2.2:** Normalized rate of consumption (NRC) of macrophytes by *P. maculata* for feeding choice experiments. The response variable was normalized to account for snail size using a modified formula from Bracken et al. (2014). The rate was calculated as total grams consumed per snail (size) per day.

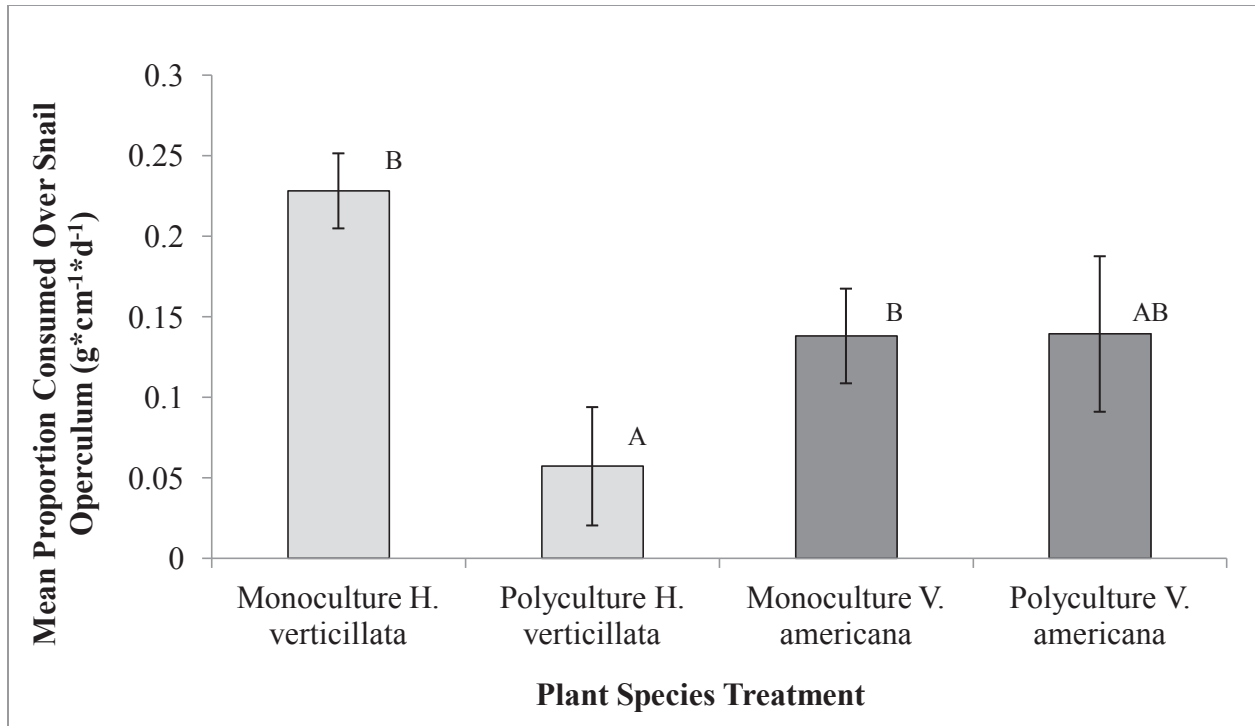
| Trial | Treatment   | Macrophyte Species     | NRC (g*cm <sup>-1</sup> *d <sup>-1</sup> ) | Tukey HSD Letter Report |
|-------|-------------|------------------------|--|-------------------------|
| 1     | Monoculture | <i>V. americana</i>    | 0.05                                       | B                       |
|       |             | <i>E. crassipes</i>    | 0.00                                       | C                       |
|       | Polyculture | <i>V. americana</i>    | 0.19                                       | A                       |
|       |             | <i>E. crassipes</i>    | 0.00                                       | C                       |
| 3     | Monoculture | <i>V. americana</i>    | 0.05                                       | AB                      |
|       |             | <i>Chara</i> sp.       | 0.04                                       | B                       |
|       | Polyculture | <i>V. americana</i>    | 0.08                                       | A                       |
|       |             | <i>Chara</i> sp.       | 0.05                                       | B                       |
| 2     | Monoculture | <i>V. americana</i>    | 0.14                                       | B                       |
|       |             | <i>H. verticillata</i> | 0.23                                       | B                       |
|       | Polyculture | <i>V. americana</i>    | 0.14                                       | AB                      |
|       |             | <i>H. verticillata</i> | 0.06                                       | A                       |



**Figure 2.5:** Normalized rate of consumption (NRC) of *E. crassipes* and *V. americana* by *P. maculata* for each macrophyte species presented in monoculture and in polyculture (Two-Way ANOVA square root transform,  $p < 0.0001$ ). Based on the Tukey HSD, snails ate significantly more *V. americana* under polyculture conditions in comparison to *V. americana* under monoculture conditions and *E. crassipes* under both polyculture and monoculture conditions. Letters indicate significantly different groups. Error bars depict standard error of the mean (SEM).



**Figure 2.6:** Normalized rate of consumption (NRC) of *Chara* sp. and *V. americana* by *P. maculata* for each macrophyte species presented in monoculture and in polyculture (Two-Way ANOVA,  $p = 0.0185$ ). Based on the Tukey HSD, snails consumed more *V. americana* under polyculture conditions compared to *Chara* sp. under monoculture and polyculture conditions. There was no difference in the amount of *V. americana* eaten under monoculture conditions compared to other treatments. Letters indicate significantly different groups. Error bars depict standard error of the mean (SEM).



**Figure 2.7:** Normalized rate of consumption (NRC) of *H. verticillata* and *V. americana* by *P. maculata* for each macrophyte species presented in monoculture and in polyculture. There was no interactive effect but plant diversity effect was significant (Two-Way ANOVA,  $p = 0.0067$ ). Based on the Tukey HSD, snails ate more *H. verticillata* under monoculture conditions compared to polyculture conditions. There was no difference between the amount of *H. verticillata* or *V. americana* eaten under polyculture conditions. Letters indicate significantly different groups. Error bars depict standard error of the mean (SEM).

## Discussion

We found that all aspects of environmental context that we examined; water temperature, salinity, and plant community context, significantly affected *Pomacea maculata* grazing rate on *Vallisneria americana*. Increasing water temperature increased grazing rate and increasing salinity decreased grazing rate. Presenting *V. americana* along with other macrophyte species generally increased grazing pressure on *V. americana* because *V. americana* was a preferred food source.

We acknowledge several limitations in our experiments. For the temperature trials we were did not test the effect of temperatures lower than 23-24°C or higher than 28-29°C on the grazing rate of *P. maculata* on *V. americana*. Gettys et al. (2008) found that the grazing and growth rates of *P. maculata* are generally lower at cooler temperatures, and in some cases the snails stop grazing at temperatures <18°C. For the feeding choice trials we tested only a subset of the macrophyte species that might co-occur with *V. americana* and *P. maculata*. Ideally, a larger variety of alternative SAV species would have been tested. Based on Baker et al. (2010) we know that there are many other native and nonnative Florida plant species that the snail will consume readily. Also, caution should be applied in extending the results of these laboratory studies to the interpretation of ecological interactions in the field. In field situations it is possible that the snails will exhibit a wider variety of behavioral responses to stressors such as salinity and temperature (Carlsson et al. 2004), and the community-level interactions between *P. maculata* populations and macrophyte populations may be more complex than the interactions we observed at the level of individual organisms. Therefore, it would be beneficial to run addition experiments under more realistic mesocosm or field settings.



Results of the temperature trial suggest that the grazing rate of *P. maculata* on *V. americana* significantly increased from moderate (23-24°C) to high (28-29°C) water temperatures. This finding builds on the results of Gettys et al. (2008), who found higher grazing rates on *H. verticillata* at 20-35°C than at 15°C but who did not resolve grazing rate differences within the 20-35°C range. The mean temperature of the upper Caloosahatchee Estuary (CE) from November 2012 – November 2014 was 27°C, with a winter minimum of 17°C and a summer maximum of 34°C (Sanibel Captiva Conservation Foundation 2015). This suggests that temperature conditions in the Caloosahatchee are generally within *P. maculata*'s optimal range for consumption of macrophytes, although consumption should occur at a somewhat reduced rate in winter. This suggests that the snail is able to graze at a high or very high rate for most of the year. Further study is needed to determine the extent to which *P. maculata* grazing rate is reduced by typical Caloosahatchee winter temperatures, but our results and those of Gettys et al. (2008) suggest that winter may be the more appropriate time to initiate *V. americana* restoration plantings or other macrophyte conservation actions vulnerable to *P. maculata* grazing.

Increases in salinity significantly reduced the grazing rate of *P. maculata*. The highest rate of grazing occurred at 0 psu, significantly reduced grazing occurred from 2 – 4 psu, and minimal grazing was observed above 6 psu. These results suggest that low levels of salinity (2 – 6 psu) in estuarine *V. americana* habitats could help suppress *P. maculata* grazing. Although the snails survived 72 hours at 10 psu, very little grazing occurred, and other signs of stress were observed, with some snails keeping their opercula shut for the duration of the experiment. In comparison with the results of similar experiments on the salinity tolerance of *P. maculata* (Ramakrishnan 2007, Martin and Valentine 2014, and Bernatis 2014), our results suggest that

although salinities < 10 psu are not acutely lethal for *P. maculata*, the snails' destructiveness is significantly reduced even at quite low salinity levels.

The results of the feeding choice trials show that *V. americana* is a preferred food source for *P. maculata* and that the grazing pressure on *V. americana* is likely to be enhanced rather than relieved by the presence of other macrophyte species. However, the macrophytes we examined do not represent the full range of possible food sources for *P. maculata*. In addition to other plant and macroalgae species, benthic microalgae and detritus may be a large part of the diet for *P. maculata*. Farther study is needed on a wider range of alternative *P. maculata* food sources to determine if producer community context can be altered to alleviate *P. maculata* grazing pressure on *V. americana*. Additional consideration should be given to the question of whether the presence of a more-preferred food source than *V. americana* would actually relieve grazer pressure on *V. americana*, or if it would enhance grazing pressure by increasing populations of *P. maculata* (apparent competition, *sensu* Conner 2008, Morrison and Hay 2011).

It may be possible to use salinity to help control or eradicate *P. maculata* populations within sea-level coastal waterways where flow and salinity are subject to manipulation by human managers, e.g., in the urban canals of Cape Coral. The fresh and saltwater canals are separated by weir systems. If the weirs were occasionally opened to allow salt water into the canals it might be possible to control the snail populations and allow for *V. americana* to recover in areas currently experiencing high herbivory stress. A possible additional benefit of opening weirs would be increasing habitat connectivity for euryhaline migratory fishes and for and blue crabs, *Callinectes sapidus*; shell-crushing predators that might also help control *P. maculata*. However, further study is needed to better predict the likely impacts of such management. Most importantly, the direct negative effects of increased salinity on *V. americana* should be weighed

against the indirect positive effects of the grazing reduction afforded by increased salinity. The outcome of this tradeoff could be assessed through mesocosm studies with live, growing *V. americana*, and through field surveys of *V. americana* and *P. maculata* occurrence along spatial or temporal gradients of salinity.

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### **Chapter 3: Mesocosm experiments evaluating the potential for estuaries to serve as refugia for the freshwater macrophyte *Vallisneria americana* from the invasive snail *Pomacea maculata*.**

#### **Abstract**

Beds of submerged aquatic vegetation (SAV) have numerous environmentally beneficial roles such as providing habitat, food, reducing erosion, and oxygenating surrounding water. Unfortunately beds of SAV have been declining worldwide as a result of being exposed to various stressors. Our research focuses on herbivory stress of the invasive freshwater gastropod *Pomacea maculata* and estuarine salinity stress on *Vallisneria americana* populations. Tapegrass, *V. americana*, is an important habitat-forming species in freshwater and in the oligohaline regions of estuaries. Loss of *V. americana* can have a number of negative impacts on the health of estuaries and potentially lead to trophic cascades. There is a need to better understand the impact and role of multiple stressors in determining the growth and health of *V. americana* populations. In this study, we manipulated salinity and snail herbivory in *V. americana* mesocosms and assessed the growth and survival of plants and snails. Snails reduced plant performance, and increasing salinity reduced both snail and plant performance. However, there was a non-additive, antagonistic effect the two stressors on *V. americana*; when snails were present, *V. americana* performed best at non-zero salinities, suggesting that low-salinity estuarine habitats may act as a refuge for *V. americana* from *P. maculata* herbivory. These results also suggest that strategic manipulation of flow and salinity could help control *P. maculata* in coastal watersheds.

## Introduction

Estuarine ecosystems are hotspots of biological productivity and biogeochemical cycling at the nexus of aquatic, terrestrial, and marine habitats, and as such they provide valuable goods and services to humans (Barnes 2005, Barbier et al. 2011). Submerged aquatic vegetation (SAV) is an important habitat feature in many estuaries, which enhances the productivity, diversity, and ecosystem functions of these environments (Wigand et al. 2000). Unfortunately, SAV is declining worldwide as estuaries and coasts are subjected increasing anthropogenic stressors, including altered shorelines and hydrology, reduced water quality, overfishing, and non-native species introductions (Orth et al. 2006).

There is a need to better understand the impact of multiple stressors and the role they play in regards to the overall health of estuarine SAV populations. Our research specifically focuses on the role of *Pomacea maculata* herbivory and salinity stress on a foundational SAV species, *Vallisneria americana* in Southwest Florida. Although our study site is focused on a Florida estuary, our research and findings can be applied any estuary that may have *V. americana* and *P. maculata* present.

The Caloosahatchee Estuary (CE), is a drowned river valley located at 26.63° N 81.85° W along the southwest coast of Florida, USA (Antonini et al. 2002). It extends 42 km from oligohaline waters near the S-79 lock and dam to the marine waters of the Gulf of Mexico (Scarlatos 1988, Xia et al. 2010). The CE supports many recreationally and commercially important fisheries species such as blue crab *Callinectes sapidus*, snook *Centropomus undecimalis*, spotted sea trout *Cynoscion nebulosus*, and red drum *Sciaenops ocellatus*. It also harbors federally endangered megafauna such as the smalltooth sawfish *Pristis pectinata* and the Florida manatee *Trichechus manatus latirostris* (Scarlatos 1988, Doering et al. 2002, Barnes



2005). These valued species are directly and indirectly supported by SAV (Ward 1984, Doering et al. 2002, Barnes 2005).

The CE supports four species of bed-forming SAV: the seagrasses *Thalassia testudinum* and *Halodule wrightii*, the euryhaline SAV *Ruppia maritima*, and the moderately salt-tolerant freshwater plant *Vallisneria americana* (Barnes 2005, Stevens et al. 2010). *V. americana* (tapegrass) was historically dominant in fresh and oligohaline regions of the Caloosahatchee, where salinities generally remained below 10 psu (Scarlatos 1988, Doering et al. 1999, Wigand et al. 2000). However, decades of hydrologic alterations in the CE watershed leading to increased temporal variability in salinity had largely eliminated the *V. americana* beds by the early 2000s (Doering et al. 1999, Barnes 2005, Douglass 2014). The introduction of an invasive herbivore, *Pomacea maculata*, in the 1990s may also have contributed to the loss of *V. americana*.

*P. maculata* is an aquatic gastropod in the Ampullariidae family and is native to warm temperate and tropical South America (Conner et al. 2008). *P. maculata* is infamous for its high reproduction and herbivory rates, displacing and outcompeting the native Florida apple snail (*Pomacea paludosa*), and feeding predominately on macrophytes (Sharfstein and Steinman 2001, Conner et al. 2008, Burks et al. 2010, Horgan et al. 2014). Ceilley et al. (2013) documented overgrazing and depletion of *V. americana* in the freshwater portion of the Caloosahatchee River where *P. maculata* is abundant (Ceilley et al. 2013). We speculate that overgrazing of *V. americana* in the freshwater Caloosahatchee has reduced the downstream transport of seeds, decreasing the recovery rate of estuarine *V. americana* beds after salinity-related dieoffs. However it is uncertain whether or not *P. maculata* can penetrate far enough into the estuary to directly harm *V. americana* there. The reported salinity tolerances of *P. maculata* (Table 3.1) do

overlap with those of *V. americana* (Table 3.2). Yet, because sub-lethal effects of salinity likely affect both organisms in this plant-herbivore system, more information is needed to predict the outcome of their interaction in the oligohaline estuary. The goal of this study is to determine the separate and combined effects of salinity and herbivory on *V. americana* growth and survivorship in a mesocosm setting. We hope to assess the likelihood of harm to estuarine populations of *V. americana* by *P. maculata*, and identify salinity regimes and management strategies that could minimize that harm.

**Table 3.1:** Reported environmental tolerances of *Pomacea maculata*

| Factor      | Range of Tolerance          | Optimal Range                                 | Study                                   |
|-------------|-----------------------------|---|---|
| Temperature | Lower limit: 6°C<br>15-35°C | -<br>Consumption: 20-35°C;<br>Growth: 20-30°C | Byers et al. 2013<br>Gettys et al. 2008 |
|             | 15.2-36.6°C                 | 20-30°C                                       | Ramakrishnan 2007                       |
| Salinity    | 0-8.0 psu                   | 0- 8.0 psu                                    | Bernatis 2014                           |
|             | 0-10 psu                    | -   | McAskill and Douglass, <i>in prep</i>   |
|             | 0-15.0 psu                  | 0-5 psu                                       | Martin and Valentine 2014               |
|             | 0-13.6 psu                  | 0-6.8 psu                                     | Ramakrishnan 2007                       |
| pH          | 5.5-9.5                     | 7.5-9.5                                       | Bernatis 2014                           |
|             | 4.0-10.5                    | 7.0-9.0                                       | Ramakrishnan 2007                       |

**Table 3.2:** Reported environmental tolerances of *Vallisneria americana*

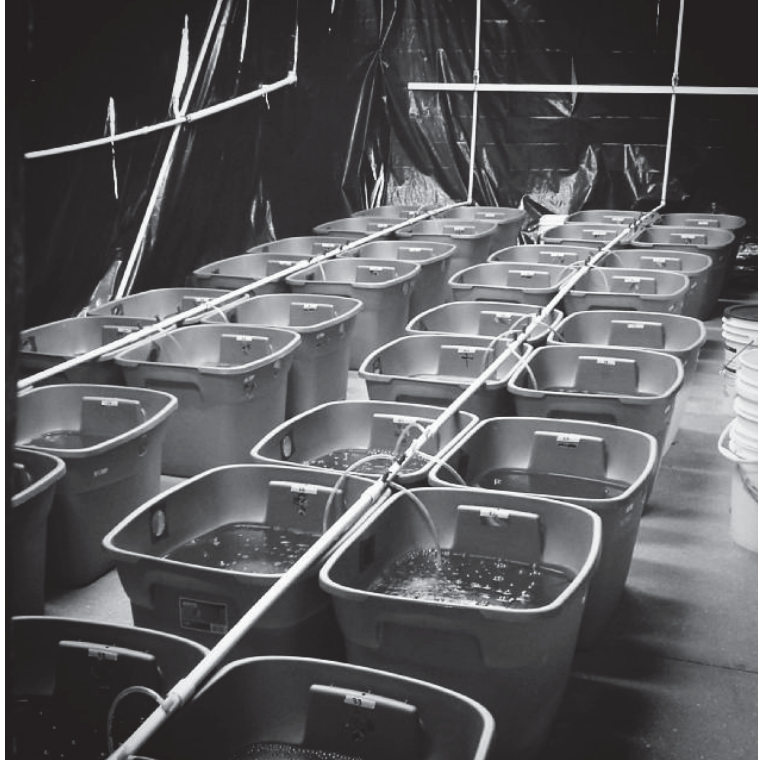
| Environmental Factor | Range of Tolerance | Optimal Range | Study                 |
|----------------------|--------------------|---------------|-----------------------|
| Temperature          | 13-38°C            | 28°C          | Bartleson et al. 2014 |
| Salinity             | 0-15 psu           | 0-3 psu       | Doering et al. 1999   |

## Methods

### *Mesocosm Experiment 1*

The experiment used a random block design with four levels of salinity (0, 2, 4, and 6 psu) crossed with two levels of herbivory (no snails present, four juvenile snails present), resulting in eight unique treatments. Each treatment was replicated five times for a total of 40 mesocosms. The sub-lethal salinity range chosen for this experiment was based on the results of Ramakrishnan (2007) who reported significant mortality of *P. maculata* at 6.8 psu.

*Vallisneria americana* for the experiment was collected from a freshwater lake in southwest Florida, Lake Trafford (26.4219°N 81.4963°W). The *V. americana* was planted in outdoor culture tanks on the Florida Gulf Coast University campus and propagated for three months there before being replanted in experimental mesocosms at the Vester Marine Field Station. Mesocosm tanks were 68 L, 60.96 x 46.67 x 40.01 cm (Fig 3.1) and were filled to 7 cm depth with clean sand and 30 cm depth with water. The mesocosm array was set up in a covered but non-air-conditioned area. Black plastic sheeting was hung around the array to minimize exposure to outside light, and overhead full spectrum florescent lights were set on a timer to simulate a 12-hour day/night cycle. The tanks were filled with tap water and then dechlorinated using 15 ml per tank of Tetra® AquaSafe Plus Aquarium Dechlorinator Water Conditioner. Air stones were added to each tank to oxygenate and circulate the water.



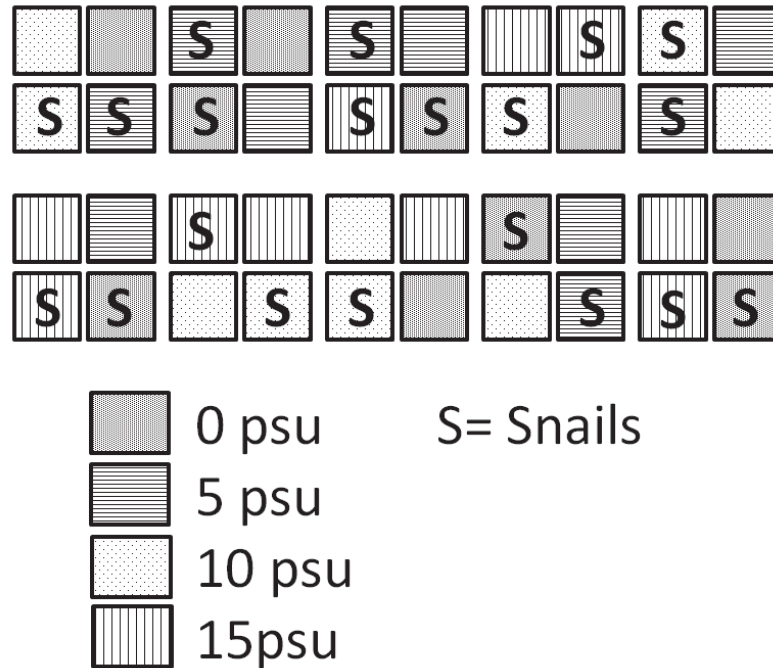
**Figure 3.1:** Mesocosm array.

Mesocosms were planted with *V. americana* on May 28, 2014. Each shoot planted was cleaned of epiphytes, debris, and dead foliage and weighed using a salad spinner as in Baker et al. (2010). All mesocosms received seven shoots of *V. americana* for a total wet biomass of 16.0-18.0 g per tank. The shoots were allowed a one week transplant acclimation period before snails were added and salinity was elevated. Prior to adding *P. maculata* the operculum width of each snail was measured to the nearest mm (Youens and Burks 2008). A total of four juvenile *P. maculata* with operculum width between 5-7 mm and total wet biomass of 0.9-1.1 g were placed in each +herbivory treatment mesocosm. The experiment ran for four weeks after snails were added, after which data were collected and analyzed as described below for mesocosm experiment 2.

## *Mesocosm Experiment 2*

The design of the second mesocosm experiment was similar to that of the first, but the experimental conditions were changed slightly to improve realism and minimize confounding factors. Since all treatments in the first mesocosm experiment experienced some reduction in *V. americana* biomass, we doubled light intensity in the second experiment to provide a better growth environment. Also, one week before the shoots were planted, 10 ml of Aqueon® Freshwater Aquarium Plant Food and 5 ml of Microbe-Lift Special Blend Aquarium Bacteria were added to each mesocosm to help promote nutrient cycling. Larger *V. americana* shoots were used, and we allowed the transplanted shoots a longer period (three weeks) to acclimate to mesocosms before salinity and herbivory treatments were initiated. The source of *V. americana* was also changed, due to concerns about alligator activity in the harvesting area at Lake Trafford. Plants were instead collected from an urban canal in Cape Coral (26.6088°N 81.9725°W) and transplanted into a outdoor culture tank at the Vester Marine Field Station at the beginning of July 2014. Shoots of *V. americana* were harvested from the culture tank and transplanted into individual mesocosms on August 18, 2014. In addition, at the start of the experiment ten, 20 x 0.7 cm artificial seagrass ribbons were planted in each tank to quantify any epiphytic algae growth that occurred. Two algae ribbons were collected every two weeks from each mesocosm and analyzed for chlorophyll *a* concentration using spectrophotometric methods (Blake and Duffy 2010).

Levels of the salinity treatment were also altered, to 0, 5, 10, and 15 psu (Fig. 3.2). These levels were based on the results of Martin and Valentine (2014), who reported the optimal range of *P. maculata* between 0-5 psu but also suggested that the snail could tolerate up to 15 psu.



**Figure 3.2:** Mesocosm array schematic with treatment designations showing blocked design.

**Table 3.3:** Summary of experimental design and conditions for mesocosm experiments.

|                                     | Experiment 1   | Experiment 2  |
|-------------------------------------|--|---|
| Levels of salinity factor           | 0,2,4,6  | 0,5,10,15   |
| Levels of snail factor              | 0 snails, 4 juvenile snails  | 0 snails, 4 juvenile snails   |
| <i>Vallisneria</i> shoot source     | Lake Trafford  | Cape Coral  |
| <i>Vallisneria</i> shoots per tank  | 7  | 6   |
| <i>Vallisneria</i> biomass per tank | 16.0-18.0 g  | 59.5-65.0 g   |
| Mean snail wet weight               | 0.9-1.1 g  | 1.9-2.6 g   |
| Mean snail operculum width          | 5-7 mm   | 4-10 mm   |
| Water treatments                    | 15 ml Tetra® AquaSafe Plus Aquarium Dechlorinator Water Conditioner                              | 15 ml of Tetra® AquaSafe Plus Aquarium Dechlorinator Water Conditioner<br>10 ml of Aqueon® Freshwater Aquarium Plant Food<br>5 ml of Microbe-Lift Special Blend Aquarium Bacteria |
| Dates run (dd/mm/yy)                | 15/6/14-1/7/14   | 8/9/14- 8/10/14   |
| Salinity ramp-up                    | None   | 2.5 psu/day   |
| Data collected                      | Salinity- every two days<br>Temperature- weekly<br>Snail mortality- daily<br>Shoot number- daily | Salinity- daily<br>Temperature- daily<br>Snail mortality- daily<br>Shoot number- daily<br>Epiphytes- biweekly<br>pH- biweekly   |

Shoot cleaning and wet weight were handled as in mesocosm experiment 1 (Baker et al. 2010). Six shoots of *V. americana* were placed into each mesocosm, but because these shoots were larger than those used in the first experiment, initial total shoot biomass for each mesocosm ranged from 59.5-65.0g. As in experiment 1, a total of four juvenile *P. maculata* were placed in each +herbivory treatment. Individual snail size ranged from 4-10 mm operculum width and initial total snail wet biomass per tank ranged from 1.9-2.6 g, approximately double the snail biomass of the first experiment.

After the three-week acclimation period for *V. americana* plantings, snails were added and salinity within each tank was increased by 2.5 psu per day until salinity had reached its intended value (i.e., 5 psu, 10 psu, 15 psu). The mesocosm experiment ran a total of four weeks. Both salinity and temperature of each mesocosm was checked and recorded on a daily basis. Salinities in each mesocosm were adjusted if needed to ensure that they remained within  $\pm 0.2$  of their intended salinity throughout the experiment.

At the end of the four-week period, snails and remaining *V. americana* shoots were collected from each tank. *V. americana* material was separated into shoots, roots, detached floating blades, and detrital blades. The sand in each tank was sieved (0.290 mm mesh size) to ensure that all below ground plant material was collected. All organisms were placed immediately on ice and brought back to the laboratory for further analysis. After recording the final wet weights of snails and *V. americana*, specimens were placed in the drying oven at 60°C for a minimum of 24hrs. Their dry weights were recorded and the organisms were then placed in a muffle furnace at 450°C for 5 hours to allow determination of ash-free dry weight (AFDW).

### *Statistical Analyses*

Statistical analyses were done similarly for both mesocosm experiments. All data were tested for normal distribution using a Goodness-of-Fit test and homogeneity of variance using O'Brien[.5], Brown-Forsythe, Levene, and Bartlett tests, and were transformed if necessary to meet the assumptions for ANOVA. One-way ANOVA was used to assess salinity treatment effects on snail response variables including final snail weight and operculum width. A nominal logistic regression was used to compare the interactive effect of time (weeks since snail addition) and salinity treatment (psu) on cumulative snail mortality. Two-way ANOVA was used to compare the interactive effects of salinity treatment and snail presence on *V. americana* responses including: the remaining number of shoots at the end of the experiment, above- and below-ground wet, dry, and ash free dry biomass, and the amounts of floating and detrital plant material per tank. Two-way ANOVA was also used to analyze epiphyte accumulation on artificial seagrass substrates. ANOVA tests were followed by post-hoc Tukey HSD pairwise comparison (Zar 1996).

## **Results**

### *Mesocosm Experiment 1*

Mean temperature for all tanks throughout the experiment was 25.19°C and salinity treatments remained within +/- 0.2 of their intended levels. Salinity treatment significantly affected snail mortality (Nominal Logistic Regression,  $p < 0.0001$ ). No snail deaths occurred within 0, 2, or 4 psu treatments but 20% of snails died after four weeks in 6 psu treatments (Fig. 3.3). Salinity significantly impacted snail growth over the four week period ( $p = 0.0290$  for final operculum width,  $p = 0.0194$  for final ash free dry weight). Based on the Tukey HSD, snail



operculum were significantly larger in 0 psu treatments than in 6 psu treatments at the end of the experiment, and snail biomass was significantly higher at 0 psu than at 4 or 6 psu (Table 3.4).

All treatments experienced some decline in *V. americana* biomass during this four week experiment, but there were significant differences among treatments in final biomass and number of shoots. The number of live shoots remaining after four weeks was significantly lower in elevated salinity treatments (Two-Way ANOVA,  $p = 0.0230$ ), and a Tukey HSD test for the main effect of salinity showed that 4 and 6 psu treatments had significantly fewer shoots remaining than 0 and 2 psu treatments. Remaining shoot number was also strongly reduced by snail presence (Two-Way ANOVA,  $p < 0.0001$ ). Further, there was a significant interactive effect of salinity treatment and snail presence on the number of remaining shoots (Two-Way ANOVA,  $p = 0.0039$ ). The Tukey HSD for the interactive effect showed that in treatments with snails, significantly more *V. americana* shoots remained at 6 psu than at 0 psu (Fig. 3.4).

Snail presence and elevated salinity tended to reduce belowground *V. americana* biomass, but these effects were marginally non-significant for ash free dry root biomass (Table 3.5). Aboveground *V. americana* biomass was significantly reduced by snails but not significantly affected by salinity treatment (Table 3.5, Fig. 3.5). The interactive effect of snail presence and salinity was also non-significant, although there was a trend that 6 psu salinity treatments had higher remaining aboveground biomass than lower salinity treatments when snails were present (Fig 3.5).

## *Mesocosm Experiment 2*

Mean temperature for all tanks throughout the experiment was 25.3°C (SD = 1.34°C). Salinity treatments remained within +/- 0.2 of their intended levels. The mean pH for all mesocosms was 8.3 (SD = 0.12). Salinity treatment significantly affected total snail survivorship (Nominal Logistic Regression,  $p < 0.0001$ ). No snail deaths occurred in 0 psu or 5 psu treatments. At 10 psu snail survivorship declined throughout the experiment, down to only 15% by week four. Snail survivorship within the 15 psu treatments declined rapidly. By the end of week two, all snails at 15 psu had died (Fig. 3.6). The effect of salinity treatment on snail operculum growth was marginally non-significant (ANOVA,  $p = 0.0971$ ). Salinity had a significant effect on final snail wet (ANOVA,  $p = <0.0001$ ) and dry (ANOVA,  $p = 0.0341$ ) biomass but not snail ash free dry biomass (ANOVA,  $p = 0.1969$ , Table 3.6). This trend conflicts with the results found in mesocosm experiment 1 where there was a significant difference in snail growth in relation to salinity treatment. This is likely due to the large number of snail deaths that occurred in mesocosm experiment 2. Since a large number of snails were dead by the second week of the experiment they no longer had operculums to be measured or flesh that could be weighed.

As in the first mesocosm experiment, all treatments in the second experiment experienced some reduction in *V. americana* biomass as compared with the biomass initially planted. However, there were significant differences among treatments in how much biomass was lost, and new shoots were gained in some treatments. Salinity affected the final number of shoots remaining in each tank (Two-Way ANOVA,  $p = 0.0014$ ), with 0 and 5 psu treatments ending with significantly more shoots than 15 psu treatments. Snail presence also significantly reduced

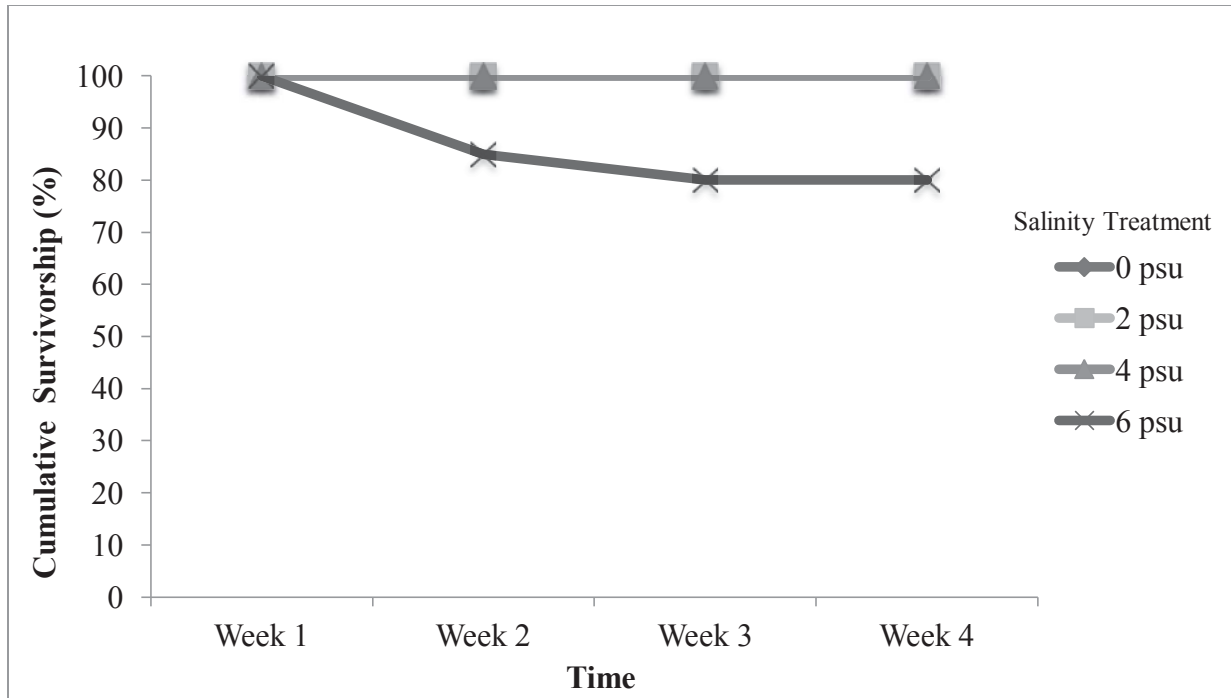
the number of shoots (Two-Way ANOVA,  $p < 0.0001$ ), and there was a significant interaction between salinity treatment and snail presence on shoot number (Two-Way ANOVA,  $p = 0.0009$ ).

Increasing salinity significantly reduced aboveground *V. americana* biomass but had no significant effect on belowground biomass. Snail presence tended to reduce root biomass, but this effect was not statistically significant for dry or ash-free dry root biomass. However, snail presence significantly reduced aboveground *V. americana* biomass, and there was a significant interactive effect of salinity and snails on aboveground biomass. Snails had stronger impacts at low salinities than at higher salinities (Fig. 3.8).

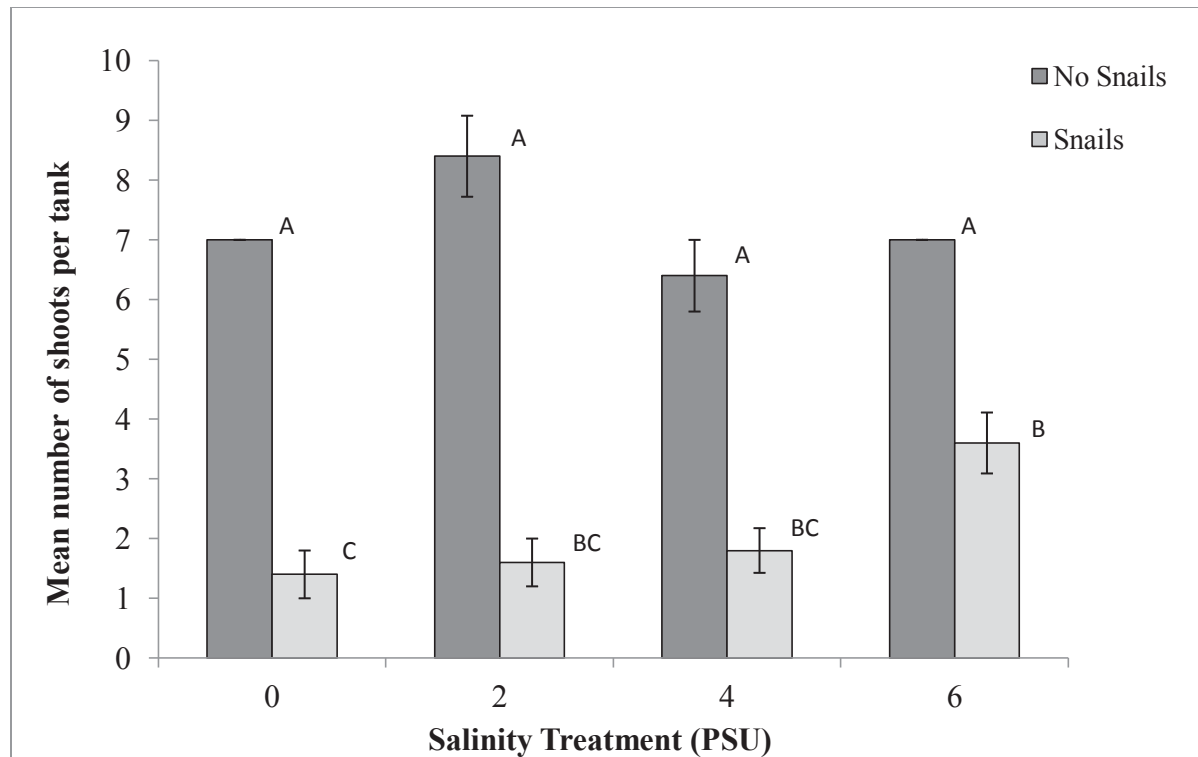
Salinity treatment significantly affected wet, dry, and ash free dry detritus biomass within each mesocosm (Two-Way ANOVA,  $p < 0.0001$ ). Both dry and ash free dry detritus biomass were log transformed to meet the assumptions of the Two-Way ANOVA test. There was more detritus in higher salinity treatments (Table 3.8). There was also a significant interactive effect of salinity treatment and snail presence on detritus biomass. The Tukey HSD test indicates that there was significantly more detrital *V. americana* in 15 psu treatments with and without snails and 10 psu treatments without snails as compared to all other treatments (Fig. 3.9). There was no significant effect of treatment on the amount of epiphytic algae ( $\mu\text{g Chl } a * \text{cm}^{-2}$ ) accumulated on the ribbons in each tank (Two-Way ANOVA,  $p = 0.0958$ , Fig. 3.10). However, epiphytes were generally less abundant at higher salinities and when snails were present.

**Table 3.4:** Mesocosm Experiment 1, one-way ANOVA results comparing snail biomass to salinity treatment. Letters indicate significant groups.

| Snail Biomass (g)                | P-value | Treatment (psu) | Tukey HSD Post Hoc Test |
|----------------------------------|---------|-----------------|-------------------------|
| Change in Wet Biomass            | 0.0088  | 0               | A                       |
|                                  |         | 2               | AB                      |
|                                  |         | 4               | B                       |
|                                  |         | 6               | B                       |
| Final Snail Dry Biomass          | 0.0215  | 0               | A                       |
|                                  |         | 2               | AB                      |
|                                  |         | 4               | AB                      |
|                                  |         | 6               | B                       |
| Final Snail Ash Free Dry Biomass | 0.0194  | 0               | A                       |
|                                  |         | 2               | AB                      |
|                                  |         | 4               | B                       |
|                                  |         | 6               | B                       |



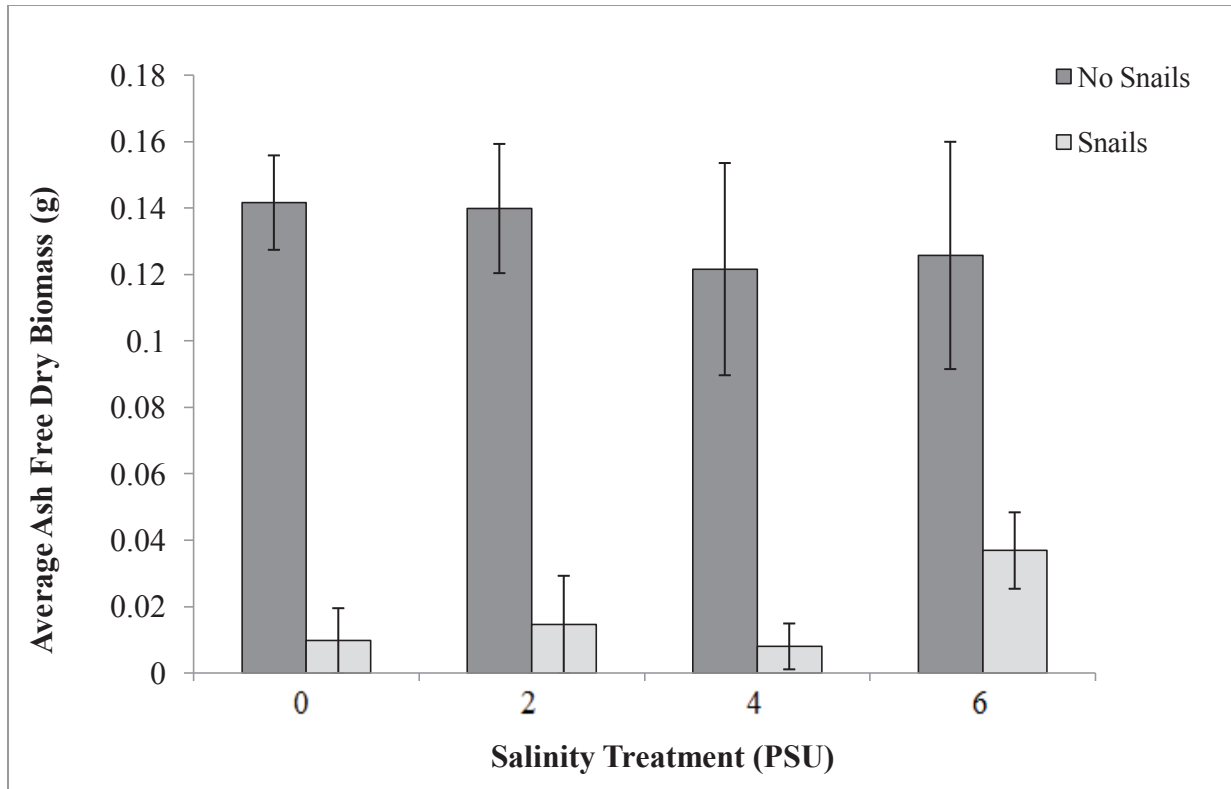
**Figure 3.3:** Cumulative snail survivorship by week and by salinity treatment in mesocosm experiment 1. Survivorship was reduced at 6 psu (nominal logistic regression,  $p < 0.0001$ ).



**Figure 3.4:** Mean number of *V. americana* shoots remaining by treatment in mesocosm experiment 1. There was a significant main effect of snail presence (Two-Way ANOVA,  $p < 0.0001$ ) and a significant interaction of salinity treatment and snail presence (Two-Way ANOVA,  $p = 0.0039$ ). Letters indicate significant groups. Error bars depict standard error of the mean (SEM).

**Table 3.5:** Mesocosm experiment 1, two-way ANOVA results for snail presence and salinity effects on *V. americana* below- and aboveground biomass. Note: Dry blade weight was log transformed.

| <b><i>V. americana</i> Belowground Biomass (g)</b> | <b>Salinity effect p value</b> | <b>Snail effect p value</b> | <b>Interactive effect p value</b> |
|--|--------------------------------|-----------------------------|-----------------------------------|
| Wet  | 0.0120                         | 0.0009                      | 0.3578                            |
| Dry  | 0.0448                         | 0.0389                      | 0.8828                            |
| Ash Free Dry                                       | 0.0512                         | 0.1166                      | 0.6970                            |
| <b><i>V. americana</i> Aboveground Biomass (g)</b> | <b>Salinity effect p value</b> | <b>Snail effect p value</b> | <b>Interactive effect p value</b> |
| Wet  | 0.2958                         | <0.0001                     | 0.0912                            |
| Dry  | 0.2451                         | <0.0001                     | 0.0571                            |
| Ash Free Dry                                       | 0.8654                         | <0.0001                     | 0.7263                            |

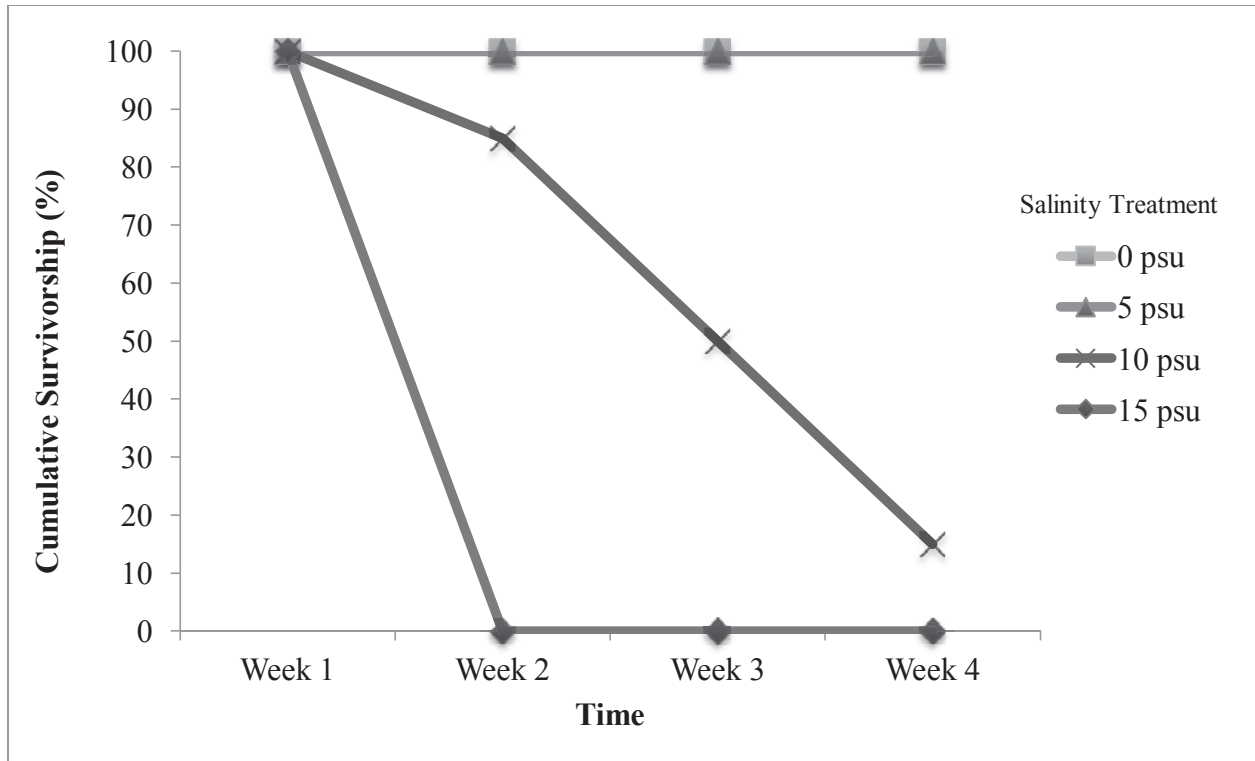


**Figure 3.5:** Mean *V. americana* aboveground biomass by treatment in mesocosm experiment 1. There was a significant effect of snail presence (Two-Way ANOVA,  $p = <0.0001$ ), but trends for the effects of salinity and the salinity x snail interaction were not significant. Error bars depict standard error of the mean (SEM).

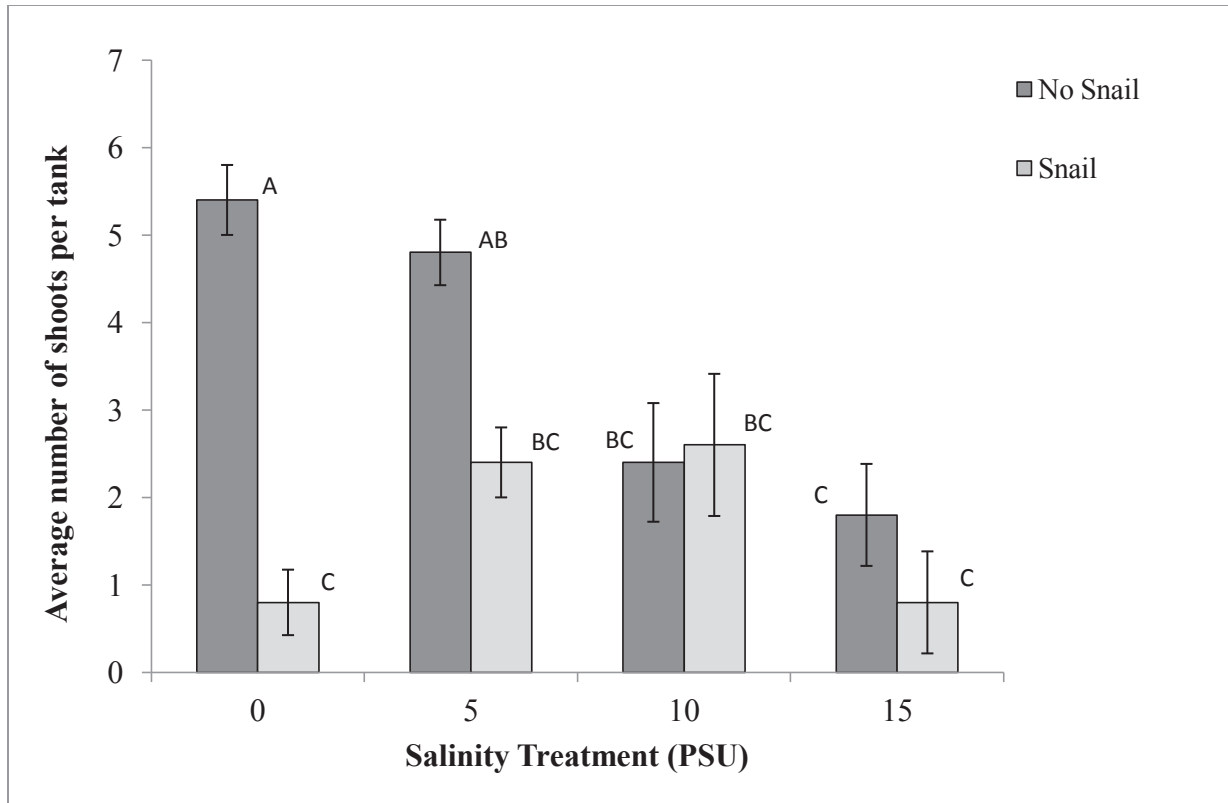


**Table 3.6:** Mesocosm experiment 2, one-way ANOVA results comparing snail biomass to salinity treatment. Letters indicate significant groups.

| Snail Biomass (g)                | p-value  | Treatment (psu) | Tukey HSD Post Hoc Test |
|----------------------------------|----------|-----------------|-------------------------|
| Change in Wet Biomass            | < 0.0001 | 0               | A                       |
|                                  |          | 5               | A                       |
|                                  |          | 10              | B                       |
|                                  |          | 15              | B                       |
| Final Snail Dry Biomass          | 0.0341   | 0               | A                       |
|                                  |          | 5               | AB                      |
|                                  |          | 10              | AB                      |
|                                  |          | 15              | B                       |
| Final Snail Ash Free Dry Biomass | 0.1969   | 0               | A                       |
|                                  |          | 5               | A                       |
|                                  |          | 10              | A                       |
|                                  |          | 15              | A                       |



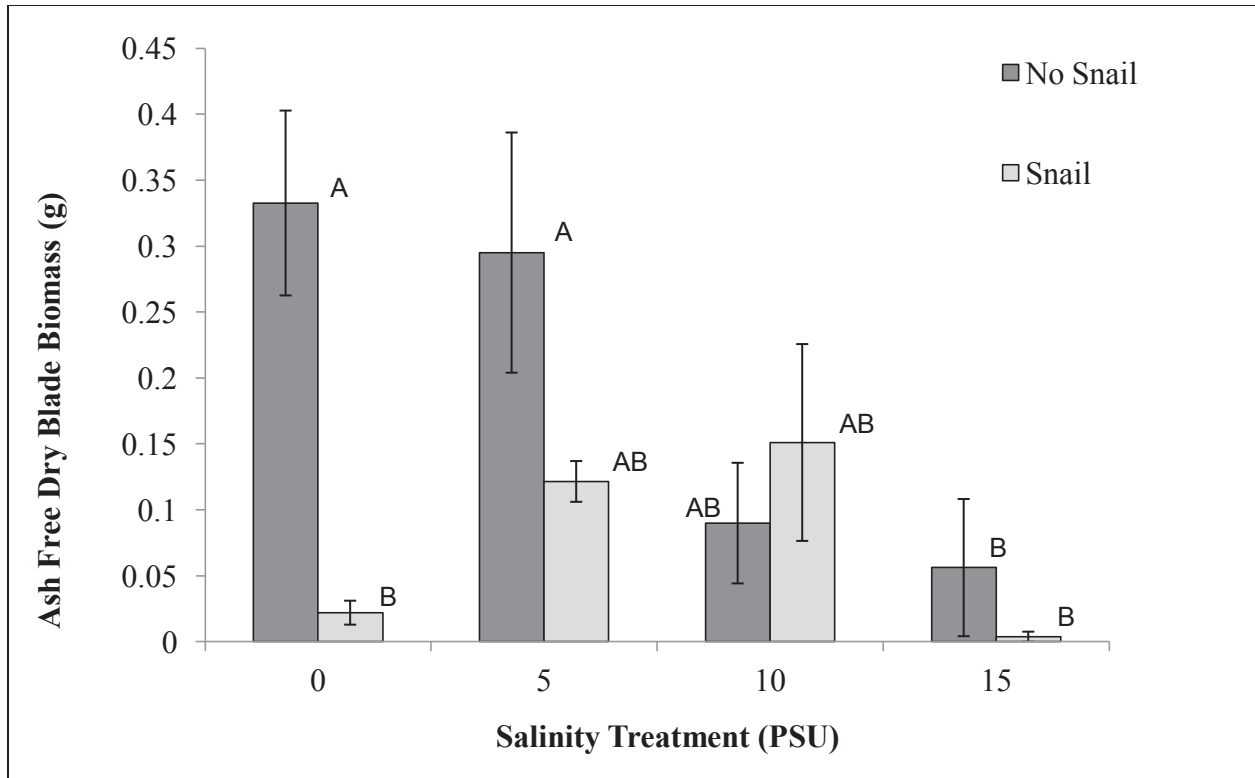
**Figure 3.6:** Cumulative snail survivorship by week and by salinity treatment in mesocosm experiment 2. However salinity treatment had a significant effect on cumulative snail survivorship (nominal logistic regression,  $p < 0.0001$ ).



**Figure 3.7:** Mean number of *V. americana* shoots remaining by treatment in mesocosm experiment 2. There was a significant main effect of salinity (Two-Way ANOVA,  $p = 0.0014$ ) and snail presence (Two-Way ANOVA,  $p < 0.0001$ ). There was a significant interactive effect of salinity and snail presence on the number of remaining shoots (Two-Way ANOVA,  $p = 0.0009$ ). Letters indicate significant groups. Error bars depict standard error of the mean (SEM).

**Table 3.7:** Mesocosm experiment 2, Two-Way ANOVA results for snail presence and salinity effects on *V. americana* below- and aboveground biomass. Mesocosm experiment 2, two-way ANOVA results comparing snail presence and salinity to *V. americana* below ground and above ground biomass per tank. Note: All *V. americana* above ground biomass is square root transformed.

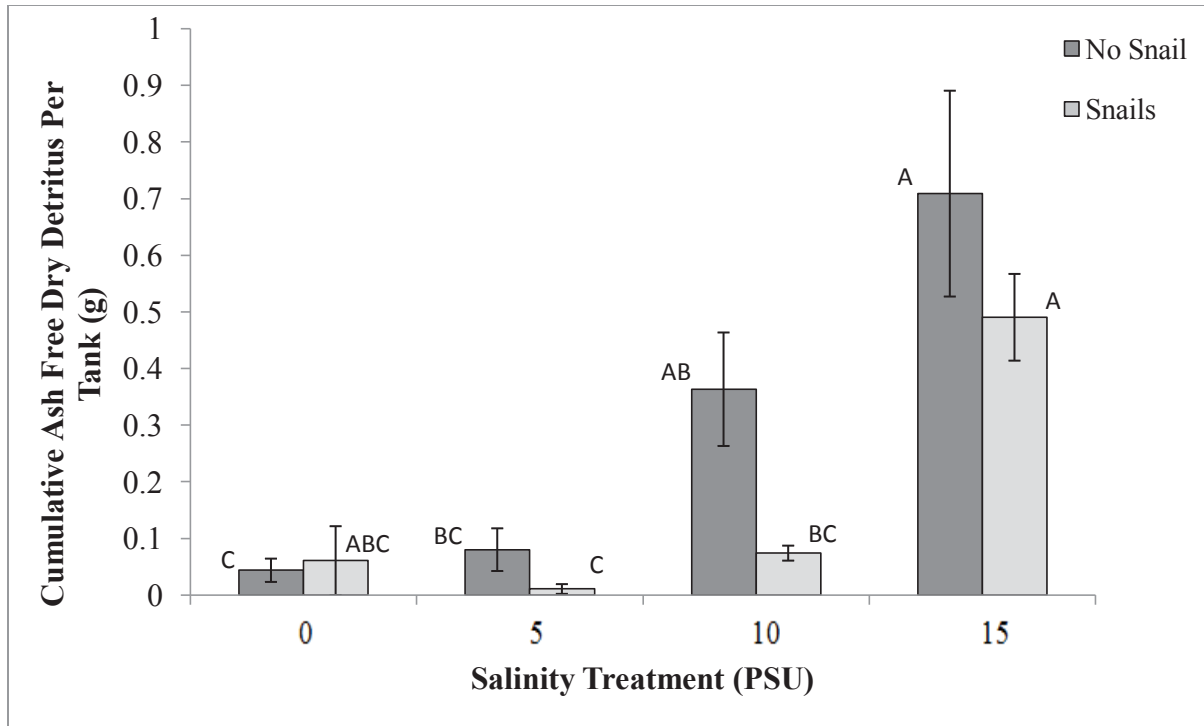
| <b><i>V. americana</i> Below Ground Biomass (g)</b> | <b>Salinity effect p value</b> | <b>Snail effect p value</b> | <b>Interactive effect p value</b> |
|---|--------------------------------|-----------------------------|-----------------------------------|
| Wet   | 0.7657                         | 0.0128                      | 0.1530                            |
| Dry   | 0.2895                         | 0.1197                      | 0.4365                            |
| Ash Free Dry  | 0.3379                         | 0.1580                      | 0.5779                            |
| <b><i>V. americana</i> Above Ground Biomass (g)</b> | <b>Salinity effect p value</b> | <b>Snail effect p value</b> | <b>Interactive effect p value</b> |
| Wet   | < 0.0001                       | 0.0026                      | 0.0001                            |
| Dry   | < 0.0001                       | 0.0005                      | 0.0002                            |
| Ash Free Dry  | 0.0003                         | 0.0030                      | 0.0087                            |



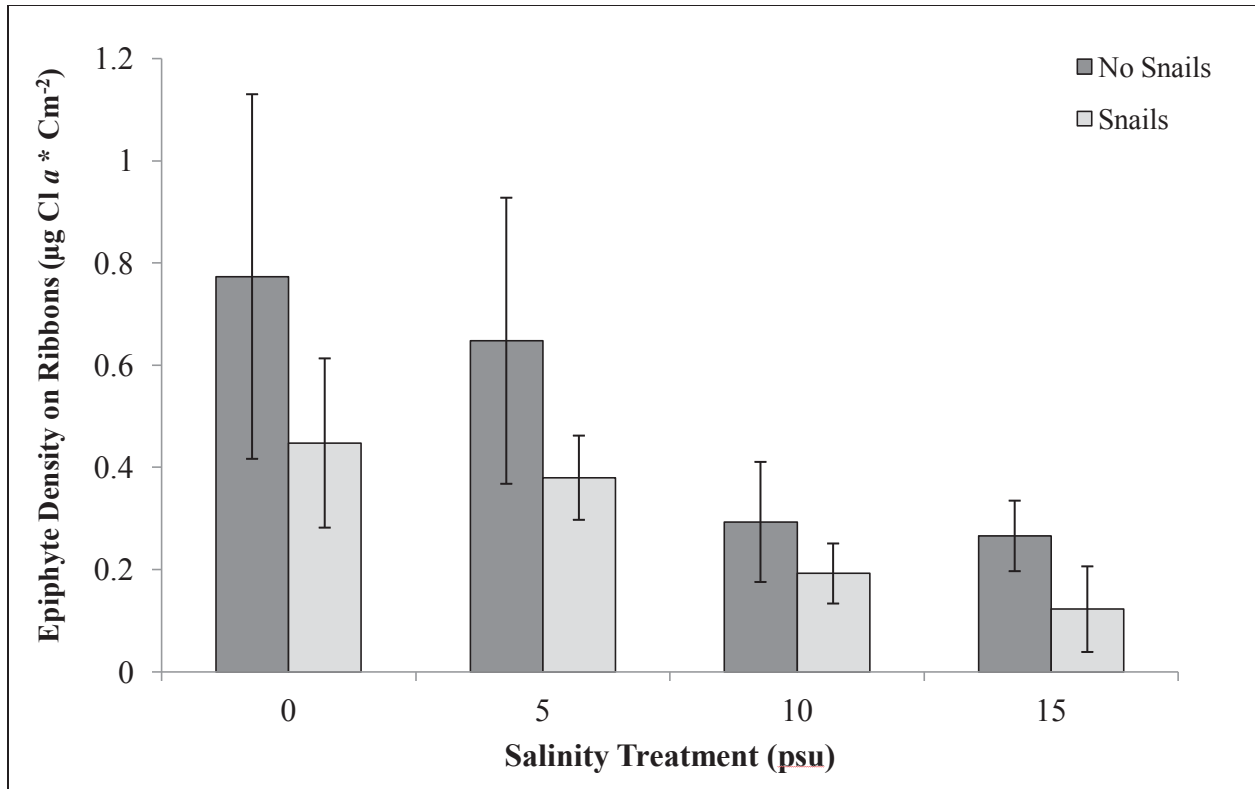
**Figure 3.8:** Mean *V. americana* aboveground biomass remaining by treatment in mesocosm experiment 2. There was a significant main effect of salinity (Two-Way ANOVA,  $p = 0.0003$ ) and snail presences (Two-Way ANOVA,  $p = 0.0030$ ). There was a significant interactive effect of snail presence and salinity treatment on total *V. americana* (Two-Way ANOVA,  $p = 0.0087$ ). Letters indicate significant groups. Error bars depict standard error of the mean (SEM).

**Table 3.8:** Mesocosm experiment 2, Two-Way ANOVA results comparing snail presence and salinity to *V. americana* detritus biomass per tank. Note: dry and ash free dry detritus biomass is log transformed.

| <b><i>V. americana</i> Detritus Biomass (g)</b> | <b>Salinity effect p value</b> | <b>Snail effect p value</b> | <b>Interactive effect p value</b> |
|---|--------------------------------|-----------------------------|-----------------------------------|
| Wet Detritus Biomass                            | < 0.0001                       | < 0.0001                    | 0.0342                            |
| Dry Detritus Biomass                            | < 0.0001                       | 0.2409                      | 0.0041                            |
| Ash Free Dry Detritus Biomass                   | < 0.0001                       | 0.5054                      | 0.0026                            |



**Figure 3.9:** Total *V. americana* detritus by treatment in mesocosm experiment 2. There was a significant main effect of salinity (Two-Way ANOVA,  $p < 0.0001$ ). There was a significant interactive effect of salinity treatment and snail presence on the amount of detritus per tank. Letters indicate significant groups. Error bars depict standard error of the mean (SEM).



**Figure 3.10:** Epiphyte density ( $\mu\text{g Chl } a * \text{cm}^{-2}$ ) on artificial seagrass ribbons at the end of mesocosm experiment 2. There was a decreasing trend with increases in salinity and with snail presence. Error bars depict standard error of the mean (SEM).



## Discussion

Our results imply that *P. maculata* is unlikely to be directly harmful to estuarine populations of *V. americana*, and that improved hydrologic connectivity between estuarine and freshwater *V. americana* populations could reduce *P. maculata* impacts. Separately, salinity and *Pomacea maculata* herbivory had direct, negative impacts on *Vallisneria americana*. However, in combination, the multiple stressors of salinity and herbivory acted in a non-additive, antagonistic manner (sensu Folt et al. 1999). That is, when snails were present, *V. americana* actually performed better at 5-10 psu than in freshwater. Based on prior results of feeding trial experiments (Gettys et al. 2008, McAskill and Douglass *in prep*), it is likely that this effect was due to a significant reduction in snail grazing rate at non-zero salinities. The snail growth and mortality data from these mesocosm experiments support the idea that snail performance is reduced at salinities well below the snails' acutely lethal limit. Sub-lethal effects of salinity on snail performance were most notable over the 0-6 psu range in experiment 1. As in Martin and Valentine (2014), no snail mortality was observed at these salinities, but increasing salinity significantly reduced snail growth as assessed by biomass and operculum width.

At 10 psu and higher in experiment 2, mortality of *P. maculata* was 85%, suggesting that 10 psu is near or above *P. maculata*'s absolute salinity tolerance limit. Snail impacts on *V. americana* were probably negligible at 10 and 15 psu, but *V. americana* performance was nevertheless poor, due to direct impacts of salinity on the plants (Doering 2002). Based on our results (Figure 3.8), salinities between 5 and 10 psu appear the most likely to afford a viable refuge for *V. americana* from snail herbivory. However, the salinity range of the refuge likely depends on other factors, such as light and nutrient availability, that affect *V. americana* growth performance. Under optimal conditions there might be a wide range of salinities (e.g., 2-10 psu)

within which *V. americana* growth could outpace *P. maculata* grazing, whereas under light-limited conditions *V. americana* growth might not outpace *P. maculata* grazing at any salinity.

Larger scale, longer term mesocosm experiments with finer gradations in salinity could help narrow down the optimum salinity for protecting *V. americana* in snail-invaded areas. Another important experiment to consider is evaluating the potential recovery of *V. americana* at different salinities after a grazing event. In general, below ground biomass was not impacted by salinity or snail presence. It is possible that as long as the rhizome remains *V. americana* can recover after being grazed by *P. maculata*. We also recommend careful monitoring of *V. americana* and *P. maculata* along estuarine-freshwater transitions zones to determine the extent to which estuarine invasion by the snail has already occurred. It is possible that in a field setting the snails will display different behavioral mechanisms to avoid salinity stress (Carlsson et al. 2004).

Fully understanding the interaction between salinity and snail herbivory on *V. americana* populations is critical to the macrophyte's survival in the estuarine habitats. While our results support the long-established fact that high salinity (> 10 psu) intrusions in the oligohaline estuary are destructive to *V. americana* (Doering 2002), they suggest that maintaining a totally freshwater environment might be counterproductive for *V. americana* survival. In conjunction with careful monitoring of *V. americana* and *P. maculata* populations, water management to occasionally increase salinity to 5-10 psu within oligohaline portions of an estuary could be a beneficial strategy to reduce *P. maculata* populations but still allow for *V. americana* survival. For example, this technique could be applied to low elevation stormwater ponds and canals that are separated from estuaries by weir systems. Of course, more research should be done before

such management actions to ensure that native freshwater macrophyte and fauna species present would not be negatively impacted by oligohaline conditions.

Ramakrishnan (2007) reported the optimal salinity range of *P. maculata* to be 0-6.8 psu and proposed that *P. maculata* be classified as a 2<sup>nd</sup> degree euryhaline limnobiont based on the classification system outlined by Remane and Schlieper (1971). Our results and those of Bernatis (2014) support Ramakrishnan's (2007) classification but are harder to reconcile with the higher tolerance reported by Martin and Valentine (2014). Martin and Valentine (2014) observed 50% survival of *P. maculata* hatchlings after a two-week exposure to 15 psu. The same or lower levels of salinity caused 100% mortality of juveniles in our study and of adults in Ramakrishnan (2007) and Bernatis et al. (2014). It is possible that hatchlings are somehow more resilient to mesohaline conditions than juveniles and adults, or that the *P. maculata* from coastal Louisiana where Martin and Valentine (2014) worked are more tolerant than those from Florida.

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## Chapter 4: Project summary and broader implications

### Summary of Research Goals:

1. Determine how sub-lethal levels of salinity affect the grazing rate of *Pomacea maculata* on *Vallisneria americana*.
2. Determine how changes in water temperature, within the range experienced seasonally in Southwest Florida, influence *P. maculata* grazing rate.
3. Compare *P. maculata* grazing rates on *V. americana* to grazing rates on other SAV species in the Southwest Florida to determine how the biotic context of plant diversity affects the vulnerability of *V. americana*.
4. Determine the separate and combined effects of salinity and herbivory on *V. americana* growth and survivorship in a mesocosm setting.
5. Assess the likelihood of harm to estuarine populations of *V. americana* by *P. maculata*, and identify salinity regimes and management strategies to minimize that harm.

### Summary of results

We found that all aspects of environmental context that we examined; water temperature, salinity, and plant community context, significantly affected *Pomacea maculata* grazing rate on *Vallisneria americana*. Increasing salinity decreased grazing rate, increasing temperature increased grazing rate, and the presence of other macrophytes increased or had little effect on grazing rate. However, the combined impacts of salinity and grazing on *V. americana* shifted from additive to nonadditive, antagonistic effects as salinity increased. Salinity reduced the harm done by grazing such that overall harm to *V. americana* was minimized at approximately 5 psu

when snails were present. Our results imply that *P. maculata* is unlikely to be directly harmful to estuarine populations of *V. americana*, and that improved hydrologic connectivity between estuarine and freshwater *V. americana* populations could reduce *P. maculata* impacts.

### *Ecological implications*

The herbivorous aquatic snail *P. maculata*, which is native to South America, has invaded the Southeastern United States (Byers et al. 2013) poses a serious threat to *Vallisneria americana*, among other aquatic plants (Baker et al. 2010, Ceilley et al. 2013, Bernatis 2014, Horgan et al. 2014). Understanding environmental factors that could be used to alleviate the impacts of *P. maculata* on *V. americana* is important due to the devastating effects the gastropod can have on macrophyte communities. Overgrazing by *P. maculata* can shift macrophyte dominated ecosystems to phytoplankton dominated ecosystems (Horgan et al. 2014). This threat is compounded by other stressors on *V. americana* populations, notably salinity fluctuations related to anthropogenic alterations in the hydrology of coastal watersheds (Scarlatos 1988, Doering et al. 1999, Barnes 2005, Douglass 2014). Shifts in primary production from benthic macrophytes to phytoplankton coupled with the destabilization of sediments can create positive feedback loops that result in further loss of macrophytes (Barnes 2005). The alteration of an ecosystem's biogenic structure over time will have negative implications for fish and invertebrate communities as well as human recreational and economic opportunities.

Biogenic structure, such as that provided by coral reefs, mangrove swamps, and submerged aquatic vegetation (SAV), is a key factor in supporting the biodiversity and productivity of aquatic and marine habitats (Ward 1984, Doering et al. 2002, Barnes 2005). In estuarine habitats, in particular, a relatively small number of so-called foundation species, which



create habitat, food, and refuge for many other organisms (Gaylord et al. 2011), may have a disproportionately strong influence on overall ecosystem function. Therefore the elimination of a single foundation species can have a devastating knock on effect on an entire ecosystem (Altieri and Witman 2006). The deep-rooted aquatic macrophyte *Vallisneria americana* is a vital foundation species worldwide, but especially in the rivers and estuaries of Florida where it feeds the endangered Florida Manatee and provides a nursery ground for numerous freshwater and euryhaline fishes and crustaceans (Doering et al. 2002, Barnes 2005, Camp et al. 2014).

### *Temperature considerations*

Results of the temperature trial suggest that the grazing pressure of *P. maculata* on *V. americana* increases with an increase in temperature, at least within the range of temperatures examined. This data coincides with the results of Gettys et al. (2008), in which *P. maculata* show a similar response to an increase in temperature when grazing on *H. verticillata*. The average annual temperature of the Caloosahatchee is 23°C, with an average winter temperature of 17°C and average summer temperature of 28°C (Scarlatos 1988). Both the overall average temperature and average summer temperature of the Caloosahatchee fall within *P. maculata*'s optimal consumption and growth range. These data suggest that the snail is able to graze at a high or very high rate in Florida for most of the year. Based on results reported by Gettys et al. (2008), *P. maculata* kept at 15°C ate less food compared to snails kept at higher temperatures and lost an average of 1.8g of body weight during the 10 day experiment. Further study is needed to determine the extent to which *P. maculata* grazing rate is reduced by typical Caloosahatchee winter temperatures, but our results suggest at least preliminarily that winter may be the more appropriate time to initiate *V. americana* restoration plantings or other macrophyte conservation actions that might be vulnerable to *P. maculata* grazing.

### *Salinity considerations*

Florida is known for its wet and dry seasons. The wet season typically extends from June to October and the dry season extends from November to May (Scarlatos 1988). Ideally, *V. americana* restoration within the Caloosahatchee Estuary should take place during the wet season because salinities are typically lower within the estuary due to increased water releases from the S-79 Lock and Dam (Buzzelli et al. 2014, Douglass 2014). Although there is a lower chance of *V. americana* encountering salinity stress during the wet season there is a higher chance for a predation by *P. maculata*. The wet season in Florida also falls within the warm summer months. We know that warmer temperatures increase *P. maculata* grazing rates which may lead to higher consumption rates of *V. americana* and other vulnerable macrophytes in the summer within the CE (Ramakrishnan 2007, Gettys et al. 2008).

In our experiments, increases in salinity significantly reduced the grazing rate of *P. maculata* with the highest level of grazing occurring at 0 psu, significantly reduced grazing from 2 – 4 psu, and minimal grazing above 6 psu. These results suggest that low levels of salinity (2 – 6 psu) in estuarine *V. americana* habitats could help suppress *P. maculata* grazing and may ultimately inhibit the snails population spread within estuarine systems. Although the snails survived 72 hours at 10 psu, very little grazing occurred, and other signs of stress were observed, with some snails keeping their opercula shut for the duration of the experiment. In comparison with the results of similar experiments on the salinity tolerance of *P. maculata* (Ramakrishnan 2007, Martin and Valentine 2014, and Bernatis 2014), our results suggest that although salinities < 10 psu are not acutely lethal for *P. maculata*, the snails' destructiveness is significantly reduced even at quite low salinity levels.

For this part of our study the snails were not acclimated to the salinity. It is possible that not acclimating the snails amplified the effects of salinity as a stressor on *P. maculata* herbivory. However, it is also important to note that changes in the amount of freshwater released from the S-79 lock and dam can create rapid, unnatural salinity fluctuations within the CE (Scarlatos 1988, Doering et al. 1999, Barnes 2005, Douglass 2014). In addition, the snails were placed in enclosed aquariums with no sediment or other debris and no space for the snail to climb out of the water. In a field or more natural setting the snails may display different behavioral mechanisms to avoid stressors (Carlsson et al. 2004).

Further study is needed to determine the other ecological impacts of using salinity for *P. maculata* management within estuaries. It is already known that too much salinity can harm *V. americana* populations as well as other organisms in the estuary (Doering et al. 1999, Doering et al. 2002, Douglass 2014). However, we also know from our research that *V. americana* populations can benefit from some salinity. Therefore, 0 psu in the upper portion of the CE may not be an ideal goal but rather having a more stable salinity regime that doesn't widely exceed the salinity tolerance valued ecosystem components. Salinity within the CE can be manipulated in a number of ways, including reducing downstream freshwater flow through locks and dams like the S-79 lock and dam or increasing upstream saltwater flow by opening barriers such as the weir systems in Cape Coral.

#### *P. maculata* tolerances and likelihood of its invading estuaries

Martin and Valentine (2014) suggested the optimal salinity range for *P. maculata* hatchlings in estuaries is between 0-5psu. Our own data supports this idea since all treatments between 0-5 psu in both mesocosm experiments had 100% juvenile snail survivorship. At 6 psu there was a 20% decline in the cumulative snail survival rate after four weeks suggesting that

salinities above 6 psu are suboptimal for snail survivorship. We also noted that at 10 psu there was an 85% decline in cumulative snail survivorship. This suggests that 10 psu is near or above *P. maculata*'s upper salinity tolerance limit. Ramakrishnan (2007) reported the optimal salinity range of *P. maculata* to be 0-6.8 psu. This was also the first study to propose *P. maculata* be classified as a 2<sup>nd</sup> degree euryhaline limnobiont, based on the classification system outlined by Remane and Schlieper (1971), which would classify the snail as having a maximum salinity tolerance somewhere between 3 and 8 psu. The optimal salinity range reported by our study as well as Bernatis (2014) are in agreement with the 2<sup>nd</sup> degree euryhaline limnobiont designation (Ramakrishnan 2007).

The highest salinity tolerance that has been reported for *P. maculata* is 15 psu. Martin and Valentine (2014) reported a 50% survival rate of *P. maculata* hatchlings in 15 psu after a two week exposure period. Although hatchlings display some degree of resilience to mesohaline conditions, it seems unlikely that juvenile and adult snail will be able to tolerate such high salinities. Ramakrishnan (2007) reported that juvenile and adult snails exposed to 13.6 psu had a 100% mortality rate within seven days of exposure. Snails exposed to higher salinities such as 14.3 died within four to seven days of exposure. We experienced a similar result with our experiment. Snails exposed to 15 psu died within as little as three days. All snails were dead within a week of exposure. A study done by Bernatis (2014) provides further evidence supporting the idea that juvenile and adult *P. maculata* have a low tolerance for mesohaline conditions. In this study, adult snails exposed to 16 psu died within 9 days and had a mean survival of 3.6 days. Juvenile snails had a mean survival of 3 days when exposed to 16 psu.

Based on the results of available literature as well as the cumulative results of both our mesocosm experiments, *P. maculata* will likely be confined to the most oligohaline portions of

estuaries. Oligohaline salinities based on the Venice System range from 0.5-5 psu. Though their grazing rates are reduced by as little as 2 psu salinity, our mesocosm results show that they graze enough to still pose a threat to estuarine macrophytes at salinities up to 5 or 6 psu. Therefore, we are proposing that the optimal salinity range for *V. americana* growth and survival is between 6 and 10 psu. However, it is also important to consider other factors such as light and nutrient availability. These factors may act as additional stressors if sufficient levels are not provided and may also play a role in determining how well *V. americana* copes with salinity and herbivory stress.

In the future another mesocosm experiment should be run to look more closely at the effects of salinity and herbivory on *V. americana* survival and growth between 5 psu and 10 psu. Another important experiment to consider is evaluating the potential recovery of *V. americana* at different salinities after a grazing event. In general below ground biomass was not impacted by salinity or snail presence it is possible that as long as the rhizome remains intact, *V. americana* can recover after being grazed by *P. maculata*. In addition, field studies on the salinity tolerance of *P. maculata* in relation to herbivory on *V. americana* or other macrophytes should be conducted within estuaries. This is important since it is possible that in a field setting the snails will display different behavioral mechanisms to avoid salinity stress (Carlsson et al. 2004).

Throughout our research we conducted pilot studies which mapping the location of *P. maculata* and *V. americana* populations within the canal systems of Cape Coral. Since there are both freshwater and salt water canals in cape coral the goal was to see how *P. maculata* and *V. americana* changed in relation to a change in the salinity gradient. However, this experiment did not work as planned since there are weir systems which separate freshwater canals from salt water canals causing an unnatural change in salinity between the two water bodies. For example,

we observed salinity 0.35 psu on freshwater side of the weir and 7.1 psu on the saltwater side of the weir. However, it is also important to note that two adult snails were found on the saltwater side of the weir at 7.1 psu. We also observed one *P. maculata* clutch which was laid on the roots of a red mangrove tree on the saltwater side of the weir. Ideally, it would have been beneficial to observe *V. americana* and *P. maculata* populations in a system where the salinity gradient is not interrupted by a weir or dam.

### *Biotic context*

The results of the feeding choice trials show that *V. americana* is a preferred food source for *P. maculata* and that the grazing pressure on *V. americana* is likely to be enhanced rather than relieved by the presence of other macrophyte species. However, the macrophytes we examined do not represent the full range of possible food sources for *P. maculata*. In addition to other plant and macroalgae species, benthic microalgae and detritus may be a large part of the diet for *P. maculata*. Further study is needed on a wider range of alternative *P. maculata* food sources to determine if producer community context can be altered to alleviate *P. maculata* grazing pressure on *V. americana*. Additional consideration should also be given to the question of whether the presence of a more-preferred food source than *V. americana* would actually relieve grazer pressure on *V. americana*, or if it would enhance grazing pressure by increasing populations of *P. maculata* (apparent competition sensu (Conner 2008, Morrison and Hay 2011)).

Another aspect of biotic community context that might help to alleviate grazing pressure on *V. americana* is the presence of snail predators. There is a strong need for studies on top-down control of *P. maculata*. It is known that the endangered snail kite, *Rostrhamus sociabilis*, preys on *P. maculata*. However, it is also known that *R. sociabilis* have a high drop rate of *P.*

*maculata* due to the snail's large size, and that the kites' handling time for the invasive snails is longer than for the native *P. paludosa* (Cattau et al. 2010). This suggests that *P. maculata* are a less suitable prey, conferring less nutrition to the kites per unit of hunting time than native *P. paludosa*. Even if individual snail kites do effectively prey on *P. maculata*, the kites have a low population size and may not be effective for widespread control of *P. maculata* populations. Therefore, alternative predators should be evaluated. Potential predators include alligators, *Alligator mississippiensis* (Delaney and Abercombie 1986, Snyder and Snyder 1971), various fish species (Darby et al. 2009), turtles (Dalrymple 1977), limpkins, *Aramus guarauna* (Snyder and Snyder 1969), white ibis, *Eudocimus albus* (Kushlan 1974), and crayfish (Snyder and Snyder 1971).

Both alternative prey species that can alleviate herbivory stress on *V. americana* and effective *P. maculata* predators could have positive indirect effects on the biogenic structure of ecosystems where *P. maculata* have invaded. If herbivory stress is alleviated on *V. americana* this should allow for larger, denser, beds to grow, increasing habitat and foraging grounds for various fish, invertebrate, and wading bird species. An additional factor to take into consideration when evaluating potential biocontrol methods for *P. maculata* is whether the species being used are native. The use of a nonnative species may have unforeseen consequences or impacts on the ecosystem. For example, Baker et al (2010) identified *Hydrilla verticillata*, a nonnative invasive, as a preferred food source for *P. maculata*. Our study also found the consumption rate of *H. verticillata* comparable to that of *V. americana*. However, using *H. verticillata* as an alternative food source is not advised since it is an invasive species and would likely end up doing more harm than good (Monette and Markwith 2014).

### *Multiple stressors and sub-lethal effects*

When an organism is exposed to multiple stressors, the effects of each stressor can combine in either an additive or a nonadditive manner. When stressors are additive the combined effect is equal to the sum of the individual stressors (Folt et al. 1999). When stressors are nonadditive, the combined effect of the two stressors is not a simple sum. Instead the stressors can have a synergistic or antagonistic interactive effect on the organism. If the interaction is synergistic, then the combined effect is greater than the sum of the two individual effects. If the interaction is antagonistic, then the combined effect is less than the sum of the two individual effects (Folt et al. 1999). The results of our mesocosm experiment suggest that the combined effect of salinity and herbivory on *V. americana* is antagonistic. Separately, salinity and snail presence had direct, negative impacts on *V. americana*. However, salinity indirectly benefited *V. americana* when snails were present. Based on prior results of feeding trial experiments, it is likely that the indirect positive effect of salinity on *V. americana* in the presence of snails was due to a reduction in snail grazing rate at sub-lethal levels of salinity. The snail growth and mortality data from our mesocosm experiments also illustrate the trend of reduced snail performance at salinities below their lethal limit. Increasing salinity had a significant negative effect on snail operculum growth and final snail biomass.

Determining the true optimum salinity for *V. americana* in the presence of snails is challenging, and we have only partially succeeded in estimating the optimum range to be 5-10 psu. In general, at salinities of 6 psu and below, the total above ground *V. americana* biomass per tank was higher in treatments that did not have snails compared to treatments with snails, whereas *V. americana* performance was equally poor with or without snail presence at salinities > 10 psu. This suggests that salinities greater than 10 psu are poor for *V. americana* growth and



survival regardless of snail presence. Figure 3.8 depicts a slight increase in *V. americana* above ground biomass for treatments between 5 psu and 10 psu with snails, in comparison to 0 psu and 15 psu treatments with snails.

### *Management implications*

Estuaries are hotspots for biological productivity and biogeochemical cycling (Barbier et al. 2011). They also provide economically important resources and ecosystem services to humans (Barnes 2005, Barbier et al. 2011). For example, the Florida Fish and Wildlife Conservation Commission have estimated that saltwater sport fishing generates approximately \$7.6 billion annually in Florida. Many of these economically important game fish such as common snook (*Centropomus undecimalis*), gag grouper (*Mycteroperca microlepis*), and spotted sea trout (*Cynoscion nebulosus*) depend on SAV beds within estuaries as nursery grounds (Barnes 2005). The blue crab, *Callinectes sapidus*, in particular, utilizes macrophytes such as *V. americana* in the oligohaline portion of estuaries as feeding and nursery grounds (Guillory et al. 2001).

Loss of foundational macrophyte communities within oligohaline portions of estuaries will not only have negative economic implications but it can also lead to increased erosion, turbidity, and reduction of nutrient residence time in the upper portion of estuaries. This can potentially cause a shift in the turbidity maximum within the estuary as well as increased eutrophication in the mid to lower parts of the estuary. Maintaining healthy macrophyte communities within oligohaline portions of an estuary is therefore critical to the overall health of the system.

We currently know that *P. maculata* is found in estuarine tributaries within Alabama (Martin and Valentine 2014) as well as Florida (Ceilley et al. 2013, McAskill and Douglass, *in*

*prep*). We know based on research done by Ceilley et al. (2013) *P. maculata* is overgrazing and depleting *V. americana* in the freshwater portion of the Caloosahatchee River. *P. maculata* hindered the restoration of *V. americana* even when grazer exclusion cages were used, as juvenile snails quickly infiltrated cages and grew to large size (Ceilley et al. 2013). Reduction of *V. americana* by *P. maculata* in the freshwater Caloosahatchee may reduce the downstream transport of seeds, indirectly contributing to the recent lack of recovery of SAV in the oligohaline estuary.

However, our current understanding of *P. maculata* herbivory on *V. americana* is mostly based on simple laboratory feeding trials where the consumption rate of the snail is examined under freshwater conditions (Gettys et al. 2008, Baker et al. 2010, Bernatis 2014). Knowing the how estuarine conditions impact *P. maculata* populations may help us find a way to protect *V. americana* from *P. maculata* herbivory within the Caloosahatchee. The goal of restoring *V. americana* populations within oligohaline portions of the estuary is to help stabilize sediments, increase fish populations, and provide food for endangered species such as the Florida manatee.

Based on our results, the interaction between salinity and snail herbivory on *V. americana* may be critical to the macrophytes' survival in the Caloosahatchee Watershed and Estuary. We suggest that salinity could be used to control *P. maculata* populations within the CE. However, both *V. americana* and *P. maculata* populations should be monitored within the CE to determine when and if using a salinity treatment is necessary. Since temperature also plays a significant role in the grazing rate of the snail and can potentially lower *P. maculata*'s grazing rate on *V. americana* within the Caloosahatchee if a salinity treatment is necessary for controlling *P. maculata* populations it would be suggested to do this during the winter (Gettys et al. 2008). In addition, salinities within the estuary tend to be higher during the winter due to

reduced freshwater releases from the S-79 lock and dam which may make using a salinity treatment to control snail populations more feasible during the winter compared to the summer months (Douglass, 2014). The construction of above ground reservoirs in the watershed is proposed to alleviate the unnatural fluctuations of salinity associated with water releases from Lake Ochechobee. The intent would be to restore the estuary to more historic patterns of water flow, and salinity fluctuation. However, exotic species have altered biotic communities in the CE, and 'historic' conditions may no longer support native biological diversity. Future water release regimes might utilize our evolving understanding of optimal, and suboptimal salinity conditions to help control invasive exotics.

We propose that salinities be kept between 5 and 10 psu within oligohaline portions of the estuary for at least a month during the winter. It would be expected that at 6 psu, the snail population would incur an 80% decrease in population due to salinity stress after four weeks. At 10 psu, it would be expected that the snail population would experience an 85% decrease in population due to salinity stress. This should sufficiently reduce *P. maculata* populations but still allow for *V. americana* survival. Similarly, this technique may work for the Cape Coral canals that are separated from the CE by weir systems. However, more research should be done on the Cape Coral canals to ensure that other freshwater macrophyte and fauna species present in the canals would not be negatively impacted by oligohaline conditions.

The future status of *P. maculata* in the CE, and other estuaries, is likely to be influenced by both temperature change and rising sea levels associated with global climate change. This further supports the need to more fully describe suboptimal temperature and salinity conditions that may help predict invasions and control this species.

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