2014

# The effects of non-native species on two life-stages of the Eastern oyster Crassostrea virginica

Wei Yuan
*University of Central Florida*

# EFFECTS OF NONNATIVE SPECIES ON TWO LIFE STAGES OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*

by

WEI SAMANTHA-WRIGHT YUAN

B.S. University of Central Florida, 2008

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2014

Major Professor: Linda J. Walters

## ABSTRACT

Since their recent introductions into Florida waters, three nonnative species [*Perna viridis* Linnaeus, 1758 (Asian green mussel), *Mytella charruana* d'Orbigny, 1846 (charru mussel) and *Megabalanus coccopoma* Darwin, 1854 (pink titan acorn barnacle)] have expanded both north and south along the Atlantic coast. Very little research has been done to understand how these nonnative species interact with the native eastern oyster (*Crassostrea virginica* Gmelin, 1791), which is a keystone species that provides important ecological services and economic benefits. To test the potential effects of *P. viridis, M. charruana* and *M. coccopoma* on *C. virginica,* I addressed the following questions: 1a) Does the presence of nonnative species decrease oyster larval settlement? 1b) Do oyster larvae avoid settling on oyster shells to which nonnative species are attached? 2a) Do nonnative species decrease survival of juvenile oysters (spat)? and 2b) Do nonnative species hinder spat growth? My manipulative experiments showed that the tested nonnative species influenced settlement, growth and survival of *C. virginica* in unique ways. *Megabalanus coccopoma* decreased the total number of settled oyster larvae, but did not influence larval preference or survival and growth of spat. *Perna viridis* negatively influenced larval settlement and oyster larvae avoided settling on shells of *P. viridis*. *Mytella charruana* had no influence on the total number of settled larvae but oyster larvae avoided settling on oyster shell with *M. charruana* or on the mussel shells themselves. Furthermore, both nonnative mussels negatively affected the survival of juvenile oysters, but only *M. charruana* reduced spat growth. These three nonnative species should be classified as invasive species because all had negative effects on the native oyster *C. virginica*.

For Julia J. Leissing, who inspired me to take a chance and always believed in me.

## ACKNOWLEDGMENTS

There are so many people to thank who helped me over the last few years. I would not have been able to finish without you. Thank you to my thesis committee Dr. Linda Walters, Dr. Eric Hoffman, Dr. John Fauth and Dr. John Scarpa for all their guidance and support on this project.

I thank all CEElab members, past and present. You are the best lab mates ever. Thank you to everyone who helped me in the field and the lab. Especially those who stayed up all night helping me set up and run my experiments.

To J. Leissing, A. Zak, M. Achenie, M. Gittings, and everyone else who listened to me talk about oysters, mussels and barnacles, thank you!

Funding was provided by USDA NIFA, the Indian River Lagoon National Estuary Program and the University of Central Florida.

## TABLE OF CONTENTS

LIST OF FIGURES ..... vi
LIST OF TABLES ..... vii
CHAPTER ONE - INTRODUCTION ..... 1
CHAPTER TWO - METHODS ..... 6
Species collection sites ..... 6
Oyster Larval Experiment: Settlement and settlement preferences ..... 6
Oyster Spat Experiment: Survival and growth ..... 9
CHAPTER THREE - RESULTS ..... 14
Oyster Larvae: Settlement ..... 14
Oyster Larvae: Settlement preference ..... 16
Oyster Spat: Survival ..... 20
Oyster Spat: Growth ..... 22
CHAPTER FOUR – DISCUSSION ..... 25
LIST OF REFERENCES ..... 32

## LIST OF FIGURES

Fig. 1. Flume layout of oyster larvae settlement for each trial ..... 7

Fig. 2. Experimental design of oyster spat survival and growth in 2012 experiment. Total of 6 treatments (n = 20). One treatment of uncaged oyster spat and five caged treatments consisting of oyster spat, oyster spat with four adult *M. charruana*, oyster spat with four adult *M. coccopoma*, oyster spat with four adult *P. viridis*, and oyster spat with four adult *G. demissa*. ..... 11

Fig. 3. Example of a frame design for 6 treatments in 2012 experiment for oyster spat survival and growth. ..... 12

Fig. 4. Average number of settled larvae per $cm^2$ on shells of *C. virginica* alone (control) or attached to oyster shells with nonnative species attached. a) 2011 mean number of settled larvae (n=3), b) 2011 mean number of settled larvae for each block, c) 2012 mean number of settled larvae (n=5), and d) 2012 mean number of settled larvae for each block. Capitalized letters in graphs indicates significant differences at $\alpha = 0.05$ as determined by Tukey's *a posteriori* tests with Bonferroni corrections. ..... 15

Fig. 5. Larval settlement preferences of nonnative species for a) 2011: *Megabalanus coccopoma* and *Perna viridis*, b) 2012: *P. viridis*, *M. coccopoma*, *Mytella charruana* and native *Geukensia demissa*. Positive values indicate oyster larvae settled more on disarticulated oyster shells with attached sessile species, while negative values indicate oyster larvae settled more on disarticulated oyster shells without attached sessile species (i.e., control)..... 17

Fig. 6. Settlement location of oyster larvae on oyster shells with native or nonnative species attached to the shell. The number of settled larvae on nonnative species is shown for a) 2011: *Perna viridis*, b) 2011: *Megabalanus coccopoma*, c) 2012: *P. viridis*, d) 2012: *M. coccopoma*, e) 2012: *Mytella charruana* and f) 2012: native *Geukensia demissa*. Native/nonnative shell indicates number of oyster larvae settled on the shells of attached invertebrates. Oyster shell indicates number of oyster larvae settled on the oyster shell with invertebrates attached. ..... 19

Fig. 7. Survival over time of oyster spat (*Crassostrea virginica*) exposed to native or nonnative species a) 2011: *Megabalanus coccopoma* and *Perna viridis* and b) 2012: *M. coccopoma. P. viridis, Mytella charruana* and *Geukensia demissa*. Cage control treatment was added in 2012. Treatments were compared using Kaplan-Meier multiple pairwise comparisons for survival over time; different letters at week 6 indicate significant differences at $\alpha = 0.05$. ..... 21

Fig. 8. Oyster spat growth of *Crassostrea virginica* after exposed to different nonnative species. a) 2011: *Megabalanus coccopoma* and *Perna viridis*. The treatments were analyzed with ANCOVA. The regression lines show best fit lines for each treatment. b) 2012: *M. coccopoma, P. viridis, Mytella charruana* and native *Geukensia demissa*. The treatments were analyzed with one-way ANOVA. Cage treatment for control was added in 2012. Treatments with different letters indicate significant differences at $\alpha = 0.05$ as determined by pairwise comparisons in 2011 and Tukey's *a posteriori* tests with Bonferroni corrections in 2012. ..... 23

## LIST OF TABLES

Table 1. Experimental blocks for larval settlement trials in (a) 2011 and (b) 2012. All treatment used oyster shell as substratum. Control: *C. virginica* shells alone. Nonnative species: *Perna viridis, Megabalanus coccopoma*, and *Mytella charruana*. Native mussel: *Geukensia demissa*… 8

Table 2. ANOVA comparison of oyster larval settlement in 2011. **Bold** and * indicates a significant difference at α = 0.05. ..... 14

Table 3. ANOVA comparison of oyster larval settlement in 2012. **Bold** and * indicates a significant difference at α = 0.05. ..... 16

Table 4. ANOVA comparison for oyster settlement preference in 2011. ..... 18

Table 5. ANOVA comparison for oyster settlement preference in 2012. **Bold** and * indicates a significant difference at α = 0.05. ..... 18

Table 6. ANOVA comparison for oyster spat growth in 2011. **Bold** and * indicates a significant difference at α = 0.05. ..... 22

Table 7. ANOVA comparison for initial oyster spat size in 2012. ..... 24

Table 8. ANOVA comparison for oyster spat growth in 2012. **Bold** and * indicates a significant difference at α = 0.05. ..... 24

Table 9. Summary of results for oyster larval and spat experiment. Settlement refers to larval choice to settle or not settle. Settlement preference refers to any location preference made by oyster larvae. ..... 26

# CHAPTER ONE - INTRODUCTION

The introduction of nonnative species is a major threat to marine ecosystems (Ruiz et al. 1997; Molnar et al. 2008; Lewis and Coutts 2010; Thomas and Ohlemüller 2010). Nonnative marine species can directly affect native species by altering biodiversity, community structure, food availability and predator-prey interactions (Pimentel et al. 2005; Lewis and Coutts 2010), and indirectly affect native species by altering water clarity, transporting diseases, and aiding in the success of other invaders (Pimentel et al. 2005; Molnar et al. 2008; Perrings et al. 2010). In addition to ecological threats, marine invaders cause economic problems to the fishing and aquaculture industries, foul ships and boats, and clog intake pipes of power plants (Ruiz et al. 1997; Ruiz and Carlton 2003; Rajagopal et al. 2006; Lewis and Coutts 2010; Perrings et al. 2010; Galil et al. 2011).

Mussels and barnacles are infamous sessile marine invaders (Pimentel et al. 2005; Lockwood et al. 2007; Perrings et al. 2010). These nonnative organisms are problematic because they can be hard to identify, difficult to eradicate, and are continuously transported globally (Ruiz et al. 1997). Their invasions date back to the 1880s, but are more common in the past 60 years due to increased maritime transport (Pimentel et al. 2005; Lewis and Coutts. 2010; Galil et al. 2011). One example of a well-documented invasive bivalve is the mussel *Mytilus galloprovincialis* (e.g., Branch and Steffani 2004). *Mytilus galloprovincialis*, native to the Mediterranean, has been repeatedly introduced to South Africa since 1979 and now accounts for approximately 74% of the mussel biomass on rocky shores (Hockey and van Erkom Schurink 1992; Branch and Steffani 2004). *Mytilus galloprovincialis* has also become established along the east and west coasts of North America (McDonald and Koehn 1988; Vario et al. 1988; Heath et al. 1995; Ramirez and Ca'ceros-Martinez 1999; Wonham 1999; Anderson et al. 2002), Hawaii

(Apte et al. 2000), Australia (McDonald et al. 1991) and northeastern Asia (Wilkins et al. 1983; Lee and Morton 1985; McDonald et al. 1990; Branch and Steffani 2004). Additionally, *M. galloprovincialis* out-competes and hybridizes with two native blue mussels, *M. trossulus* and *M. edulis* (Branch and Steffani 2004), where their ranges are sympatric. An example of an invasive barnacle is *Chthamalus proteus* (e.g., Zabin and Altieri 2007). *Chthamalus proteus* is native to the Caribbean and western Atlantic (Southward et al. 1998), but was introduced to Hawaii in 1973 and has since displaced most species in the intertidal zone (Zabin and Altieri 2007; Zabin 2009). Very few individuals can coexist with *C. proteus* on rocky shorelines (Zabin 2009). This invasive barnacle has also been found in Guam, the Mariana Islands, and French Polynesia (Zabin 2009). *Chthamalus proteus* not only displaced native species but has also out-competed other nonnative barnacles, including *Amphibalanus reticulatus, Balanus amphitrite* and *Balanus reticulatus* (Zardus and Hadfield 2005; Zabin and Altieri 2007).

Marine sessile invaders can have an impact on larval and juvenile stages of native invertebrates, which can consequently affect the native species through adulthood (Diederich 2005; Diederich 2006). One example is the native blue mussel *Mytilus edulis* in the Wadden Sea. *Mytilus edulis* was predominantly found in dense beds at intertidal and subtidal zones; these mussel beds have been replaced by oyster reefs composed of invasive *Crassostrea gigas* (Reise 1998; Diederich 2006). Diederich (2005) found that *C. gigas* recruited twice as much in number of larvae as *M. edulis*. Another study found that survival of juvenile mussels was 15% less when invasive *C. gigas* was present as compared to *M. edulis* alone (Markert et al. 2010).

Within the last thirteen years, the pink titan acorn barnacle, *Megabalanus coccopoma* (Darwin, 1854), the Asian green mussel *Perna viridis* (Linnaeus, 1758) and the charru mussel *Mytella charruana* (d'Orbigny, 1846) have all been introduced into the southeastern Atlantic

coastline of the United States (Power et al. 2004; Boudreaux and Walters 2006; Spinuzzi et al. 2013). A brief history of these invasions follows: The barnacle *M. coccopoma* is native to North and Central America's Pacific coast from southern California to Ecuador (Newman and McConnaughey 1987). The first documented introduction was to Brazil in the 1970s where this barnacle displaced existing barnacles (Lacombe and Monteiro 1974; Newman and McConnaughey 1987; Kerckhof 2002). A few decades later, *M. coccopoma* was found along the coasts of Japan and Belgium (Kerckhof 2002; Yamaguchi et al. 2009; Gilg et al. 2010; Kerckhof et al. 2010). In 2006, *M. coccopoma* was documented along the Atlantic coastline of central Florida, and is now established from Georgia to central Florida (Spinuzzi et al. 2013).

The mussel *P. viridis* is native to the Indo-Pacific region from the Arabian Gulf to southern China and southern Japan (Sidall 1980). *Perna viridis* has been globally introduced through aquaculture and ballast water (Vakily 1989; Rajapopal et al. 2006; Baker et al. 2007). These invasions have resulted in unintentional harm in many marine ecosystems in Asia and have led to local economic losses (Vakily 1989; Rajapopal et al. 2006). *Perna viridis* was first observed in the United States in Tampa Bay, Florida in 1999, where it was observed attached to the cooling system of an electrical power plant (Power et al 2004; Baker et al. 2007). This nonnative mussel can now be found along the Atlantic coastline from northern Georgia to central Florida, and along the Gulf of Mexico from Florida to Texas (Baker et al. 2007; Spinuzzi et al. 2013; V. Encomio, pers. comm.).

The mussel *Mytella charruana* is native to the Atlantic coast of South America and the Pacific coast of Central America from Mexico to Ecuador (Lee 1987; Boudreaux and Walters 2006). The first U.S. report of *M. charruana* was at the Northside Generator Power Plant in Jacksonville, FL in 1987 (Lee 1987). This mussel clogged the intake pipes until it disappeared in

the spring of 1987 (Lee 1987). *Mytella charruana* was next found on intertidal oyster reefs in Mosquito Lagoon in 2004, 212 km south of Jacksonville (Boudreaux and Walters 2006). Currently, *M. charruana*, which has a broad salinity tolerance, can be found along the Atlantic coastline from northern Georgia to central Florida (Yuan et al. 2010; Spinuzzi et al. 2013).

One concern related to the recent invasions of *M. coccopoma, P. viridis*, and *M. charruana* to the southern United States, is whether or not these nonnative species negatively impact the native eastern oyster *Crassostrea virginica* (Gmelin, 1791). The eastern oyster is a keystone species and ecosystem engineer that creates three-dimensional habitats used by numerous species that depend on oysters for food, refuge, and water filtration (Kennedy et al. 1996). In light of research on other marine invaders, it is likely that one, two, or all three of these recent nonnative species are negatively impacting larvae or juvenile stages of native oysters. We know from other studies how detrimental invasive species can be on oysters (Kimbro et al. 2009; Sanford et al. 2014). Therefore, I examined potential effects of *P. viridis, M. coccopoma* and *M. charruana* on larval and juvenile (spat) stages of *C. virginica* in two manipulative experiments.

In the first experiment, I investigated the effects of nonnative species on the larval stage of oysters. This larval stage experiment had two objectives. First, I sought to determine whether oyster settlement was altered by the presence of any of these nonnative species. I hypothesized that the introduced species would negatively influence oyster larval settlement. This prediction was based on space availability (Zabin 2009), as the nonnative species will decrease the available area for oyster settlement. Second, I investigated whether there were any settlement location preferences for the oyster larvae that did indeed settle. Here, I hypothesized that a reduction of larval settlement would occur on oyster shells with attached introduced species and on the shells of the introduced species themselves. This prediction was based on similar studies

of barnacle larvae (Denley and Underwood 1979; Underwood and Denley 1984). Some barnacle species prefer to settle near conspecifics but would avoid settling if crowded conditions occur (Crisp 1961; Denley and Underwood 1979; Underwood and Denley 1984; Minchinton and Scheibling 1993).

For the second experiment, I investigated how oyster spat would be impacted by the presence of the three nonnative species. Specifically, I investigated how spat survival and growth were impacted by the presence of the nonnative species. I hypothesized that the presence of introduced species would physically limit the resources and, thus, have a negative effect on survival and growth of oyster spat. This prediction was based on observed detrimental effects that the zebra mussel *Dreissena polymorpha* has on the native species upon which they colonize (Nalepa et al. 1995). For example, the weight of epibiont zebra mussels prevents native clams from opening their shells to feed, eventually leading to death of the clams (Schloesser and Nalepa 1994). The present study will provide basic understanding how specific nonnative species interact with the native *Crassostrea virginica* and whether these nonnative species should be considered invasive species.

# CHAPTER TWO - METHODS

## Species collection sites

For all experiments, *Megabalanus coccopoma* Darwin, 1854 (pink titan acorn barnacle) were collected from Sea Love Marina at Ponce Inlet, FL (28.083082 N, 80.935111 W). *Perna viridis* Linnaeus, 1758 (Asian green mussel) were collected from two floating docks at Euclid Avenue in St. Augustine, FL in 2011 (29.949781 N, 81.310227 W) and Sea Love Marina in 2012. Due to low numbers of *Mytella charruana* d'Orbigny, 1846 (charru mussel), this species was not included in any of the 2011 experiments. In 2012, *M. charruana* were collected from a floating dock at Arlington Marina, Jacksonville, FL (30.334568 N, 81.612135 W). The native *Geukensia demissa* Dillwyn, 1817 (ribbed mussel) was added as a treatment in 2012 to test for the biotic effect of native neighbors. This mussel was collected off rocks in Mosquito Lagoon adjacent to the Marine Discovery Center in New Smyrna Beach, FL (29.030700 N, 80.917042 W).

All experiments used disarticulated shells of *Crassostrea virginica* Gmelin, 1791 collected from Mosquito Lagoon. All fouling organisms and biofilm were scrubbed from the oyster shells and the shells were returned to the lagoon for at least one month to establish a natural biofilm. All oyster shells were randomly checked with the aid of a dissecting microscope for any new live macro-organisms one day before the experiment; any new macro-organisms were removed with forceps.

## Oyster Larval Experiment: Settlement and settlement preferences

Settlement experiments were conducted at the University of Central Florida Marine Field Laboratory (Fellers House Field Station) in Canaveral National Seashore (28.906818 N, 80.821216 W). All oyster settlement experiments were conducted within a 24 h period in 2011

and 2012. Each experiment was performed using a recirculating, raceway flume (20 cm wide, 120 cm long with two semicircular ends as described in Tamburri et al. 1996, Fig. 1) with an overall flow rate of 5 cm $s^{-1}$, which is the mean average mainstream flow rate in Mosquito Lagoon, FL (Boudreaux et al. 2009). A motor-driven paddle wheel was used to create the flow in the flume. Water was collected from Mosquito Lagoon and filtered through a plankton collector with nominal 25 µm opening (Aquarium Pro). Water depth was 10 cm for each experiment. Water salinity for each experiment was matched to the salinity in which oyster hatchery larvae were received. For the summer 2011 experiment, oyster larvae were obtained from the Virginia Institute of Marine Science in Gloucester Point, VA, at salinities ranging from 16 to 18. For the 2012 experiment, oyster larvae were obtained from Research Aquaculture, Inc. in Stuart, Florida, at a salinity of 27. All treatment species were acclimated to the experimental salinities for a minimum of 12 hours.

One batch of oyster larvae (~ 150,000 eyed larvae in 2011, ~ 2,500,000 eyed larvae in 2012) was used for each block of trials. A total of three trials per block were conducted in 2011 and five trials per block in 2012 (Table 1). Oyster larvae were observed using a dissecting microscope to ensure larval activity before each trial.

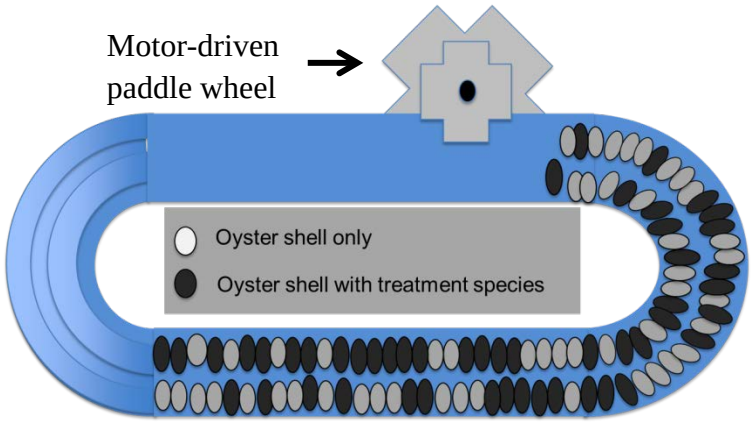

Fig. 1. Flume layout of oyster larvae settlement for each trial

Table 1. Experimental blocks for larval settlement trials in (a) 2011 and (b) 2012. All treatment used oyster shell as substratum. Control: *C. virginica* shells alone. Nonnative species: *Perna viridis, Megabalanus coccopoma,* and *Mytella charruana*. Native mussel: *Geukensia demissa*.

a) 2011

| **Trial** | **Block 1** | **Block 2** | **Block 3** |
|---|---|---|---|
| 1 | Control | *M. coccopoma* | *P. viridis* |
| 2 | *M. coccopoma* | *P. viridis* | Control |
| 3 | *P. viridis* | Control | *M. coccopoma* |

b) 2012

| **Trial** | **Block 1** | **Block 2** | **Block 3** | **Block 4** | **Block 5** |
|---|---|---|---|---|---|
| 1 | *P. viridis* | *G. demissa* | *M. coccopoma* | *M. charruana* | Control |
| 2 | *G. demissa* | *P. viridis* | Control | *M. coccopoma* | *M. charruana* |
| 3 | *M. coccopoma* | *M. charruana* | *G. demissa* | Control | *P. viridis* |
| 4 | *M. charruana* | Control | *P. viridis* | *G. demissa* | *M. coccopoma* |
| 5 | Control | *M. coccopoma* | *M. charruana* | *P. viridis* | *G. demissa* |

Each trial within a block was comprised of a mix of 50 disarticulated oyster shells and 50 disarticulated oyster shells with one attached live sessile invertebrate treatment (i.e., *M. coccopoma*, *P. viridis*, *M. charruana,* or *G. demissa*), with the exception of the control trials. Each control trial consisted of 100 oyster shells with nothing attached. The position and orientation of each oyster shell in the flume and the order of each shell (i.e., control or treatment) within a trial were randomized. Each trial was run for 1 h.

For treatments with *M. coccopoma,* one barnacle was attached to each oyster shell using Gorilla Glue™. Gluing was necessary because *M. coccopoma* cannot naturally re-attach to substrates after they have been removed from their original substrate. Excess glue was removed after drying with a scalpel. A preliminary test of the effect of Gorilla Glue™ on survival of *M. coccopoma* (n = 6) found 100% survival after 2 weeks (W. Yuan, pers. obs.).

For all mussel treatments, I placed individual mussels onto oyster shells for a minimum of 2 h in separate tanks. This allowed each mussel to naturally attach to the oyster shell via byssal thread production. The orientation of mussels was not accounted for because the mussels

move using their foot and repeatedly attach/re-attach with their byssal threads. Mussels remained attached to oyster shells throughout each trial.

After each trial ended, all oyster shells and the shells of attached species were examined with the aid of a dissecting microscope (LEICA EZ4) for newly settled oysters. The surface area of all oyster shells and all attached invertebrate shells were traced using a permanent ultra-fine point marker onto plastic transparency sheets. The tracings were digitized using ImageJ software (Abramoff et al. 2004) to calculate surface areas. This process standardized total surface area among treatments and blocks. To calculate the number of settled larvae per $cm^2$/trial, I first found total number of larvae that settled in each trial and then divided by the total surface area of oyster shells and shells of treatment species in that trial.

Total oyster settlement data was analyzed using randomized-block ANOVA in JMP statistical software (SAS Institute 2009). Multiple comparisons among treatments were analyzed using Tukey's *a posteriori* tests with Bonferroni corrections with $\alpha = 0.002$. As no block effects were found for either the 2011 and 2012 oyster settlement preference experiments [Random block ANOVA: 2011 ($F_{2, 4} = 0.4$, $p = 0.6817$); 2012 ($F_{4, 16} = 0.3$, $p = 0.8849$)], these data were analyzed with one-way ANOVA. Additionally, t-tests were used to analyze the difference between the number of settled larvae on oyster shells and shells of the treatment species barnacles and mussels.

**Oyster Spat Experiment: Survival and growth**

The survival and growth experiment evaluated whether nonnative species negatively impacted oyster spat. Oyster spat and all live species were prepared in the laboratory and

transferred to floating docks at the New Smyrna Beach Marine Discovery Center. All three nonnative species were already present at this study site (Spinuzzi et al. 2013).

Spat of *Crassostrea virginica* used in this experiment came from different sources in 2011 and 2012. In 2011, I used spat that were naturally attached to disarticulated oyster shells collected in Mosquito Lagoon. In 2012, I used spat reared by Research Aquaculture, Inc. (Stuart, FL) using oysters spawned from St. Augustine, FL. Hatchery spat were used due to the low number of spat found in Mosquito Lagoon in 2012. The 2012 spat were cultchless (i.e., unattached to any substrate) and were glued to oyster shells using Gorilla Glue™. All excess glue was removed to prevent confounding factors such as bumps or ridges. Prior to my trials, all spat were acclimated to field salinity for 5 days. I tested whether glued spat had different growth and survival as compared to naturally attached spat. Glued spat were placed in the field for one week. No significant differences (t-test, $N = 20$, $p = 0.6874$) were found for growth and survival (100%), indicating that the glue was not negatively influencing the glued spat as compared to naturally attached spat.

In 2011, I conducted the trial between 25 June and 6 August. There were three treatments: 1) oyster spat, 2) oyster spat with four adult *M. coccopoma,* and 3) oyster spat with four adult *P. viridis*. For the 2012 experiment, I conducted the trial between 28 July and 8 September. The experiment conducted in 2012 consisted of six treatments, one treatment of uncaged oyster spat and five caged treatments consisting of: 1) oyster spat, 2) oyster spat with four adult *M. charruana*, 3) oyster spat with four adult *M. coccopoma*, 4) oyster spat with four adult *P. viridis,* and 5) oyster spat with four adult *G. demissa* (Fig. 2).

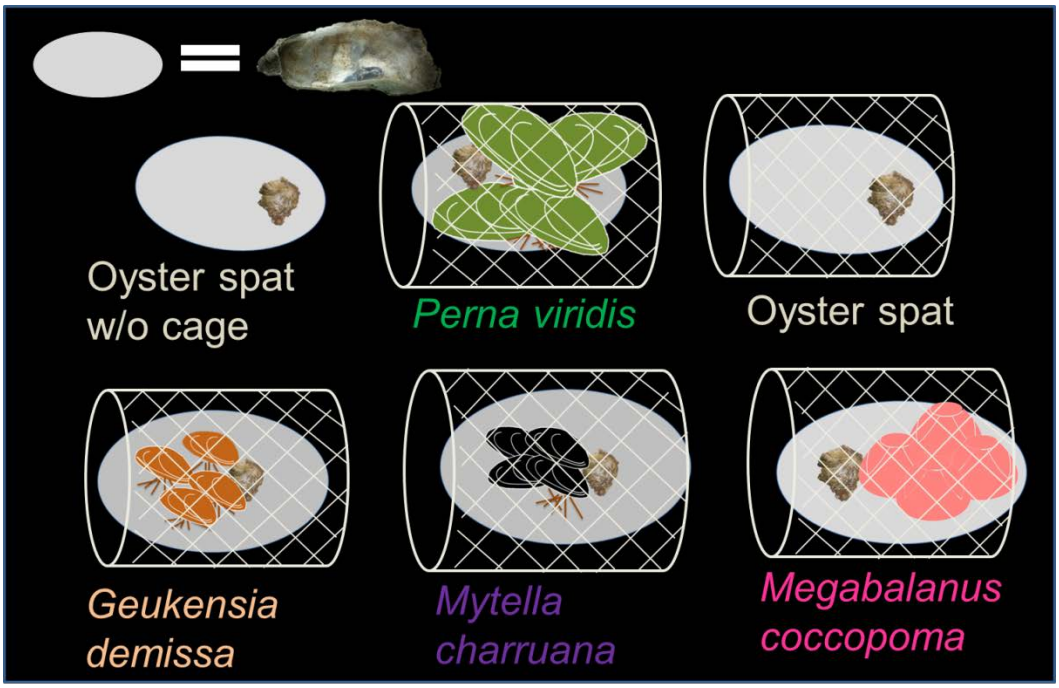


Fig. 2. Experimental design of oyster spat survival and growth in 2012 experiment. Total of 6 treatments (n = 20). One treatment of uncaged oyster spat and five caged treatments consisting of oyster spat, oyster spat with four adult *M. charruana*, oyster spat with four adult *M. coccopoma*, oyster spat with four adult *P. viridis*, and oyster spat with four adult *G. demissa*.

Mussels are gregarious; therefore, I used clusters of four mussels in both 2011 and 2012, as this was a better representation of the natural environment than a solitary mussel (Bayne et al. 1976). For each mussel treatment, mussels were allowed to attach via byssal threads to a disarticulated oyster shell with one oyster spat. The mussels all attached within 1 cm of the spat. Mean shell lengths and standard deviation of mussels used in these trials were: *M. charruana* (n = 20, 48 ± 7 mm), *P. viridis* (n = 40, 113 ± 28 mm), and *G. demissa* (n=20, 54 ± 12 mm). Similarly, the *M. coccopoma* (n = 20, mean height: 68 ± 8 mm) treatment consisted of a cluster of four barnacles glued onto a disarticulated oyster shell within 5 mm of the previously settled or glued oyster spat.

Cages were added in 2012 to reduce crab predation after a juvenile stone crab was found in the 2011 experiment. These cages were made of aquaculture grade Vexar$^{TM}$ mesh with 1.5 cm openings and constructed to fit within the experimental mesh bags. Oyster shells of each treatment were placed individually in aquaculture grade, 1.5 cm opening diamond-oriented-mesh bags (bag dimensions: 16 x 16 x 19 cm). One bag of each treatment was placed in a randomized order on PVC frames (50 x 50 cm) hung under a floating dock in New Smyrna Beach. Each PVC frame was 0.5 $m^2$ and all bags were attached to the PVC pipe that bisected the frame; bags were separated by 5 cm (Fig. 3). A total of 20 PVC frames floated on the surface with all experimental bags hanging at a depth of 25 cm. Oyster spat survival was recorded weekly for 6 weeks. Any dead barnacles and mussels were replaced once a week thoughout the 6-week experimental period.

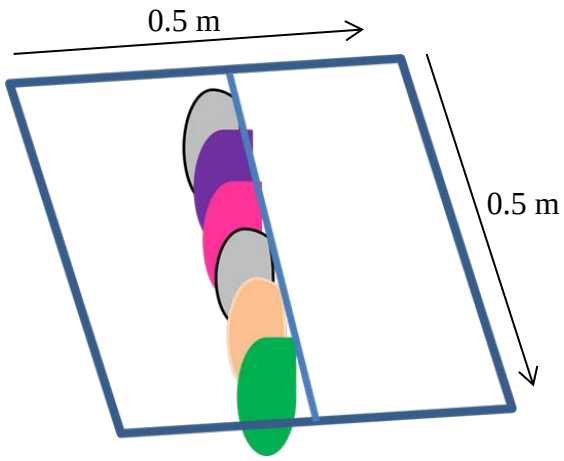


Fig. 3. Example of a frame design for 6 treatments in 2012 experiment for oyster spat survival and growth.

Growth was calculated for surviving oysters using the difference between the initial and final surface areas for each individual. I traced surface areas at the start and end of each experiment with a permanent, ultra-fine point marker onto plastic transparency sheets. The initial

and final surface area of each oyster spat were then digitized and calculated using ImageJ software (Abramoff et al. 2004).

Survival and growth data were analyzed in JMP statistical software (SAS Institute 2009). Kaplan-Meier survival analysis was used to compare survivorship (slopes of weekly survival over 6 weeks) and pairwise comparisons were performed to determine if significant differences were present in slopes among treatments (SAS Institute 2009). Final survival data at week 6 were analyzed with logistic regression. I analyzed the oyster spat growth data in the 2011 experiment with ANCOVA. This analysis incorporated the significant variation among the initial spat sizes (i.e., covariate). In the 2012 experiment, initial spat size was not significantly different among treatments (one-way ANOVA, $_{F\,2,\,41} = 0.8401$, $p = 0.439$). Therefore, one-way ANOVA was used to analyze the growth. Growth data was log transformed to meet test assumptions.

## CHAPTER THREE - RESULTS

### Oyster Larvae: Settlement

In 2011, oyster settlement was significantly affected by the presence of nonnative species in the flume (randomized-block ANOVA: $F_{2, 4} = 7.5$, $p = 0.0445$; Fig 4a; Table 2). Tukey's *a posteriori* tests showed the number of settled larvae was significantly reduced in treatments with the Asian green mussel *Perna viridis* (Linnaeus, 1758) and the pink titan acorn barnacle, *Megabalanus coccopoma* (Darwin, 1854) when compared to control shells of the eastern oyster, *C. virginica* (Germin, 1791). A difference among blocks was also found in the 2011 experiment (randomized-block ANOVA: $F_{2, 4} = 34.0$, $p = 0.0031$), coinciding with differences in batches of larvae received (Fig. 4b).

Table 2. ANOVA comparison of oyster larval settlement in 2011. **Bold** and * indicates a significant difference at $\alpha = 0.05$.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 2 | 24.0 | 12.0 | 7.5 | **0.0445*** |
| Block | 2 | 109.4 | 54.7 | 34.0 | **0.0031*** |
| Error | 4 | 6.4 | 1.6 | | |
| Total | 8 | 139.8 | | | |

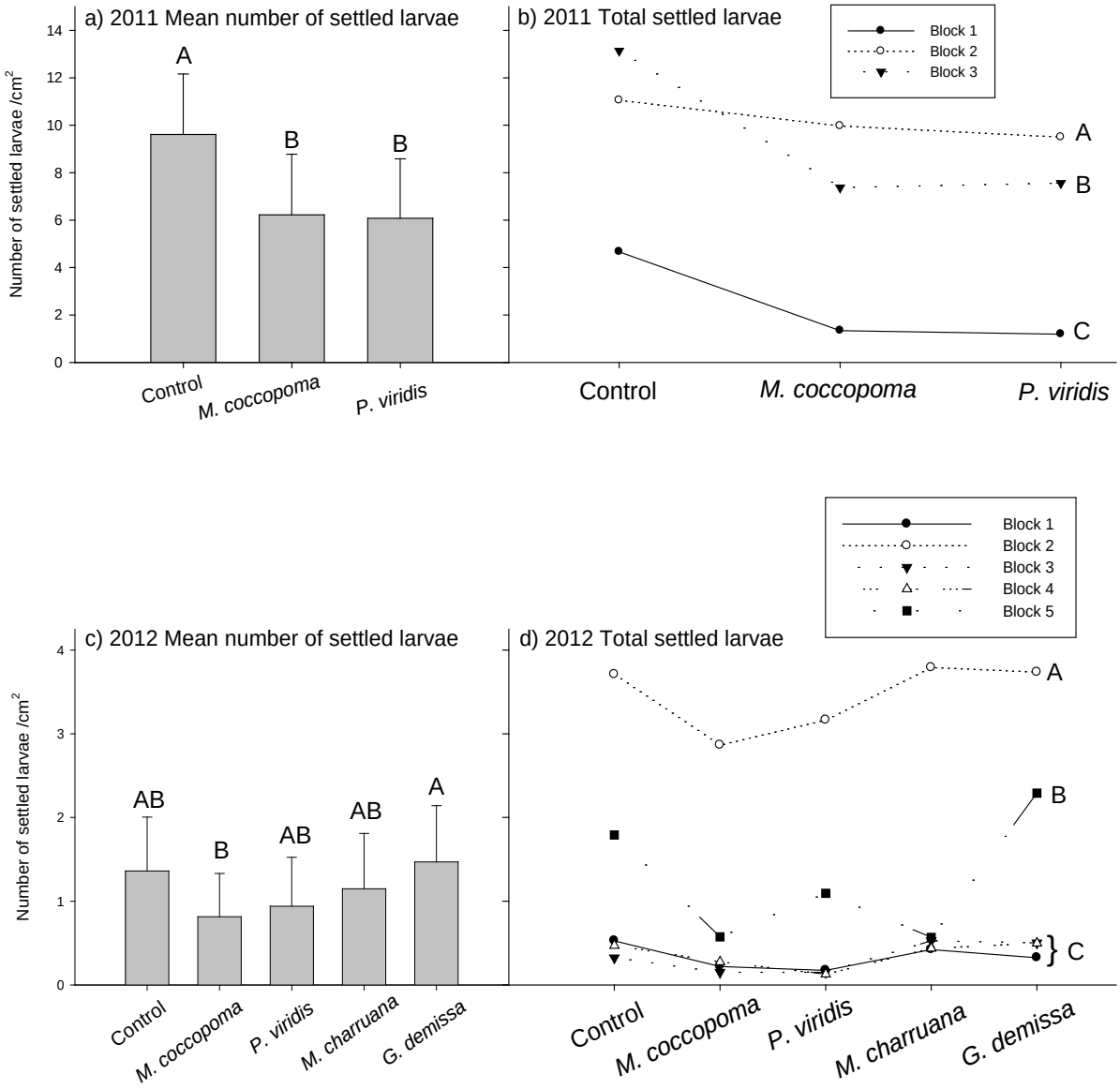


Fig. 4. Average number of settled larvae per $cm^2$ on shells of *C. virginica* alone (control) or attached to oyster shells with nonnative species attached. a) 2011 mean number of settled larvae (n=3), b) 2011 mean number of settled larvae for each block, c) 2012 mean number of settled larvae (n=5), and d) 2012 mean number of settled larvae for each block. Capitalized letters in graphs indicates significant differences at $\alpha = 0.05$ as determined by Tukey's *a posteriori* tests with Bonferroni corrections.

Similarly, in the 2012 experiment, oyster settlement was significantly affected by the presence of nonnative species in the flume (randomized-block ANOVA: $F_{4, 16} = 3.3$, $p = 0.0364$; Table 3, Figs. 4c). Tukey's *a posteriori* tests showed the number of settled larvae was significantly reduced by *M. coccopoma* as compared to *Geukensia demissa* (Dillwyn, 1817), but not as compared to the charru mussel *Mytella charruana* (d'Orbigny, 1846), *P. viridis* or the control shells of *C. virginica* only. A difference among blocks was also found in the 2012 experiment (randomized-block ANOVA: $F_{4, 16} = 80.1$, $p = 0.0001$; Table 3, Figs. 4d).

Table 3. ANOVA comparison of oyster larval settlement in 2012. **Bold** and * indicates a significant difference at $\alpha = 0.05$.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 4 | 1.5 | 0.4 | 3.3 | **0.0364*** |
| Block | 4 | 36.4 | 9.1 | 80.1 | **0.0001*** |
| Error | 16 | 1.8 | 0.1 | | |
| Total | 24 | 39.7 | | | |

**Oyster Larvae: Settlement preference**

For larvae that settled, there was no preference or avoidance of shells of *C. virginica* with attached mussels or barnacles in the 2011 experiment (One-way ANOVA: $F_{2, 6} = 3.3$, $p = 0.1100$; Fig. 5a; Table 4). However, in the 2012 experiment a significant difference was found among treatments in the number of settled oysters on control shells and shells with attached native or nonnative invertebrates (One-way ANOVA: $F_{4, 20} = 2.9$, $p = 0.0490$; Table 5]. Tukey's *a posteriori* test showed that shells with *M. charruana* had significantly less settlement than shells with attached *G. demissa* (Fig. 5b).

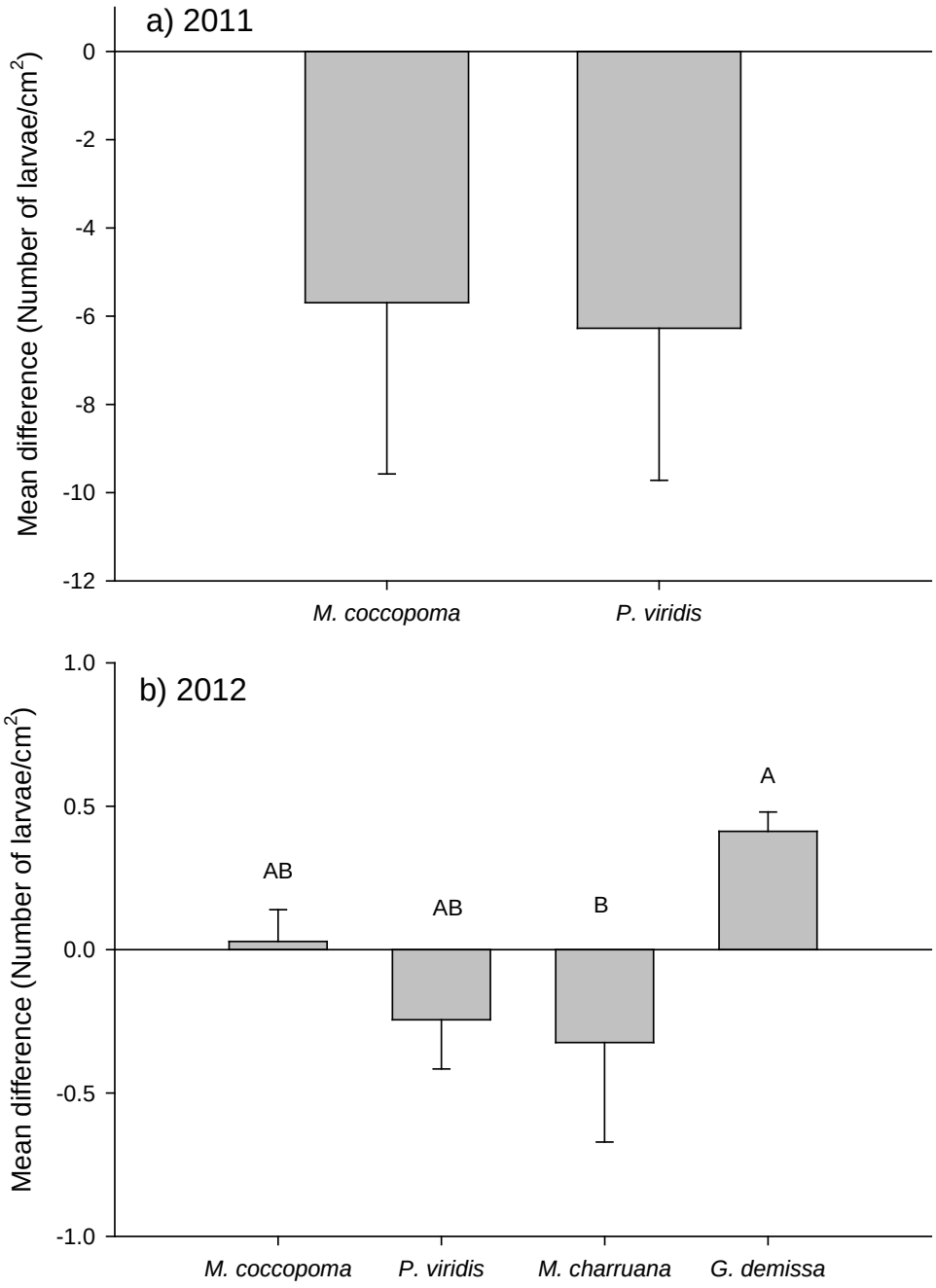


Fig. 5. Larval settlement preferences of nonnative species for a) 2011: *Megabalanus coccopoma* and *Perna viridis*, b) 2012: *P. viridis*, *M. coccopoma*, *Mytella charruana* and native *Geukensia demissa*. Positive values indicate oyster larvae settled more on disarticulated oyster shells with attached sessile species, while negative values indicate oyster larvae settled more on disarticulated oyster shells without attached sessile species (i.e., control).

Table 4. ANOVA comparison for oyster settlement preference in 2011.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 2 | 72.1 | 36.1 | 3.3 | 0.1100 |
| Error | 6 | 64.8 | 11.0 | | |
| Total | 8 | 137.9 | | | |

Table 5. ANOVA comparison for oyster settlement preference in 2012. **Bold** and * indicates a significant difference at $\alpha = 0.05$.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 4 | 1.7 | 0.4 | 2.9 | **0.0490*** |
| Error | 20 | 2.9 | 0.1 | | |
| Total | 24 | 4.5 | | | |

Further analyses were conducted on oyster shells with attached invertebrates by comparing the number of larvae settled on the oyster shell versus the shell of the barnacles and mussels (Fig. 6). In the 2012 experiment, oyster larvae preferred to settle on oyster shell over the shells of *M. charruana* (t-test: N= 250, p = 0.0159; Fig. 6e), *G. demissa* (t-test: N= 250, p = 0.0053; Fig. 6f) and *P. viridis* (t-test: N=250, p = 0.0410; Fig. 6c). However, the shells of *P. viridis* was not preferred in 2011 experiment (t-test: N = 150, p = 0.1468; Fig. 6a). The shells of *M. coccopoma* was not preferred or avoided by oyster larvae either year (Figs. 6b, d).

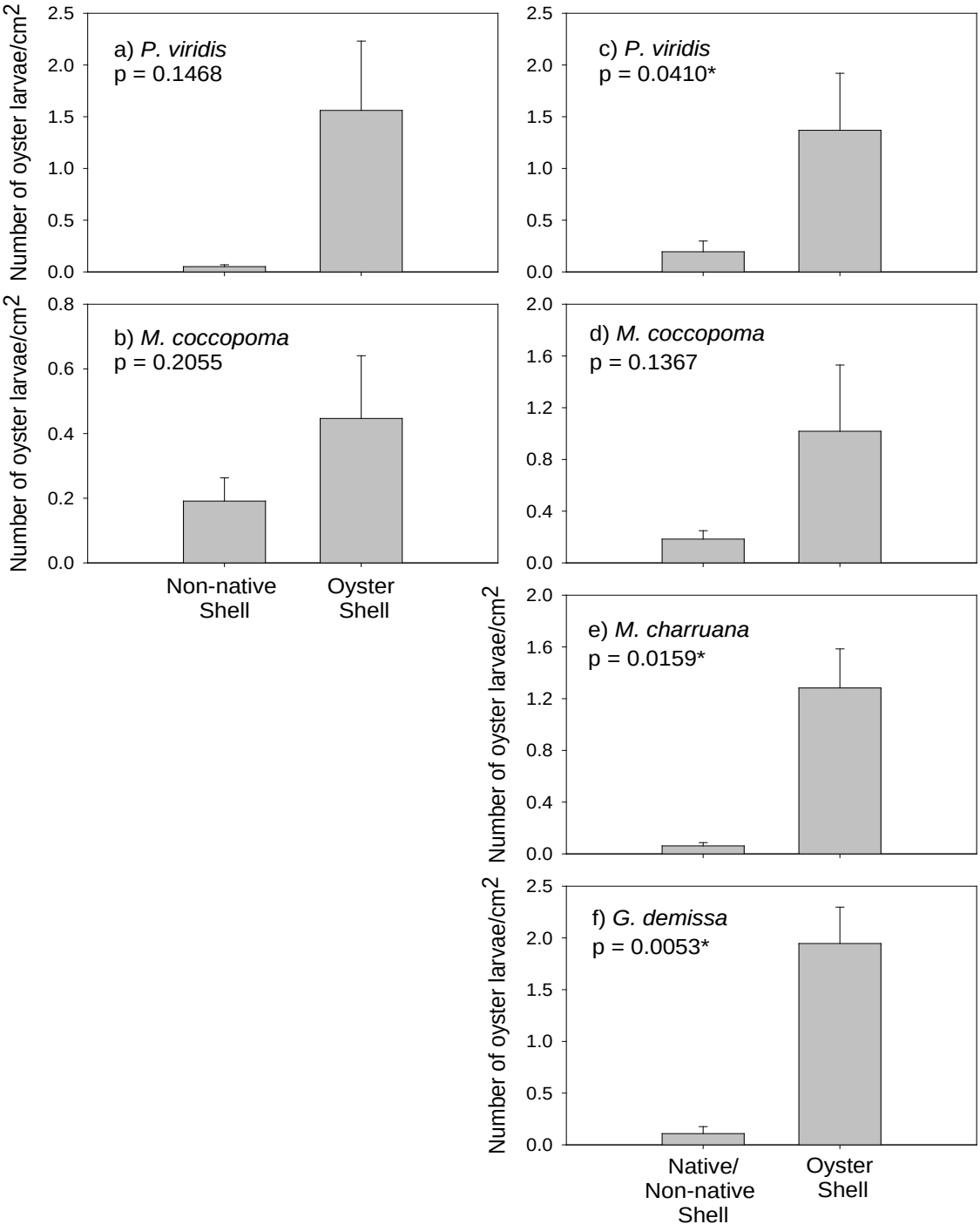


Fig. 6. Settlement location of oyster larvae on oyster shells with native or nonnative species attached to the shell. The number of settled larvae on nonnative species is shown for a) 2011: *Perna viridis*, b) 2011: *Megabalanus coccopoma*, c) 2012: *P. viridis*, d) 2012: *M. coccopoma*, e) 2012: *Mytella charruana* and f) 2012: native *Geukensia demissa*. Native/nonnative shell indicates number of oyster larvae settled on the shells of attached invertebrates. Oyster shell indicates number of oyster larvae settled on the oyster shell with invertebrates attached.

**Oyster Spat: Survival**

For the 2011 experiment, there was a significant difference in final oyster survival among all tested treatments (logistic regression: r = 0.1879, $chi^2$ = 13.4, p = 0.0002). Spat on the oyster shell - only treatment had a 90% survivorship at the end of experiment. The survivorship of spat on oyster shell was not significantly different from oyster shells with *M. coccopoma* (85% survivorship), but different from oyster shells with *P. viridis* (45% survival, Fig. 7a). In the 2012 experiment, there was also a significant difference in final survival among treatments (logistic regression: r = 0.0269, $chi^2$ = 4.35, p = 0.0411). Caged-control (60% survivorship) and uncaged-control (55% survivorship) treatments were not significantly different, suggesting that cages had no effect on oyster spat survival ($chi^2$ = 0.1023, p = 0.749). Spat surrounded by clusters of *P. viridis* had the lowest survival; only 20% of spat survived. In the presence of *M. charruana, M. coccopoma* and *G. demissa*, 25, 35, and 45% of oysters survived, respectively (Fig. 7b).

Kaplan-Meier survival analysis for the 2011 experiment showed that there was a significant difference (p = 0.0006) in spat survival slopes among the treatments over six weeks (Fig. 7a). Pairwise analyses then showed that there was a significant difference (p = 0.0061) between spat-only treatment and spat with *Perna viridis*. However, no significant difference in survivorship (p = 0.5537) was shown between control and spat in the *M. coccopoma* treatment over the course of the experiment (Fig. 7a). In 2012, the survival analysis again showed that there was a significant overall difference (p = 0.0060) in survivorship of spat among the treatments over six weeks. The two nonnative mussels, *P. viridis* and *M. charruana,* had the greatest impact (20 and 25% survived, respectively) on oyster spat survival. The survivorship curve for the spat with *M. charruana* showed a large decrease during the third to fourth week (Fig. 7b). The spat in the uncaged control treatment, the caged control treatment, and in the

native mussel treatment (*Geukensia demissa*) had similar survival curves with final survival of 55, 60, and 45% (Fig. 7b). The spat in the *Megabalanus coccopoma* treatment had a steady decline over the course of six weeks with a final survival of 35% (Fig. 7b).

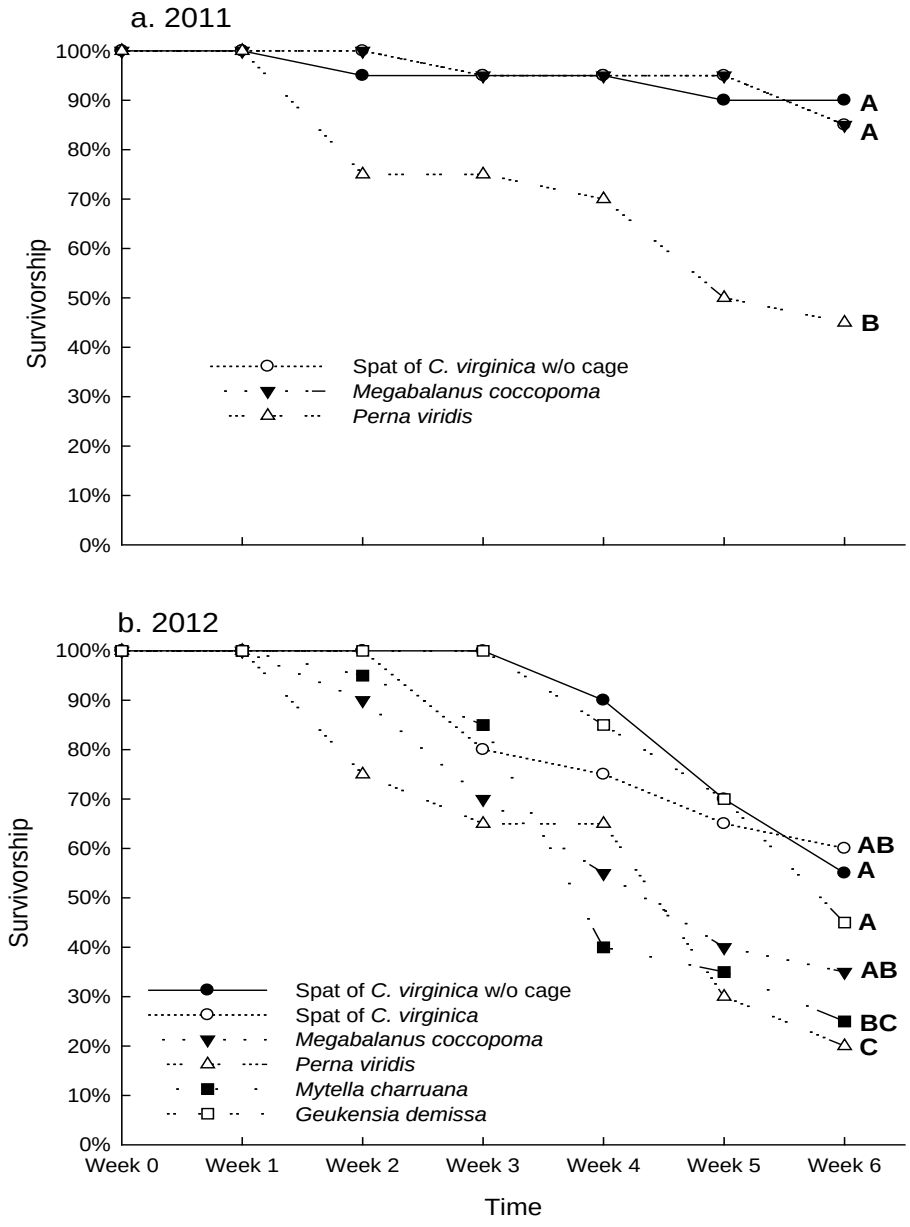


Fig. 7. Survival over time of oyster spat (*Crassostrea virginica*) exposed to native or nonnative species a) 2011: *Megabalanus coccopoma* and *Perna viridis* and b) 2012: *M. coccopoma. P. viridis*, *Mytella charruana* and *Geukensia demissa*. Cage control treatment was added in 2012. Treatments were compared using Kaplan-Meier multiple pairwise comparisons for survival over time; different letters at week 6 indicate significant differences at $\alpha = 0.05$.

**Oyster Spat: Growth**

With regard to oyster spat overall growth in the 2011 experiment, the initial spat size was significantly different among treatments (ANCOVA, $F_{1, 40} = 10.7$, $p = 0.0020$; Table 6; Fig. 8a) and treatment did not have an effect on growth ($F_{1, 40} = 2.3$, $p = 0.11$, Table 6; Fig. 8a) because the initial size of oyster spat influenced growth. The interaction of initial spat size and treatments was not significant (ANCOVA, $F_{2, 38} = 0.57$, $p = 0.57$); therefore, an interactive effect was not included in this analysis.

Table 6. ANOVA comparison for oyster spat growth in 2011. **Bold** and * indicates a significant difference at $\alpha = 0.05$.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Initial size (covariate) | 1 | 36.4 | 36.4 | 10.7 | **0.0020*** |
| Treatment | 2 | 15.6 | 7.8 | 2.3 | 0.1100 |
| Error | 40 | 135.8 | 3.4 | | |
| C. Total | 43 | 180.1 | | | |

Initial spat size was not significantly different among the tested treatments in 2012 experiment (One-way ANOVA, $F_{5, 114} = 0.0563$, $p = 0.9979$; Table 7). There was a significant difference in the growth of spat among treatments (One-way ANOVA, $F_{5, 42} = 3.5$, $p=0.0098$; Fig. 8b; Table 8). Tukey's *a posteriori* showed oyster growth in the presence of *M. charruana* was significantly slower than uncaged oyster spat, caged oyster spat, and spat with *G. demissa*. In 2012, cages had no effect on spat growth as no significant difference was detected between spat with and without cages (Tukey's *a posteriori:* $p = 0.7337$).

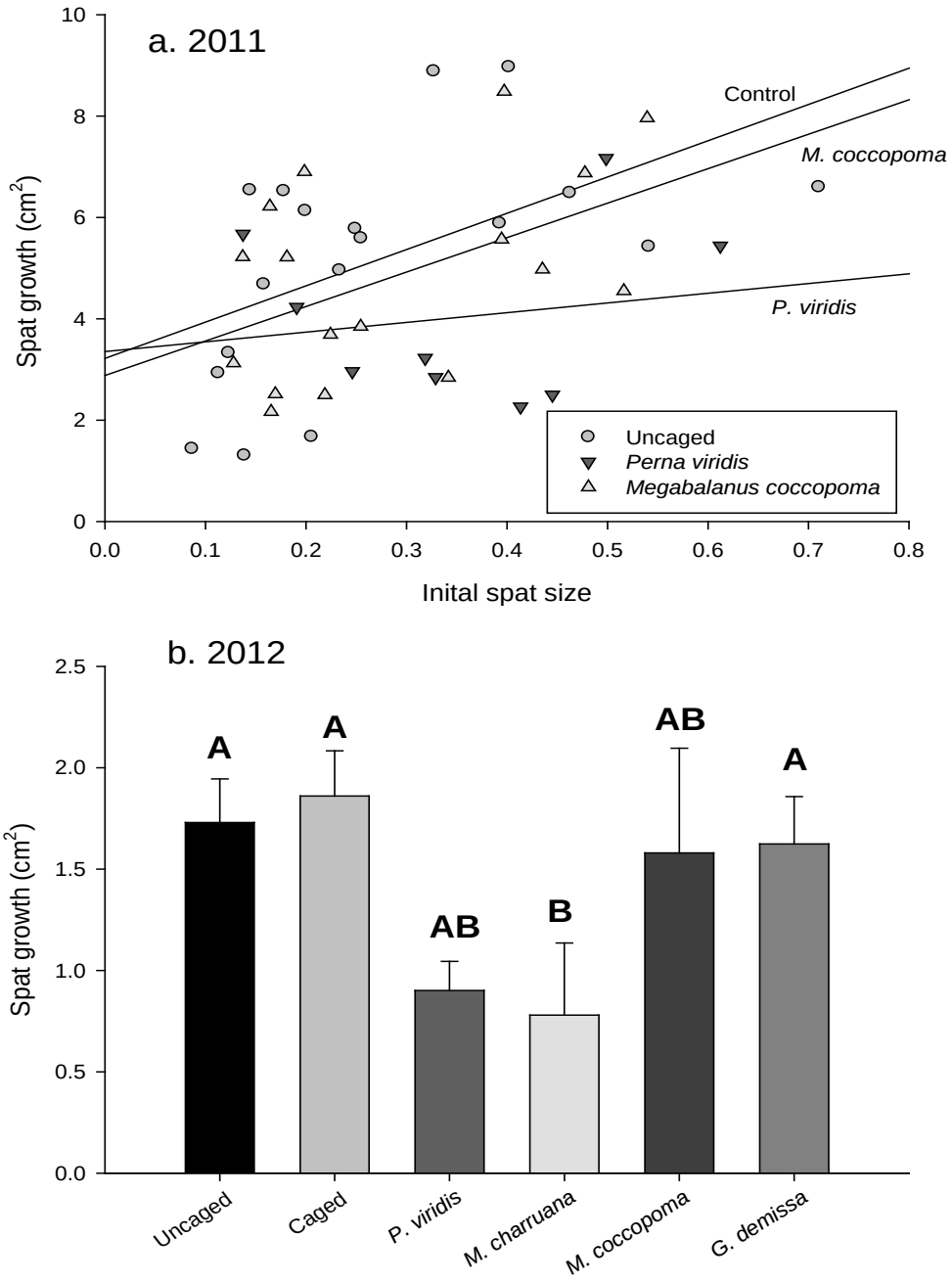


Fig. 8. Oyster spat growth of *Crassostrea virginica* after exposed to different nonnative species. a) 2011: *Megabalanus coccopoma* and *Perna viridis.* The treatments were analyzed with ANCOVA. The regression lines show best fit lines for each treatment. b) 2012: *M. coccopoma, P. viridis, Mytella charruana* and native *Geukensia demissa.* The treatments were analyzed with one-way ANOVA. Cage treatment for control was added in 2012. Treatments with different letters indicate significant differences at $\alpha = 0.05$ as determined by pairwise comparisons in 2011 and Tukey's *a posteriori* tests with Bonferroni corrections in 2012.

Table 7. ANOVA comparison for initial oyster spat size in 2012.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 5 | 0.0002 | 0.000038 | 0.1 | 0.9979 |
| Error | 114 | 0.1 | 0.000682 | | |
| Total | 119 | 0.1 | | | |

Table 8. ANOVA comparison for oyster spat growth in 2012. **Bold** and * indicates a significant difference at $\alpha = 0.05$.

| | DF | SS | MS | F-ratio | Prob > F |
|---|---|---|---|---|---|
| Treatment | 5 | 1.1 | 0.2 | 3.5 | **0.0098*** |
| Error | 42 | 2.5 | 0.1 | | |
| Total | 47 | 3.6 | | | |

## CHAPTER FOUR – DISCUSSION

This study sought to investigate how three recent marine invaders [(*Megabalanus coccopoma* (Darwin, 1854), *Perna viridis* (Linnaeus, 1758), and *Mytella charruana* (d'Orbigny, 1846)] impact two life history stages (larvae and spat) of the native eastern oyster *Crassostrea virginica* (Gmelin, 1791). I found that all three tested nonnative species negatively affected in some manner *C. virginica*. Two of the three species, *M. coccopoma* and *P. viridis,* reduced larval settlement and all mussel species [*M. charruana*, *P. viridis* and *Geukensia demissa* (Dillwyn, 1817)] influenced the attachment location for larvae that did settle (Table 9). In addition, spat survival was reduced by *M. charruana* and *P. viridis,* while spat growth was reduced when surrounded by *M. charruana* (Table 9). Below I discuss how these three nonnative species impact oyster settlement, survival and growth of oyster spat and compare these species to other known marine invaders. This study concludes by discussing the risks these species pose to the native oyster populations in the southeastern United States.

Table 9. Summary of results for oyster larval and spat experiment. Settlement refers to larval choice to settle or not settle. Settlement preference refers to any location preference made by oyster larvae.

| | **Larvae of *Crassostrea virginica*** | | | | **Spat of *Crassostrea virginica*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Settlement** | | **Settlement Preference** | | **Survival** | | **Growth** | |
| | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** |
| *M. coccopoma* | * | ** | X | X | X | X | X | X |
| *P. viridis* | * | X | X | * | * | * | X | X |
| *M. charruana* | - | X | - | * | - | * | - | * |
| *G. demissa* | - | X | - | * | - | X | - | X |

* indicates a significant difference between nonnative species and control.
** indicates a significant difference between nonnative species and native *G. demissa* only.
X indicates no statistical difference was found between treatment and control.
\- indicates not tested in 2011.

Chemicals that repel oyster settlement and the consumption of oyster larvae before settlement are two mechanisms that have been shown to reduce oyster settlement (Kennedy 1996). First, allelopathic chemicals (surface-bound or excreted) produced by benthic species have been found to repel larvae from settling on or near defended species (Goodbody 1961). For example, Johnson and Strathmann (1989) found larvae of *Balanus glandula* avoid settling on and near the snail *Nucella lamellosa*, a predator of barnacles, because of chemicals contained in the mucus they produced. Based on my results, the release of chemicals that prevented oyster larvae from settling is possible only for *P. viridis.* It is unlikely that *M. coccopoma* released deterrent chemicals because larvae were found both on and near *M. coccopoma*. Second, some large sessile species are known to prey on oyster larvae. Benthic predators such as sea anemones, barnacles, and ascidians are all predators of oyster larvae and, thus, reduce oyster larval settlement (MacKenzie 1977, Steinberg and Kennedy 1979, Osman et al. 1989; Kennedy 1996).

Steinberg and Kennedy (1979) observed that barnacles ingested as well as partially digested larvae found in their guts. In my study, predation by *M. coccopoma* and *P. viridis* was potentially a cause of the reduction of larval settlement. Egan and Anderson (1986) documented that adult *Balanus amphitrite* used their cirral fans to capture and prey on zooplankton, including oyster larvae. *Megabalanus coccopoma* was approximately twice the size of the barnacles used in the study by Egan and Anderson (1986), so it is possible that *M. coccopoma* was also able to consume oyster larvae. In other studies, predation by adult barnacles decreased larval settlement of benthic species by 65 - 100% (Anderson 1994, Navarrete and Wieters 2000). Much like barnacles, large marine mussels can ingest zooplankton, including larvae of invertebrates (Lehane and Davenport 2002). *Perna canaliculus,* a congener to *P. viridis,* has been found to consume zooplankton up to 430 μm in size (Zeldis et al. 2004), which is larger than the average size of oyster larvae (250 μm) used in my study.

For the oyster larvae that settled, individuals avoided shells of mussels and showed no settlement preference or avoidance of barnacle and oyster shells. This difference was not likely due to the composition of the shells because the shells of oysters, barnacles and mussels are all composed of calcium carbonate (Bourget 1987; Osman and Whitlatch 1995; Hamester et al. 2012). Preference for specific shell surface topographic complexity is a plausible explanation for the oyster larvae that settled on barnacle shells, but not mussel shells (Steinberg and Kennedy 1979; Osman and Whitlatch 1995). Surface topography often regulates the location where larvae settle (Walters and Wethey 1991, Walters 1992, Tamburri et al. 2008). Oyster and barnacle shells shared similar surface roughness and texture; creating small crevices for oyster larvae to settle (Osman and Whitlatch 1995). Additionally, Diederich (2005) found larvae of the Pacific oyster *C. gigas* preferred shells of oysters over mussels or a mixture of both. Even larvae of the

gregarious barnacle *Elminius modestus* preferred to settle on the shells of oysters over mussels as substratum (Kochmann et al. 2008).

In my study, the reductions in survival and growth of oyster spat may be, in part, the results of weight from *P. viridis* and *M. charruana* on oyster spat, as well as from byssal threads produced by the mussels that covered the shells of the oyster spat. Competition from epibiont organisms is one of the main causes of mortality and reduction in growth for some juvenile basibionts (Osman et al. 1989; Kennedy et al. 1996). First, calcareous epibionts can increase weight and decrease accessibility to resources (food, space) for basibionts (Wahl 1989). For example, Royer et al. (2006) found that epibionts, such barnacles, mussels, polychaetes and ascidians, can increase the total weight (oyster and epibionts) up to 35.2% within a six month period. Second, studies have shown that the production of byssal threads of mussels can restrict the amount of food availability for basibionts; this includes oysters and mussels (Zardi et al. 2006; Wahl 2008). In these studies, the epibionts decreased growth and eventually caused mortality of the basibionts (Zardi et al. 2006; Wahl 2008). Koganezawa (1972) found that clusters of three and seven mussels reduced oyster growth by 20% and 40%, respectively in the field. Similar to Kogenazawa (1972), I placed clusters of mussels (4/cluster) around each oyster spat. Additionally, I expected *P. viridis* to have equal or greater effect on oyster spat because *P. viridis* was twice the size of *M. charruana*. However, the reduction of spat growth was not observed with *P. viridis.* Although the lack of observable impact of *P. viridis* on oyster spat growth was counter to what I expected, this result was likely due to the fact that *P. viridis* affected spat differentially that resulted in spat mortality. This is evident from high mortality occurring within the first two weeks of the experiment in treatments with *P. viridis*, whereas, spat with *M. charruana* experienced higher mortality after week 3. Another surprising result was

that only *M. charruana* reduced oyster growth rate even though *M. charruana* and *G. demissa* were similarly sized. Invasive *M. charruana* may have greater detrimental effects on oyster spat than native *G. demissa* due to a higher production of byssal threads (Brodsky et al. 2011). Brodsky et al. (2011) found that *M. charruana* produced twice as many byssal threads as *G. demissa* at 23°C in 7 days. When sufficient numbers are present, byssal threads from mussels can decrease food consumption of basibionts by restricted bivalves from opening as well as hindering water flow (Haag et al. 1993).

In my study, I found that barnacles had no effect on the survival or growth of oyster spat. These results are counter to results from Boudreaux et al. (2009), also working in Mosquito Lagoon, who found that the presence of the native barnacle *B. eburneus* and the nonnative barnacle *B. amphitrite* reduced oyster spat growth. This result was independent of barnacle density. In the same study, oyster survival was negatively affected by high barnacle density but not by the presence of barnacles (Boudreaux et al. 2009). It is possible that higher densities of *M. coccopoma* than those used in in my study (clusters of 4/spat) are needed to impact oyster survival.

Differences in larval settlement and differences in spat survival of control treatments were found between experiments run in 2011 and 2012. First, higher density of oyster larvae settled in 2011 compared to 2012 in the larval experiment using a recirculating flume with local water. This was surprising as there were 10X more larvae used in 2012. It is possible that the brown tide alga *Aureoumbra lagunensis* influenced oyster settlement in 2012 as cell densities of *A. lagunensis* reached 3.2 billion/liter in Mosquito Lagoon during my study (St. Johns River Water Management District 2013). My larval settlement experiment in 2012 coincided with the first appearance of *A. lagunensis* in the Mosquito Lagoon, the source of the water used in my

experiment. Although the water used in the flume was filtered with a nominal opening of 25 μm, the smaller *A. lagunensis* (4-5 μm) would not be completely filtered out (Gobler and Sunda 2012). The variation between years in the proportions of settled larvae could also be due to differences in larval sources or the age of the larvae when tested. The larvae came from two different hatcheries; Virginia was the source of larvae for the 2011 experiment and Florida was the source in 2012. The hatcheries shared the same basic protocols but there were unavoidable differences in handling, equipment and shipping time. It is possible that the oyster larvae in the 2011 experiment were more competent to settle than in 2012. Second, one surprising conclusion was that there were observed year-to-year differences in how *P. viridis* impacted oyster larval settlement (Fig. 4). A statistically significant reduction was found in 2011 experiment, but not in 2012. However, the overall trend did show a small reduction in larval settlement in the presence of *P. viridis* ($p = 0.3199$). Third, there was a 35% difference in spat survival of the control treatments for the field experiment between the two years (Fig. 7). The additive effects of *A. lagunensis* plus high salinity (40 during my experiment) likely contributed to the differences in spat survival and growth. Gobler et al. (2013) found that *A. lagunensis* was associated with a significant reduction in the size of *C. virginica* that settled in summer of 2012 when compared to previous 3 years. Unlike the larval experiment in the flume where I was able to modify salinity, I was unable to adjust the salinity for the field experiment for spat survival and growth.

Like other sessile invaders, *M. coccopoma*, *P. viridis* and *M. charruana* are now established along the southeastern US and it would be almost impossible to eradicate all three populations (Thresher and Kuris 2004; Molnar et al. 2008). The densities of these nonnative species depend on environmental factors such as temperature and salinity (Yuan et al. 2010; Spinuzzi et al. 2013; Yuan et al. unpublished data) as well as propagule pressure (number of

introductions which is currently unknown). Years with warmer winters yielded higher counts of nonnative species (Spinuzzi et al. 2013); global climate change will likely influence the number of these nonnative species in the introduced range as the projections for temperature are increasing over time (Molnar et al. 2008; Lewis and Coutts 2010). The negative effects of nonnative species competing for resources can amplify the global stress already present for reefs of *C. virginica* (Beck et al. 2011; zu Ermgassen et al. 2013). My study showed that the three tested nonnative invertebrates do, in fact, negatively affect *C. virginica* during two different life-stages (i.e., larval and early juvenile). Given these negative effects, all three of these nonnative species should be categorized as invasive according to Executive Order 13112 (1999), whereby invasive species are defined as nonnative species that either cause economic or environmental harm or impact to human health. The combined impact of these challenges can put oysters in a vulnerable state, which may leave them more susceptible to the effects of other abiotic and biotic stressors. This study provides important insights that will help conserve remaining oyster reef habitats, as well as underscore the threats that invasive species pose to the conservation of this imperiled ecosystem.

## LIST OF REFERENCES


Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics international 11:36-43

Anderson AS, Bilodeau AL, Gilg MR, Hilbish TJ (2002) Routes of introduction of the Mediterranean mussel (*Mytilus galloprovincialis*) to Puget Sound and Hood Canal. J Shellfish Res 21:75– 79

Anderson DT (1994) Barnacles: structure, function, development and evolution. St. Edmundsbury Press Limited, Suffolk

Apte S, Holland BS, Godwin LS, Gardner JPA (2000) Jumping ships: a stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. Biol Invasions 2:75–79

Baker P, Fajans JS, Arnold WS, Ingrao DA, Marelli DC, Baker SM (2007). Range and dispersal of a tropical marine invader, the Asian green mussel, *Perna viridis*, in subtropical waters of the southeastern United States. J Shellfish Res 26:345-355

Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC, Lenihan HS, Luckenbach MW, Toropova CL, Zhang G, Guo X (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. BioScience 61:107-116

Boudreaux ML, Walters LJ (2006) *Mytella charruana* (Bivalvia: Mytilidae): A new, invasive bivalve in Mosquito Lagoon, Florida. Nautilus 120:34-36

Boudreaux ML, Walters LJ, Rittschof D (2009) Interactions between native barnacles, non-native barnacles, and the Eastern oyster *Crassostrea virginica*. Bull Mar Sci 84:43-57

Bourget, E (1987) Barnacle shells: composition, structure and growth. In: Southward AJ (ed) Barnacle Biology. 1st edn. A.A. Balkema, Netherlands, pp 267-285

Branch GM, Steffani CN (2004) Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J Exp Mar Biol Ecol 300:189-215

Brodsky S, Walters L, Schneider K, Hoffman E (2011) Cold Temperature effects on byssal thread production by the native mussel *Geukensia demissa* versus the non-native mussel *Mytella charruana*. Univ Central Fla Undergrad Res J 5:1-10

Crisp DJ (1961) Territorial behaviour in barnacle settlement. J Exp Biol 38:429-446

Denley EJ, Underwood AJ (1979) Experiments on factors influencing settlement, survival, and growth of two species of barnacles in New South Wales. J Exp Mar Biol Ecol 36:269-293

Diederich S (2005) Differential recruitment of introduced Pacific oysters and native mussels at the North Sea coast: coexistence possible? J Sea Res 53:269-281

Diederich S (2006) High survival and growth rates of introduced Pacific oysters may cause restrictions on habitat use by native mussels in the Wadden Sea. J Exp Mar Biol Ecol 328: 211-227

Egan EA, Anderson DT (1986) Larval development of *Balanus amphitrite* Darwin and *Balanus variegatus* Darwin (Cirripedia, Balanidae) from New South Wales, Australia. Crustaceana 51:188-207

Executive Order 13112 (1999) Invasive species. Federal Register 64:6183-6186

Galil BS, Clark PF, Carlton JT (eds) (2011) In the wrong place-alien marine crustaceans: distribution, biology and impacts. Invading Nature – Springer Series in Invasion Ecology 6. Doi: 10.1007/978-94-007-0591-3

Gilg MR, Lukaj E, Abdulnour M, Middlebrook M, Gonzalez E, Turner R, Howard R (2010) Spatio-temporal settlement patterns of the non-native titan acorn barnacle, *Megabalanus coccopoma*, in northeastern Florida. J Crustac Biol 30:146-150

Gobler CJ, Sunda WG (2012) Ecosystem disruptive algal blooms of the brown tide species, *Aureococcus anophagefferens* and *Aureoumbra lagunensis*. Harmful Algae 14:36-45

Gobler, CJ, Koch F, Kang Y, Berry DL, Tang YZ, Lasi M, Walters L, Hall L, Miller JD (2013) Expansion of harmful brown tides caused by the pelagophyte, *Aureoumbra lagunensis* DeYoe et Stockwell, to the US east coast. Harmful Algae 27:29-41

Goodbody I (1961) Continuous breeding in three species of tropical ascidian. P Zool Soc Lond 136:403-409

Haag WR, Berg DJ, Garton DW, Farris JL (1993) Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can J Fish Aquat Sci 50:13-19

Hamester MRR, Balzer PS, Becker D (2012) Characterization of calcium carbonate obtained from oyster and mussel shells and incorporation in polypropylene. Mat Res 15:204-208

Heath DD, Rawson PD, Hilbish TJ (1995) PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. Can J Fish Aquat Sci 52:2621-2627

Hockey PAR, van Erkom Schurink C (1992) The invasive biology of the mussel *Mytilus galloprovincialis* on the southern African coast. Trans R Soc S Afr 48:123-139

Johnson LE, Strathmann RR (1989) Settling barnacle larvae avoid substrata previously occupied by a mobile predator. J Exp Mar Biol Ecol 128:87-103

Kennedy VS, Newell RI, Eble AF (1996) The eastern oyster: *Crassostrea virginica*. Maryland Sea Grant College, College Park, MD

Kerckhof F (2002) Barnacles (Cirripedia, Balanomorpha) in Belgian waters, an overview of the species and recent evolutions, with emphasis on exotic species. Bull Kon Belg Inst Natuurwet Biologie 72:93-104

Kerckhof F, Haelters J, Degraer S (2010) The barnacles *Chirona* (Striatobalanus) *amaryllis* (Darwin 1854) and *Megabalanus coccopoma* (Darwin 1854) (Crustacea, Cirripedia): two invasive species new to tropical West African waters. Afr J Mar Sci 32:265-268

Kimbro DL, Grosholz ED, Baukus AJ, Nesbitt NJ, Travis NM, Attoe S, Coleman-Hulbert C (2009) Invasive species cause large-scale loss of native California oyster habitat by disrupting trophic cascades. Oecologia, 160:563-575

Kochmann J, Buschbaum C, Volkenborn N, Reise K (2008) Shift from native mussels to alien oysters: differential effects of ecosystem engineers. J Exp Mar Biol Ecol 364:1-10

Koganezawa A (1972) Ecological studies at a seed oyster production area. II. Studies on the spawning condition of the pacific oyster in Matsushima Bay. Bull Jpn Soc Sci Fish 38:1315-1324

Lacombe D, Monteiro W (1974) Balanídeos como indicadores de poluição na Baía de Guanabara. Ver Bras Biol Rio de Janeiro 34:633-644

Lee HG (1987) Immigrant mussel settles in Northside Generator. Jacksonville Shell Club website. Url: http://www.jaxshells.org/immigran.htm Accessed 10 October 2013

Lehane C, Davenport J (2002) Ingestion of mesozooplankton by three species of bivalve; *Mytilus edulis, Cerastoderma edule* and *Aequipecten opercularis*. J Mar Biol Ass U.K. 82:615-619

Lewis JA, Coutts ADM (2010) Biofouling Invasions, In: Dürr S, Thomason CJ (eds) Biofouling, Wiley-Blackwell, Oxford pp 348-365

Lockwood LJ, Hoopes MF, Marchetti MP (2007) Invasion ecology. Blackwell, Malden, MA

Mackenzie CL (1977) Sea anemone predation on larval oysters in Chesapeake Bay (Maryland). In Proc Natl Shellfish Assoc 67:113-117

Markert A, Wehrmann A, Kröncke I (2010) Recently established *Crassostrea*-reefs versus native *Mytilus*-beds: differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight). Biol Invasions 12:15-32.

Marsden JR (1991) Responses of planktonic larvae of the serpulid polychaete *Spirobranchus polycerus* var. *augeneri* to an alga, adult tubes and conspecific larvae. Mar Ecol Prog Ser 71:245-251

McDonald JH, Koehn RH (1988) The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Mar Biol 99:111-118

McDonald JH, Seed R, Koehn RH (1991) Allozyme and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar Biol 111:323–335

Minchinton TE, Scheibling RE (1993) Free-space availability and larval substratum selection as determinants of barnacle population-structure in a developing rocky intertidal community. Mar Ecol Prog Ser 95:233-244

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front in Ecol Environ 6:485-492

Nalepa TF, Fahnenstiel GL (1995) *Dreissena polymorpha* in the Saginaw Bay, Lake Huron ecosystem: overview and perspective. J Great Lakes Res 21:411-416

Navarrete SA, Wieters EA (2000) Variation in barnacle recruitment over small scales: larval predation by adults and maintenance of community pattern. J Exp Mar Biol Ecol 253:131-148

Newman WA, McConnaughey RR (1987) A tropical eastern pacific barnacle, *Megabalanus coccopoma* (Darwin), in southern California, following El Niño 1982-83. Pac Sci 41:1-4

Osman RW, Whitlatch RB (1995) The influence of resident adults on recruitment: a comparison to settlement. J Exp Mar Biol Ecol 190:169-198

Osman RW, Whitlatch RB, Zajac RN (1989) Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. Mar Ecol Prog Ser 54:61-73

Perrings C, Burgiel S, Lonsdale M, Mooney H, Williamson M (2010) International cooperation in the solution to trade-related invasive species risks. Annals NY Acad Sci 1195:198-212

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Eco Econ 52:273-288

Power AJ, Walker RL, Payne K, Hurley D (2004) First occurrence of the nonindigenous green mussel, *Perna viridis* (Linnaeus, 1758) in coastal Georgia, United States. J Shellfish Res 23:741-744

Rajagopal S, Venugopalan VP, van der Velde G, Jenner HA (2006) Greening of the coasts: a review of the *Perna viridis* success story. Aquat Ecol 40:273-297

Ramirez SC, Ca´ceros-Martı´nez J (1999) Settlement of the blue mussel *Mytilus galloprovincialis* Lamarck on artificial substrates in Bahı´a de Todos Santos, B.C., Me´xico. J Shellfish Res 18:33–39

Reise K (1998) Pacific oysters invade mussel beds in the European Wadden Sea. Senckenb Marit 28:167–175

Royer J, Ropert M, Mathieu M, Costil K (2006) Presence of spionid worms and other epibionts in Pacific oysters (*Crassostrea gigas*) cultured in Normandy, France. Aquaculture 253:461-474

Ruiz GM, Carlton JT (eds) (2003) Invasive species: vectors and management strategies. Island Press, Washington, DC

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Am Zool 37:621–32

Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014) Ocean acidification increases the vulnerability of native oysters to predation by invasive snails. P Roy Soc B: Biol Sci 281:20132681

SAS Institute Inc. 2009. JMP 10. Cary, NC

Schloesser DW, Nalepa TF (1994) Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. Can J Fish Aquat Sci 51:2234-2242

Sidall SE (1980) Clarification of the genus *Perna*. Bull Mar Sci 30:858-870

Southward AJ, Burton RS, Coles SL, Dando PR, DeFelice R, Hoover J, Parnell PE, Yamaguchi T, Newman WA (1998) Invasion of Hawaiian shores by an Atlantic barnacle. Mar Ecol Prog Ser 165:119-278

Spinuzzi S, Schneider KR, Walters LJ, Yuan WS, Hoffman EA (2013) Tracking the distribution of non-native marine invertebrates (*Mytella charruana*, *Perna viridis* and *Megabalanus coccopoma*) along the south-eastern USA. Marine Biodiversity Records 6:55-68

St. Johns River Water Management District (2013) Brown tide bloom in northern lagoon. Current lagoon news. Url: http://floridaswater.com/itsyourlagoon/currentnews.html Accessed 25 January 2014

Steinberg PD, Kennedy VS (1979) Predation upon *Crassostrea virginica* (Gmelin) larvae by 2 invertebrate species common to Chesapeake Bay oyster bars. Veliger 22:78-84

Tamburri MN, Finelli CM, Wethey DS, Zimmer-Faust RK (1996) Chemical induction of larval settlement behavior in flow. Biol Bull 191:367-373

Tamburri MN, Luckenbach MW, Breitburg D L, Bonniwell SM (2008) Settlement of *Crassostrea ariakensis* larvae: Effects of substrate, biofilms, sediment and adult chemical cues. J Shellfish Res 27:601-608

Thomas CD, Ohlemüller R (2010) Climate change and species' distributions: an alien future? In: Perrings C, Mooney H, Williamson M (eds) Bioinvasions and Globalization: Ecology, Economics, Management and Policy. Oxford University Press, Oxford, pp 19-29

Thresher RE, Kuris AM (2004) Options for managing invasive marine species. Biol Invasions 6:295-300

Underwood AJ, Denley EJ (1984) Paradigms, explanations, and generalizations in models for the structure of intertidal communities on rocky shores. In: Simberloff D, Strong R, Abele L, Thistle AR (eds) Ecological communities: conceptual issues and the evidence. Princeton Univ Press, New Jersey pp 151-180

Vakily JM (1989) The biology and culture of mussels of the genus *Perna*. Internal Center for Living Aquatic Recourses Management, Manila, Philippines

Vario SL, Koehn RK, Vaino la R (1988) Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar Biol 98:51– 60

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. Mar Ecol Prog Ser 58:175-189

Wahl M (2008) Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. Biofouling 24:427-438

Walters LJ (1992) Post-settlement success of the arborescent bryozoan *Bugula neritina* L.: the importance of structural complexity. J Exp Mar Biol Ecol 164:55-71

Walters LJ, Wethey DS (1991) Settlement, refuges, and adult body form in colonial marine invertebrates: a field experiment. Biol Bull 180:112-118

Wonham MJ (1999) Factors limiting the spread of the introduced Mediterranean mussel *Mytilus galloprovincialis* on Washington's outer coast. In: Peterson J (ed) Marine Bioinvasions. Proceedings of the First National Conference. Massachusetts Institute of Technology, Cambridge, pp 127– 129

Yamaguchi T, Prabowo RE, Ohshiro Y, Shimono T, Jones D, Kawai H, Otani M, Oshino A, Inagawa S (2009) The introduction to Japan of the Titan barnacle, *Megabalanus coccopoma* (Darwin, 1854) (Cirripedia: Balanomorpha) and the role of shipping in its translocation. Biofouling 25:325-333

Yuan W, Walters LJ, Schneider KR, Hoffman EA (2010) Exploring the survival threshold: a study of salinity tolerance of the nonnative mussel *Mytella charruana.* J Shellfish Res 29:415-422

Zabin CJ (2009) Battle of the barnacle newcomers: niche compression in invading species in Kaneohe Bay, Oahu, Hawaii. Mar Ecol Progr Ser 381:175-182

Zabin CJ, Altieri A (2007) A Hawaiian limpet facilitates recruitment of a competitively dominant invasive barnacle. Mar Ecol Progr Ser 337:175-185

Zardi GI, Nicastro KR, McQuaid CD, Rius M, Porri F (2006) Hydrodynamic stress and habitat partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels: constraints of an evolutionary strategy. Mar Biol 150:79-88

Zardus JD, Hadfield MG (2005) Multiple origins and incursions of the Atlantic barnacle *Chthamalus proteus* in the Pacific. Mol Ecol 14:3719-3733

Zeldis J, Robinson K, Ross A, Hayden B (2004) First observations of predation by New Zealand Greenshell mussels (*Perna canaliculus*) on zooplankton. J Exp Mar Biol Ecol 311:287-299

zu Ermgassen PSE, Spalding MD, Grizzle RE, Brumbaugh RD (2013) Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. Est Coast 36:36-43