Spring 2016

Effects of Non-Native Species on Two Life Stages of the Olympia Oyster, Ostrea Lurida, in the Elkhorn Slough Estuary

Pamela Ann Neeb Wade
California State University, Monterey Bay

Follow this and additional works at: http://digitalcommons.csumb.edu/caps_thes

Recommended Citation

This Master's Thesis is brought to you for free and open access by Digital Commons @ CSUMB. It has been accepted for inclusion in Capstone Projects and Theses by an authorized administrator of Digital Commons @ CSUMB. Unless otherwise indicated, this project was conducted as practicum not subject to IRB review but conducted in keeping with applicable regulatory guidance for training purposes. For more information, please contact digitalcommons@csumb.edu.
EFFECTS OF NON-NATIVE SPECIES ON TWO LIFE STAGES OF THE OLYMPIA OYSTER, *OSTREA LURIDA*, IN THE ELKHORN SLOUGH ESTUARY

A Thesis
Presented to the
Faculty of the
Moss Landing Marine Laboratories
California State University Monterey Bay

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Marine Science

by
Pamela Ann Neeb Wade
Spring 2016
CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the

Thesis of Pamela Ann Neeb Wade:

EFFECTS OF NON-NATIVE SPECIES ON TWO LIFE STAGES OF THE OLYMPIA OYSTER, *OSTREA LURIDA*, IN THE ELKHORN SLOUGH ESTUARY

_____________________________________________
Jonathan Geller, Professor
San Jose State University, Moss Landing Marine Laboratories

_____________________________________________
Scott Hamilton, Assistant Professor
San Jose State University, Moss Landing Marine Laboratories

_____________________________________________
Kerstin Wasson, Research Coordinator
Elkhorn Slough National Estuarine Research Reserve

Digitally signed by Kris Roney, Ph.D.

Kris Roney, Dean
Associate VP of Academic Programs

_____________________________________________
Approval Date
DEDICATION

This work is dedicated to my grandmother Catherine Harner, who will always be an inspiration to me. She shared with me her passion for nature, hard work, education and lifelong learning.
“Eventually man, too, found his way back to the sea. Standing on its shores, he must have looked out upon it with wonder and curiosity, compounded with an unconscious recognition of his lineage. He could not physically re-enter the ocean as the seals and whales had done. But over the centuries, with all the skill and ingenuity and reasoning powers of his mind, he has sought to explore and investigate even its most remote parts, so that he might re-enter it mentally and imaginatively.”

— Rachel Carson, *The Sea Around Us*
ABSTRACT

EFFECTS OF NON-NATIVE SPECIES ON TWO LIFE STAGES OF THE OLYMPIA OYSTER, OSTREA LURIDA, IN THE ELKHORN SLOUGH ESTUARY

by

Pamela Ann Neeb Wade
Master of Science in Marine Science
California State University Monterey Bay, 2016

Marine and estuarine systems worldwide have been altered due to introductions of non-native species. A common mode of non-native species transport is in ship ballast water that, when released, deposits larvae in new locations. Once established, non-native species compete for resources, reduce native species populations and alter ecosystem services. Some non-native species have been found to be ecosystem engineers, encouraging additional non-native species settlement. Many studies have looked at the competitive interaction between non-native and native species, but few have examined the possibility of a facilitative effect or how additional environmental stressors, such as desiccation, may modify species interactions.

This study examined the effect of non-native species on two life stages (adult and juvenile) of the Olympia oyster, Ostrea lurida, a species in decline at two different tidal heights in a central California estuary. In separate experiments, adult and juvenile O. lurida were outplanted on settlement plates in Elkhorn Slough, and two treatments were maintained: (1) manual removal of non-natives every two weeks from half the plates or (2) control treatments that allowed non-native species to colonize and persist. Every two weeks, photographs were taken of each plate in order to calculate changes in percent cover of non-native species and to measure growth of O. lurida in response to the experimental treatment conditions. Results indicated that in the presence of the Australian tubeworm, Ficopomatus enigmaticus, and other non-native species, adult O. lurida exhibited an ~50% increase in area (cm^2) on average across the plates. The presence of non-natives consistently resulted in a neutral or positive facilitative effect on oyster growth at two different life stages. The results of this study show that adult O. lurida are able to survive and even benefit from the presence of other species currently found in Elkhorn Slough both above and below MLLW. Juvenile O. lurida demonstrated high initial growth regardless of treatment, with increased growth below MLLW. With this increased understanding, there is much that is unknown about the long-term affect of introduced species on ecosystems and it is important to continue to study their impacts. In particular, the effect of non-natives on oyster recruitment requires further investigation because the earliest life stages are most likely to be adversely affected by a rapid growing non-native that can monopolize available bare space for settlement. If O. lurida recruitment and juvenile survivorship is further reduced, the local Elkhorn Slough population may be lost.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER I- EFFECTS OF NON-NATIVE SPECIES ON ADULT OLYMPIA OYSTERS</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>8</td>
</tr>
<tr>
<td>Study location</td>
<td>8</td>
</tr>
<tr>
<td>Survey</td>
<td>8</td>
</tr>
<tr>
<td>Field experiment (adult <em>Ostrea lurida</em> with <em>Ficopomatus enigmaticus</em>)</td>
<td>9</td>
</tr>
<tr>
<td>Field experiment (adult <em>Ostrea lurida</em> with non-native species)</td>
<td>12</td>
</tr>
<tr>
<td>Genetic analysis</td>
<td>13</td>
</tr>
<tr>
<td>Results</td>
<td>15</td>
</tr>
<tr>
<td>Discussion</td>
<td>26</td>
</tr>
<tr>
<td>CHAPTER II- EFFECTS OF NON-NATIVE SPECIES ON JUVENILE OLYMPIA OYSTERS</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>30</td>
</tr>
<tr>
<td>Methods</td>
<td>32</td>
</tr>
<tr>
<td>Laboratory</td>
<td>33</td>
</tr>
<tr>
<td>Field</td>
<td>35</td>
</tr>
<tr>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>41</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>44</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>50</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Railroad bridge vertical quadrat samples ................................................................. 16
Table 2. Community distribution on settlement plates ......................................................... 22
Table 3. Molecular identifications of organisms ................................................................. 51
LIST OF FIGURES

PAGE

Figure 1. Representation of experimental layout at Hudson’s Landing ..........................................................11
Figure 2. Adult *Ostrea lurida* with *Ficopomatus enigmaticus* on settlement plates .................................12
Figure 3. Adult *Ostrea lurida* with non-native species on settlement plates .........................................13
Figure 4. Percent cover of *Ficopomatus enigmaticus* ............................................................................17
Figure 5. *Ostrea lurida* area (cm²) in the presence and absence of *Ficopomatus enigmaticus* ..........18
Figure 6. Percent change in area (mm²/d) of *Ostrea lurida* in presence and absence of *Ficopomatus
enigmaticus* ........................................................................................................................................19
Figure 7. Percent change in area (mm²/d) of *Ostrea lurida* in presence and absence of *Ficopomatus
enigmaticus*, after *F. enigmaticus* reached >50% at -0.3 m MLLW ................................................20
Figure 8. Non-native species succession ......................................................................................................21
Figure 9. Percent cover of non-native species ..............................................................................................23
Figure 10. *Ostrea lurida* area (cm²) in the presence and absence of non-native species ....................24
Figure 11. Percent change in area (mm²/d) of *Ostrea lurida* in the presence and absence of non-native
species .....................................................................................................................................................25
Figure 12. Percent change in area (mm²/d) of *Ostrea lurida* in presence and absence of *Ficopomatus
enigmaticus*, after *F. enigmaticus* reached >50% at -0.3 m MLLW ................................................26
Figure 13. Broodstock and sump tanks .........................................................................................................34
Figure 14. Juvenile *Ostrea lurida* settlement plates (laboratory) .............................................................35
Figure 15. Juvenile *Ostrea lurida* settlement plates (field experiment) ......................................................36
Figure 16. Percent cover of *F. enigmaticus* on juvenile *Ostrea lurida* settlement plates ..................38
Figure 17. Juvenile *Ostrea lurida* area (cm²) in presence and absence of *F. enigmaticus* ..........39
Figure 18. Percent change in area (cm²/d)) of juvenile *Ostrea lurida* in presence and absence of *Ficopomatus enigmaticus* ..................................................................................................40
Figure 19. Percent change in area (cm²/d)) of juvenile *Ostrea lurida* in presence and absence of *Ficopomatus enigmaticus*, after *F. enigmaticus* reached >25% at -0.3 m MLLW ..............................41
ACKNOWLEDGEMENTS

This research was made possible by the funds from the James Nybakken award and the David and Lucile Packard Foundation. I would like to thank my advisor Jonathan Geller for his support and for giving me the opportunity to pursue a Master’s degree while working full time. Thank you to my committee members Scott Hamilton and Kerstin Wasson. I greatly appreciated Scott’s guidance with the data analysis. Thank you to Kerstin for introducing me to field sites in Elkhorn Slough and for introducing me to the world of oyster research. I would like to thank Chela Zabin for letting me join her in the field and for sharing materials. Thank you to Jillian Bible and Brian Cheng from Bodega Marine Laboratories for their guidance on oyster broodstock and encouragement.

Thank you to all my colleagues, friends and former students who joined me in the mud collecting data, no matter what time of day or type of weather. Thank you to Nicole Barbour and UROC.

Thank you to the students of the MLML Invertebrate Zoology Laboratory for their encouragement and friendship, and to Melinda Wheelock and Tracy Campbell for helping me with the genetic analysis. I have been so grateful to have the support and understanding of my colleagues in the Education Department at the Monterey Bay Aquarium. Thank you to my friend JR Sosky for photographing my fieldwork.

Thank you to my dear friends and family for their unending support. Thank you most of all to my husband Patrick and to our family. This work wouldn’t have been possible without your continuous love, patience, and support.
CHAPTER I

EFFECTS OF NON-NATIVE SPECIES ON ADULT OLYMPIA OYSTERS

INTRODUCTION

Worldwide, many ecosystems are highly invaded and are undergoing changes due to the presence and proliferation of non-native species (Ruiz et al. 1997; Ruiz et al. 1999). Non-native species are responsible for reducing the size of the native populations, altering community structure and ecosystem function (Mooney and Drake 1986; OTA 1993; Ruiz et al. 1997). In the waters around the continental United States alone, there are approximately 400 non-native species in marine and estuarine systems (Cohen and Carlton 1998; Ruiz et al. 1997; Ruiz et al. 1999) and the rate of invasions is expected to increase as transport continues on a global scale (Elton 1958; Carlton 1979; Carlton 1989; Carlton 1992; Ruiz et al. 1997).

Estuaries and bays are among the most highly invaded coastal marine habitats (Ruiz et al. 1999). This result is most commonly explained by estuaries and bay being more disturbed by human activities and thus, the number of new species introductions are significantly higher in protected waters than along the outer coast (Williamson 1996; Wasson et al. 2005). In Elkhorn Slough, located along the central California coast, 10 percent more non-native species have been recorded than in the nearby rocky coastline (Wasson et al. 2005). Estuarine systems may also be more likely to contain non-native species due to the presence of man-made structures, which add hard substrate to soft sediment environments and aid in settlement (Glasby et al. 2007).

Non-native species are introduced into ecosystems in two different ways, through unintentional and intentional invasions (Carlton 1992). Examples of unintentional invasions
include transportation in ship ballast water (Carlton 1992; Carlton and Geller 1993), transportation on ship hulls (Ruiz et al. 1997; Ruiz et al. 1999; Bax et al. 2003), and through connected waterways including canals (Ruiz et al. 1997). Release of organisms for open seas fisheries enhancement (Carlton 1992) and aquaculture, are examples of intentional introductions (Carlton 1992; Ruiz et al. 1997).

In recent years, the most significant mechanism of transportation of marine non-native species has been through ships' ballast water (Carlton 1985; Carlton and Geller 1993; Ruiz et al. 1997; Wasson et al. 2001; Bax et al. 2003). Ships travel around the world bringing plankton, including larvae of benthic species, with them to be discharged in entirely new locations (Carlton and Geller 1993). Once non-native species have become established in a new ecosystem, secondary transport may occur to disperse individuals further from the source of introduction (Wasson et al. 2001). Forms of secondary transport include natural mechanisms such as dispersal of adults or larvae in water currents, and human mediated methods such as transplanting of eel grass, *Zostera marina* from one estuary to another (Wasson et al. 2001). Not all introduced species are able to successfully establish in a new location. Factors that limit establishment include, inappropriate climate, predation, competition, and disease (Lodge 1993; Byers 2000).

The successful establishment of non-native species can have detrimental effects by changing community structure, introducing disease (Ruiz et al. 1997; Molnar et al. 2008), changing levels of primary production, altering nutrient cycles, causing extinctions of native species (Grosholz et al. 2000), and decreasing biodiversity and available resources (Bax et al. 2003). An example of a predatory non-native species in California is the European green crab, *Carcinus maenas*, which became established in the Bodega Bay harbor and altered the
community structure and function of the system by reducing populations of the native species through predation (Grosholz et al. 2000).

In some estuarine systems, native populations of benthic species have been disrupted and replaced by introduced species (Carlton et al. 1990; Wu and Culver 1991; Alpine and Cloern 1992; Cloern 1996; McIssac 1996; Ruiz et al. 1997). For example, the native population of the common mud snail, Cerithidea californica has been virtually eradicated in the Elkhorn Slough, following the invasion of the Asian horn snail, Batillaria attramentaria (Byers 2000). When native species are displaced, non-native species often fundamentally alter the community structure and function of affected estuaries (Carlton et al. 1990; Wu and Culver 1991; Alpine and Cloern 1992; Cloern 1996; McIssac 1996; Ruiz et al. 1997). The introduction of non-native species has also been shown to affect resource availability (Schwindt et al. 2001). For example, the Zebra mussel, Dreissena polymorpha, was introduced into the Great Lakes in North America (Hebert et al. 1989, MacIsaac 1996). Once it became established, zebra mussels modified the environment by filter feeding on large quantities of phytoplankton, resulting in enhanced water clarity, and increasing plant productivity through higher irradiance levels (McIssac 1996).

Competition and other negative effects of non-native species have been well studied, however recent research describes potential for facilitative effects of some non-native species on marine and terrestrial communities (Bruno et al. 2003). In order for an effect to be facilitative, one of the organisms involved needs to benefit from the interaction, while the others either benefit or experience a neutral effect (Bertness et al. 1999). Examples of facilitative effects in the intertidal include, amelioration of thermal stress through shading or desiccation resistance, reductions in predation pressure through associational refuges, and nutritional symbiosis
(Bertness et al. 1999). Many species modify the local environment through their presence. For example, intertidal algae on the rocky shore may serve to reduce desiccation stress for invertebrates during low tides by retaining water in and around their blades and holdfasts, thus allowing those invertebrates to expand their vertical range to higher tidal heights (Bruno et al. 2003).

High-density populations of sessile organisms typically experience competitive interactions, although a shift to facilitative interactions can occur as physical stress increases (Bertness and Callaway 1994; Bertness and Leonard 1997; Callaway and Walker 1997; Bertness et al. 1999). In stressful environments, neighbors are capable of reducing physical stress. This idea was explored by Bertness et al. (1999), during density dependent studies of the northern Acorn barnacle, *Semibalanus balanoides* in Cape Cod, Massachusetts. It was found that barnacles living in high-density populations with neighbors were able to survive at higher tidal heights because the proximity of others reduced the overall thermal stress (Lively and Raimondi 1987; Bertness 1989; Bertness et al. 1999). The opposite effect was observed for barnacles living at lower population densities, where individuals experienced high mortality at the higher tidal heights (Barnes and Powell 1950; Roughgarden et al. 1985; Bertness et al. 1999).

Elkhorn Slough, located in the Monterey Bay, along the central coast of California is an 11km long tidal slough and estuary. It is located in a 1700-acre National Estuarine Research Reserve and is characterized by having the largest tidal salt marsh outside of San Francisco Bay. The habitats within the Elkhorn Slough provide a nursery for juvenile fish, habitat for threatened and endangered species, and an crucial resting and feeding location for 200 different species of birds during their annual migration. The Elkhorn Slough also serves as an important buffer
between the terrestrial and marine habitats, by filtering water, sequestering carbon and reducing flooding.

The Elkhorn Slough has undergone many anthropogenic changes, including cutting an opening through the sand dunes in the 1940’s, allowing boats direct access from the Monterey Bay into the Moss Landing Harbor (Silberstein et al. 2002; Van Dyke and Wasson et al. 2005). Other changes included building of culverts, outplanting non-native oysters for aquaculture (Wasson et al. 2001), and the unintentional introduction of non-native species. The Moss Landing Harbor is a commercial fishing harbor which experiences boat traffic from vessels that travel up and down the coast. Many of the boats docked in Moss Landing Harbor have also traveled to San Francisco Bay (Wasson et al. 2001), which is used as a commercial fishing and international shipping port (Molnar et al. 2008; Ruiz et al. 2011). Secondary transport by small boats is a likely mechanism of introduction for non-native species to Elkhorn Slough (Wasson et al. 2001). Of the 56 non-native species documented in Elkhorn Slough, 51 are also found in San Francisco bay (Wasson et al. 2001).

A second mode of introduction of non-native species into Elkhorn Slough occurred along with the transport of the Atlantic oyster, *Crassostrea virginica* and the Pacific oyster, *Crassostrea gigas*, which were brought to California for aquaculture in the early 1900’s (Carlton 1979; Ruiz et al. 1997; Wasson et al. 2001). Of the 56 non-native species residing in Elkhorn Sough today, 38 were potentially transported with oysters (Wasson et al. 2001). One example is the Asian horn snail, *Batillaria attramentaria* that was introduced to estuaries in northern California during the early 1900’s through aquaculture of *C. gigas* (Bonnot 1935; Byers 2000). This non-native snail is now abundant in Elkhorn Slough outcompeting native species (Bonnot
1935; Byers 2000).

An example of a non-native species that acts as an ecosystem engineer in Elkhorn Slough is *Ficopomatus enigmaticus*, a reef building polychaete native to Australia (Carlton 1979; Heiman 2006). This non-native species has only been reported in one other location along the west coast, the San Francisco Bay, where it was first observed 80 years ago (Carlton 1979; Wasson et al. 2001; Heiman 2006). This tubeworm is found in the upper intertidal zone where it suspension feeds, and requires hard substrate to initiate settlement (Carlton 1979; Schwindt et al. 2001; Wasson et al. 2001). After reproducing, newly settled juvenile worms grow upon adult tubes, thereby increasing the size of the reef (Dittmann et al. 2009). The space between the intertwined calcareous tubes creates habitat for many other species, which feed on the trapped organic matter (Heiman 2006). Total species abundance is often greater on *F. enigmaticus* reefs, than those dominated by native oysters or in the surrounding soft sediments (Heiman 2006; Heiman et al. 2008).

In Elkhorn Slough the native Olympia oyster, *Ostrea lurida*, and *Ficopomatus enigmaticus* both live at intertidal elevations and are more abundant at the northern end of the slough (Heiman 2006). In addition, they both act as ecosystem engineers providing hard substrate in soft sediment environments facilitating other invertebrates (Heiman et al. 2008). In Elkhorn Slough, *O. lurida* has an average maximum shell length of 5 cm and can reach densities of 340 oysters m² (Heiman et al. 2008). It is found attached to and growing on stable, hard substrates including wood and rocks (Heiman 2006). Surveys of the Elkhorn Slough suggest that there are approximately 5000 oysters remaining from this once abundant native population (Wasson 2010).
It is important to know what led to the decline of *O. lurida* and which factors are keeping the population from recovering (Wasson 2010). Along California’s coast, *O. lurida* is found in San Francisco Bay, 154 km to the north, and Point Mugu, 451 km to the south of Elkhorn Slough (Polson and Zacherl 2009). The loss of *Ostrea lurida* from Elkhorn Slough could result in numerous severe consequences. First, the Elkhorn Slough population serves as a stepping stone for gene flow between northern and southern populations, which would become genetically isolated (Polson and Zacherl 2009). The loss of oysters would also have a negative impact on estuaries, and result in the loss of the ecosystem services they provide (Wasson 2010). Oysters are responsible for creating habitat, filtering large quantities of water (Bible et al. 2012), modifying the environment by adding hard substrate (Heiman et al. 2008), and moving nutrients from the water column to the benthos through suspension feeding and excretion (Ruesink et al. 2005).

The objective of the study presented here is to explore the effect of the non-native species *Ficopomatus enigmaticus* on the Olympia oyster, *Ostrea lurida* in the Elkhorn Slough estuary by evaluating the effects of *F. enigmaticus* on the growth and survival of oysters (1) at different intertidal heights, (2) across different life stages, and (3) in the presence of other non-native species. The Elkhorn Slough estuary was chosen because it is home to *O. lurida* and 56 identified non-native species. It was predicted that *O. lurida* would grow more slowly in the presence of *F. enigmaticus* or other non-native species than without non-native species. I also predicted *F. enigmaticus* would settle more quickly and have a greater competitive effect on *O. lurida* at deeper tidal depths below mean lower low water (MLLW).
METHODS

Study location

The study site was located at Hudson’s Landing in the upper Elkhorn Slough, Watsonville, CA (36°51.23”N, 121°45.18”W). This site is tidally influenced and consists of a narrowing channel, flanked by mud flats on either side. A railroad bridge runs through the site, with wooden pilings extending into the substrate. Extensive *Ficopomatus enigmaticus* reefs extend along the northern bank for 25 meters in length, continuing under the railroad bridge. A culvert at the farthest end of the site controls water flow into a retention pond.

This site was chosen for my experiments because both the native *O. lurida* and non-native *F. enigmaticus* are present at the site and because this location is accessible by walking through a mud flat for approximately 30 meters. In addition, the railroad pilings were accessible and a secondary hard substrate that could be surveyed to understand patterns of benthic community composition. Hudson’s Landing is separated from the main channel of the Elkhorn Slough, which is heavily used for recreation, this limiting the risk of human disturbance to the experimental array.

Initial community surveys

In order to characterize the species composition of sessile invertebrate assemblages in Hudson’s Landing, I conducted an initial community survey of 6 wooden pilings under the railroad bridge during May 2014. In Elkhorn Slough, Olympia oysters range from the mid intertidal to shallow subtidal (Polson and Zacherl 2009; Wasson et al. 2014). For my experiments, I chose two tidal heights (-0.3 m and +0.3 m) mean lower low water (MLLW) to
test whether competitive effects of non-native species on *O. lurida* varied by tidal height. Quadrats were used to sample the distribution pattern of *Ostrea lurida*, *Ficopomatus enigmaticus* and other sessile invertebrates at the two tidal heights. A 1-m\(^2\) quadrat with 25-point contacts was used to sample 6 wooden pilings at the two tidal heights during a negative tide and the organisms under each point were identified to the lowest taxonomic level. Percent cover of each species was calculated to provide a picture of the patterns of relative abundance of different taxa at the study location.

*Field Experiments*

A. Adult oysters with *Ficopomatus enigmaticus*

To test the effect of *Ficopomatus enigmaticus* on adult *Ostrea lurida*, oyster growth was measured over a 9-month period (July 2014- March 2015) at two tidal heights (-0.3 m and +0.3 m MLLW). These two tidal heights were chosen because they are within the range of *O. lurida* and *F. enigmaticus* settlement. Oysters in the subtidal portions of the Slough are more susceptible to sedimentation, as fine grain mud can decrease their feeding ability and capacity to compete with other fouling species (Wasson et al. 2014). At the upper tidal height, physical factors such as high air temperatures during low tide and decreased salinity during rainy months may have a greater impact on oyster survival than the biological interactions that dominate at lower tidal heights (Wasson et al. 2014). I expected to see greater competition between non-native species and *O. lurida* at the lower tidal height. There they remain underwater, with the exception of extreme low tides, have increased feeding opportunities, and less stress due to physical factors. At the upper tidal height +0.3 m MLLW, I expected to see less settlement of
non-native species and less competition between *O. lurida* and non-native species resulting in greater oyster growth.

To obtain adult oysters for this study, settlement plates containing *Ostrea lurida* ranging in size from 3 to 5 cm in length were collected at the completion of a separate 2-year study in Elkhorn Slough. The plates were brought to the laboratory where they were cleaned by submerging them in a 30-minute freshwater soak, followed by brushing with a steel brush, and scraping with a metal putty knife to remove all fouling organisms, leaving only the oysters. The settlement plates were then standardized by removing oysters with a putty knife until only one oyster remained per plate. The settlement plates and extra individual oysters were temporarily housed in tanks with flowing seawater in the MLML aquarium facility.

In July 2014, 32 settlement plates with one adult *Ostrea lurida* each were deployed at two tidal heights (-0.3 m and +0.3 m MLLW), on oyster racks made of 1.9 cm diameter PVC, 1.22 m tall, with a cross bar of 91 cm in length. The higher and lower racks were deployed parallel to one another (Fig. 1). Each rack held four-10 cm x 10 cm ceramic settlement plates, distributed at intervals of 10 cm. Plates were attached horizontally to the underside of the rack (Hopkins 1935) by a 6.4 mm stainless steal bolt and wing nut. A random number generator was used to assign the placement of treatment and control plates throughout the array.
Figure 1. Representation of the experimental layout at Hudson’s Landing. Blue lines represent 16-oyster racks at two tidal heights (-0.3 m and +0.3 m MLLW) parallel to one another.

The settlement plates were left for two weeks to allow for recruitment of fouling species. Every two weeks during low tide, the plates were removed, photographed, and the length, width, and depth of each oyster were measured with calipers to the nearest mm. Each time point, half of the plates were cleared of all fouling species to create and maintain the “F. enigmaticus removed” treatment using a steel wool brush and a scraper, leaving only the oyster on the plate (Fig. 2a). On the second half of the plates, fouling species other than F. enigmaticus were removed, using forceps, a soft brush, and scraper, creating and maintaining the “F. enigmaticus present” treatment (Fig. 2b).
Photo analysis software (ImageJ64 and PhotoQuad) was used to calculate growth rates of the oysters in the presence and absence of *F. enigmaticus* (by measuring change in oyster area, cm²) and percent cover of *F. enigmaticus* through time. Using ImageJ64, I traced the outline of each oyster shell three separate times and determined the mean of the measurements. This was used to reduce sampling error. PhotoQuad was set to use a 100-point uniform quadrat to determine percent cover on the entire settlement plate. A smaller quadrat was also drawn around the edge of the oyster in the interaction zone. Here a 25-point random quadrat was used to determine percent cover on the oyster and around the edges of the oyster. This was used to determine what was happening in the interaction zone surrounding the oyster. The same method of photo analysis was used in both adult experiments *(see next section)*.

![Figure 2. Adult Ostrea lurida with Ficopomatus enigmaticus on settlement plates A) F. enigmaticus removed B) F. enigmaticus present.](image)

B. Adult oysters with non-native species (other than *F. enigmaticus*)

To test the effect of non-native species other than *F. enigmaticus* on adult *Ostrea lurida*, oyster growth was measured over an 8-month period (October 2014- May 2015) at two tidal
heights (-0.3 m and +0.3 m MLLW). The settlement plates were left for two weeks to allow for recruitment of non-native species. Every two weeks during low tide, the plates were removed, cleaned, photographed, and the oysters measured with calipers. Half of the plates were cleared of all non-native species to create and maintain the “non-native species removed” treatment, by using a steel wool brush and a scraper, leaving only the oyster on the plate (Fig. 3a). On the second half of the plates, *Ficopomatus enigmaticus* was carefully removed using forceps, leaving the oyster and other non-native species intact to create and maintain the “non-native species present” treatment (Fig. 3b).

![Figure 3](image.png)

Figure 3. Adult *Ostrea lurida* with non-native species on settlement plates A) non-native species removed B) non-native species present (other than *F. enigmaticus*).

*Genetic identification of non-native species*

Genetic methods were used to confirm the identity of one mussel and several tunicates on the *Ostrea lurida* settlement plates, when morphological identification was not adequate. A sample of DNA was extracted using Qiagen (DNeasy- Blood and Tissue Kit 250) DNA
extraction kit following the manufacturer’s protocol. Polymerase chain reaction (PCR) was used to amplify the COI region with primers, jgHCO2198 and jgLCO1490 (Geller et al. 2013) using a three-step thermo-cycler program. PCR products were sent for Sanger sequencing (Elim Biopharmaceuticals). Analysis of sequences used Geneious© software (by Biomatters Ltd.) and samples were compared to known sequences in Genbank (National Center for Biotechnology Information).

Data analysis

The response variables for the experiments were oyster growth, measured as area (cm²) and as percent change in area (mm²/d). Percent change in area controls for differences in oyster size among the settlement plates. Increase in oyster area was calculated for the entire experiment and again once *F. enigmaticus* reached >50% cover at -0.3 m MLLW. Examining it with the two methods demonstrated that the oysters always increased in area in response to the *F. enigmaticus* or other non-native present treatments, although the second measurement is a better reflection of the actual treatment conditions, because it is limited to the period in time when the interaction is occurring.

A Repeated Measures Analysis of Variance (RM-ANOVA) in JMP Pro 12 was used to examine relationships between percent cover of *F. enigmaticus* or other non-native species at two tidal heights over time and to measure oyster growth by tidal height and time. One sample date per month was used to span the trajectory of the study. The Greenhouse-Geisser (G-G) adjustments of degrees of freedom were used when the assumption of within-class sphericity was not met, following the recommendations of Von Ende (2001).
A two-way Analysis of Variance (ANOVA) in JMP Pro 12 was used to examine the relationship of tidal height, non-native species removal treatment and the interaction of these two factors on average oyster growth in the field experiments. The time period was 9 months for the first experiment, from July 2015- March 2014. The second experiment was 8 months, from October 2014- May 2015. The length of time was chosen to allow for settlement of *F. enigmaticus* and other non-native species to greater than 50 percent of the settlement plates. This was necessary to test for an interaction between the non-natives and *O. lurida*.

RESULTS

A survey of the fouling community at Hudson’s Landing revealed four species on the wooden pilings. Three of the species are non-native and one is native to Elkhorn Slough. Two of the non-native species (*Ficopomatus enigmaticus* and *Hymeniacidon sinapium*) were observed at both tidal heights, although they are present in higher abundance at -0.3 m MLLW. The non-native barnacle (*Balanus amphitrite*) and the native oyster (*Ostrea lurida*) were only present at +0.3 m MLLW (Table 1).
Table 1. Railroad bridge vertical quadrat samples. Vertical wooden pilings were sampled with a 1m² 25-point quadrat at -0.3 m and +0.3 m MLLW.

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Lowest Taxonomic Level</th>
<th>% Cover</th>
<th>Native Status</th>
<th>Tidal Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annelida</td>
<td><em>Ficopomatus enigmaticus</em></td>
<td>65%</td>
<td>Non-native</td>
<td>-0.3</td>
</tr>
<tr>
<td>Porifera</td>
<td><em>Hymeniacidon sinapium</em></td>
<td>9%</td>
<td>Non-native</td>
<td>-0.3</td>
</tr>
<tr>
<td>Annelida</td>
<td><em>Ficopomatus enigmaticus</em></td>
<td>41%</td>
<td>Non-native</td>
<td>+0.3</td>
</tr>
<tr>
<td>Mollusca</td>
<td><em>Ostrea lurida</em></td>
<td>3%</td>
<td>Native</td>
<td>+0.3</td>
</tr>
<tr>
<td>Porifera</td>
<td><em>Hymeniacidon sinapium</em></td>
<td>3%</td>
<td>Non-native</td>
<td>+0.3</td>
</tr>
<tr>
<td>Arthropoda</td>
<td><em>Balanus amphitrite</em></td>
<td>6%</td>
<td>Non-native</td>
<td>+0.3</td>
</tr>
</tbody>
</table>

*IEffects of Ficopomatus enigmaticus on adult Ostrea lurida*

In this experiment, 32 adult *Ostrea lurida* were outplanted on separate settlement plates. Three mortalities occurred during the first month in the field, prior to the settlement of *Ficopomatus enigmaticus* and were removed from the analysis. Thus, 29 individual *Ostrea lurida* survived on settlement plates for 9 months in Elkhorn Slough, divided between two tidal heights (-0.3 m and +0.3 m).

Three measurements of percent cover of *F. enigmaticus* were calculated for each plate; first, on the entire settlement plate; second, in contact with the oyster; and third, around the oyster’s edge. These measurements revealed the percent cover of *F. enigmaticus* was higher below MLLW at -0.3 m than at the upper tidal height of +0.3 m for all three areas measured (Fig. 4a-c). For that reason total percent cover was further examined using a RM-ANOVA. A significant increase of *F. enigmaticus* settlement over time was observed at both tidal heights (G-G ε = 0.268, F₈,₃₅ = 95.346, P < .0001; Fig. 4a). There was also a significant difference between Time*Tidal height (G-G ε = 0.268, F₈,₃₅ = 27.327, P < .0001; Fig. 4a). The percent
cover of *F. enigmaticus* at -0.3 m ranged from 68% (total percent cover) to 47% (around the oyster’s edge) at the end of the experiment, compared to +0.3 m MLLW, where the mean percent cover ranged from 34% (in contact with oyster) to 19% (around the oyster’s edge) (Fig. 4d).

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

**Figure 4.** Percent cover of *Ficopomatus enigmaticus*. Mean of settlement plates calculated by sampling date. Error bars ±SE. A) total settlement plate B) in contact with oyster, C) around oyster’s edge D) comparison of final percent cover at both tidal heights the last sampling date.

On the plates deployed at -0.3 m MLLW, oysters grew and increased in size in both treatments (RM-ANOVA, Time: G-G ε = 0.307, F_{8,35} = 51.806, P < .0001) In addition,
there was a significant difference in the oyster growth trajectories between the two treatments (RM-ANOVA, Time*Treatment: G-G $\epsilon = 0.307$, $F_{8,35} = 7.798$, $P = 0.001$; Fig 5a). Oysters started off at the same size, but increased in size faster in the presence of *F. enigmaticus* than in the absence of this invasive tubeworm. On the plates deployed at $+0.3$ m MLLW oysters also increased in size in both treatments (RM-ANOVA, Time: G-G $\epsilon = 0.130$, $F_{8,35} = 6.503$, $P = 0.024$). In contrast, there were no significant effects of treatment on the oyster growth trajectories over the course of the experiment (RM-ANOVA, Time*Treatment: G-G $\epsilon = 0.130$, $F_{8,35} = 0.534$, $P = 0.485$; Fig 5b). By random chance, oysters on the *F. enigmaticus* removal plates started off larger in size than the oysters in the *F. enigmaticus* present plates. While not statistically significant, the initial difference in oyster size among treatments narrowed over the course of the experiment, suggesting that oysters tended to grow faster in the *F. enigmaticus* present treatments at $+0.3$ m MLLW, similar to the pattern observed at the lower tidal level.

![Figure 5](image)

**Figure 5.** *Ostrea lurida* area (cm$^2$) in the presence and absence of *Ficopomatus enigmaticus*. A) -0.3 m MLLW B) +0.3 m MLLW.
To control for potential differences in initial oyster size on subsequent growth, I calculated a second response variable as the percent change in area per day, when in the presence of *F. enigmaticus* versus when *F. enigmaticus* was removed (Fig. 6). Averaged over the full course of the experiment, a two-way ANOVA showed no effect of tidal height ($F_{1,25} = 0.622$, $P = 0.438$), no effect of treatment ($F_{1,25} = 2.572$, $P = 0.121$) when *F. enigmaticus* was present or removed, and no interaction between height and treatment ($F_{1,25} = 0.251$, $P = 0.621$). The statistically non-significant result was likely due to the large variation in oyster growth at the +0.3 m tidal height. However, the trends indicated that if anything, *O. lurida* grew faster in the treatments where *F. enigmaticus* was present than those where the invasive tubeworm was removed.

![Figure 6. Percent change in area (mm$^2$/d) of Ostrea lurida in presence and absence of Ficopomatus enigmaticus ±SE.](image)
Similarly to the results presented in (Fig. 6), which evaluated growth changes over the full course of the experiment, oysters consistently exhibited a greater increase in area when growth was calculated from the point at which *F. enigmaticus* reached greater than 50% cover at the -0.3m MLLW tidal height. This threshold was chosen as the point at which *F. enigmaticus* was likely to be directly interacting with oysters on each plate. However, a two-way ANOVA showed no effect of tidal height ($F_{1,23} = 0.379$, $P = 0.544$); no effect of treatment ($F_{1,23} = 2.213$, $P = 0.150$) due to a large variation in oyster area, and no interaction of height and treatment ($F_{1,23} = 0.050$, $P = 0.824$; Fig. 7).

Figure 7. Percent change in area of *Ostrea lurida* in presence and absence of *F. enigmaticus* ±SE, after *F. enigmaticus* reached >50% cover at -0.3 m tidal height (December 2014-March 2015).
Effect of non-native species on adult Ostrea lurida

In the second manipulative experiment, *F. enigmaticus* was removed from the settlement plates to test for an effect of other non-native species on *O. lurida* growth. A succession of settlement was observed on the settlement plates at -0.3 m MLLW, with *Bugula neritina* settling first in high abundance, followed by other sessile invertebrates in low densities. The last species to settle was unidentified brown alga, which grew rapidly and covered >50% of the area on the plates by the end of the study (Fig. 8a). The brown alga was the most abundant at +0.3 m MLLW, followed by *Bugula* (Fig. 8b). Two species of invertebrates (*Molgula manhattensis* and *Mytilus trossulus*) were identified by genetic methods as represented by the asterisk (Table 2).

![Figure 8. Non-native species succession over 8 months A) -0.3 m MLLW B) +0.3 m MLLW.](image-url)
Table 2. Community distribution on settlement plates. This table represents the mean percent cover of all treatment plates on the final day of the study at -0.3 and +0.3 m MLLW.

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Lowest Taxonomic Level</th>
<th>% Cover</th>
<th>Native Status</th>
<th>Tidal Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryozoa</td>
<td><em>Bugula neritina</em></td>
<td>28%</td>
<td>Non-native</td>
<td>-0.3</td>
</tr>
<tr>
<td>Phaeophyta</td>
<td>Brown algae</td>
<td>69%</td>
<td>Unknown</td>
<td>-0.3</td>
</tr>
<tr>
<td>Bryozoa</td>
<td>Encrusting bryozoan</td>
<td>2%</td>
<td>Unknown</td>
<td>-0.3</td>
</tr>
<tr>
<td>Chordata</td>
<td><em>Molgula manhattensis</em></td>
<td>1%</td>
<td>Non-native</td>
<td>-0.3</td>
</tr>
<tr>
<td>Bryozoa</td>
<td><em>Bugula neritina</em></td>
<td>4%</td>
<td>Non-native</td>
<td>+0.3</td>
</tr>
<tr>
<td>Phaeophyta</td>
<td>Brown algae</td>
<td>93%</td>
<td>Unknown</td>
<td>+0.3</td>
</tr>
<tr>
<td>Mollusca</td>
<td><em>Mytilus trossulus</em></td>
<td>1%</td>
<td>Native</td>
<td>+0.3</td>
</tr>
<tr>
<td>Bryozoa</td>
<td>Encrusting bryozoan</td>
<td>2%</td>
<td>Unknown</td>
<td>+0.3</td>
</tr>
</tbody>
</table>

In this experiment, 32 adult *Ostrea lurida* were outplanted on individual settlement plates for 8 months in the Elkhorn Slough. Three measurements of percent cover of non-native species were calculated for each plate, as in the first experiment. The settlement rate of non-native species was high at both tidal heights (-0.3 m and +0.3 m), with the greatest increase in growth occurring during the first three months, October-December 2014 (Fig. 9a-c). After the initial growth, there was a decrease in percent cover of non-native species on the plates during January 2015. During the remainder of the study, the percent cover of non-natives continued to increase, reaching 95-100 percent cover, overgrowing the oysters on the settlement plates in May 2015 (Fig. 9a-c).

Non-native species settled and rapidly covered the plates regardless of tidal height. A RM-ANOVA examined the association between tidal height and total percent cover of non-native species. There was a significant increase in non-native species over time, (G-G ε = 0.339,
F_{7,27} = 85.182, P < .0001; Fig. 9a), but there was no significant difference in the trajectories of non-native species cover as a function of Time*Tidal height (G-G ε = 0.339, F_{7,27} = 1.865, P = 0.165; Fig. 9a).

Figure 9. Percent cover of non-native species. Mean of settlement plates calculated by sampling date. Error bars ±SE. A) total settlement plate B) percent cover in contact with the oyster C) around the oyster’s edge D) comparison of final percent cover at both tidal heights the last sampling date.
An increase in oyster growth was observed in the presence of non-native species. *O. lurida* growth at -0.3 m MLLW revealed, a significant increase in area (cm²) over time (RM-ANOVA, Time: $\text{GG} \varepsilon = 0.253$, $F_{7,27} = 46.397$, $P < .0001$) throughout the experiment. In addition, the growth trajectories diverged between the two treatments with oyster sizes increasing more rapidly in the treatments with non-native species present than those where non-native species were removed, despite starting off at larger sizes by random chance (RM-ANOVA, Time*Treatment: $\text{GG} \varepsilon = 0.253$, $F_{7,27} = 8.982$, $P = 0.002$; Fig. 10a).

*O. lurida* growth trajectories as measured in area (cm²) showed less increase at the upper tidal height +0.3 m MLLW. There was a significant increase in size over time (RM-ANOVA, Time: $\text{GG} \varepsilon = 0.219$, $F_{7,27} = 62.885$, $P < .0001$), however at this tidal height there were no significant differences in the growth trajectories of oysters as a function of the presence or absence of non-native species (RM-ANOVA, Time*Treatment: $\text{GG} \varepsilon = 0.219$, $F_{7,27} = 1.804$, P = 0.193; Fig. 10b).

Figure 10. *Ostrea lurida* area (cm²) by date in the presence and absence of non-native species A) -0.3 m MLLW B) +0.3 m MLLW.
Over the course of the entire experiment growth, measured as the percent change in area per day, did not differ as a function of tidal height ($F_{1,28} = 0.001, P = 0.976$). On average, oysters on settlement plates with non-native species (other than *F. enigmaticus*) exhibited a significantly faster percent increase in area ($F_{1,28} = 5.731, P = 0.024$) when compared to those cleared of non-native species (Fig. 11). In addition, there was no interaction of tidal height and treatment ($F_{1,28} = 1.002, P = 0.325$), indicating that the pattern of faster growth on tiles including non-native species was consistent across the two tidal heights.

![Figure 11. Percent change in area of *Ostrea lurida* in presence and absence of non-native species +SE. Mean percent change in area (mm$^2$/d).](image)

The change in area of adult *O. lurida* was also calculated once the percent cover of non-native species reached a threshold of greater than 50 percent cover on the plates at the lower tidal height -0.3 m, which occurred in November 2014. The threshold was used to signify the time period at when there were direct species interactions between oysters and non-natives. Similar to previous results, the oysters continued to show an increase in area (mm$^2$/d) in the presence of
non-native species compared to those where the non-native species were removed from the plates. A two-way ANOVA showed no effect of tidal height ($F_{1,28} = 0.203, P = 0.656$, a significant effect of treatment ($F_{1,28} = 5.332, P = 0.029$), and no interaction of height and treatment ($F_{1,28} = 0.077, P = 0.783$; Fig. 12).

![Bar graph showing percent change in area of Ostrea lurida in presence and absence of non-native species with standard error (SE) after non-native species reached >50% cover at -0.3 m tidal depth (November 2014-May 2015).]

**DISCUSSION**

The objective of this study was to provide insight on how well adult *O. lurida* will survive in the presence of *F. enigmaticus* and other non-native species in the Elkhorn Slough. The results ultimately contradicted the original hypothesis that oyster growth would be negatively affected by the presence of invasive *F. enigmaticus* and other non-native species. Instead, oyster growth either did not differ or exhibited a positive response to the presence of invasive suspension feeders. Due to the high variability of growth among individual oysters, a
power analysis (with statistical power = 0.80) indicated that a sample size of 47 oysters would be needed to demonstrate a statistically significant positive effect of *F. enigmaticus* cover on oyster growth at +0.3 m MLLW, given the effect size observed in the experiments. This suggests that with greater replication, a positive facilitative effect of *F. enigmaticus* on solitary adult *O. lurida* may have been observed. It was also determined that the settlement of *F. enigmaticus* was directly affected by tidal height, with increased settlement occurring below MLLW.

This study found a positive facilitative effect of non-native species on the growth of adult *Ostrea lurida* above and below MLLW. This spatial pattern suggests the presence of non-natives species may provide a benefit to solitary adult *O. lurida* in the intertidal zone, mitigating some of the environmental stress. Challenges of living in the low intertidal include increased predation and competition for resources, while stress in the high intertidal includes desiccation, higher temperatures, and limited filter feeding opportunities. As observed in previous studies, facilitation is important for organisms living in environments with high physical stress (Clements 1936; Bertness and Leonard 1997; Bertness et al. 1999; Bruno and Kennedy 2000; Stachowicz 2001). For example, habitat modification occurs when one species changes the local environment ameliorating it for other species (Stachowicz 2001). This has been described in the rocky intertidal zone in New England, where the canopy forming algae *Fucus sp.* acts as a habitat ameliorator for the red alga *Chondrus crispus* (Stachowicz 2001). *Fucus* act to reduce temperatures and desiccation stress of the local environment, and as a result *C. crispus* grows higher in the intertidal (Stachowicz 2001).

In Elkhorn Slough, above MLLW competition is low and desiccation stress is high, while below MLLW fouling species may have an increased competitive effect on oyster growth
(Wasson 2010). However, my experiments did not result in a negative effect of non-native species on oysters at either tidal height. Instead, *F. enigmaticus* appeared to have no negative effect on oyster growth (although statistical power was low and the trends suggested some positive effects) while the other non-native species appeared to have a positive facilitative effect. The solitary oysters likely benefited from having other species in close proximity. *O. lurida* is sensitive to high air temperature at low tide, although Elkhorn Slough rarely reaches high temperatures (Wasson et al. 2014; Wasson et al. 2015). The presence of other filter feeding species below MLLW may have enhanced water flow through their filtering activities, inadvertently bringing more food to the oysters. It was observed that *F. enigmaticus* and other non-native species settled on the oysters at both tidal heights, but they were not abundant enough to prevent feeding. Above MLLW, the presence of neighbors could reduce temperature and desiccation stress.

The results of this study are similar to those of Wasson et al. 2015, stating in Elkhorn Slough there were no negative effects of other sessile species on density or growth of *O. lurida* (Wasson et al. 2015). Comparably, research on *O. lurida* in San Francisco and Tomales Bays has shown comparable results. Deck (2007) reported non-native species had little competitive effect on adult *O. lurida* growth. The greatest effect of non-native species was seen in post recruitment juveniles in Tomalas Bay. There oysters without competitors were two times larger than those with competitors, while in San Francisco Bay competitors reduced the total number of recruits (Deck 2007).

In conclusion, this study found no negative effects of *F. enigmaticus* and a facilitative effect of several non-native species on adult *O. lurida* above and below MLLW. Since the effect
of *F. enigmaticus* was unclear due to constraints of sample size, it requires further investigation.

Even though this study demonstrates the facilitative effect of non-natives in this context, this is not to say that non-native species are beneficial to ecosystems. The benefit observed was suspected to be a result of having neighbors, which reduced the stress environmental stress. The anthropogenic invasion of non-native species is ongoing and some non-natives are responsible for the facilitation of other non-natives, increasing their ability to establish (Heiman and Micheli 2010). There is much that is unknown about the long-term affect of introduced species on ecosystems and it is important to continue to study their impact. Additionally, the effect of non-natives on oyster recruitment needs further investigation. If *O. lurida* recruitment and juvenile survivorship is further reduced, the local Elkhorn Slough population may be lost. It is imperative that we increase our understanding of the effects of non-native species on *O. lurida* populations so that mitigation and restoration can be designed and implemented.
CHAPTER II
EFFECTS OF NON-NATIVE SPECIES ON JUVENILE OLYMPIA OYSTERS

INTRODUCTION

A wealth of studies has sought to quantify the impacts of non-native species on the structure and function of native species in many ecosystems (Grosholz 2002). However, fewer studies have examined the effects of invasive species across multiple life stages of native species (Callaway and Walker 1997; Schiffers and Tielborger 2006; Deck 2007).

In Chapter 1, I found neutral to positive effects of non-native species on adult Ostrea lurida growth. However, it is unclear whether juvenile oysters will respond similarly or differently than adults. It is expected that recently settled juveniles are more vulnerable and poorer competitors making them more susceptible to competition and predation.

Previous studies by Deck (2011), have demonstrated sessile organisms including bryozoans and tunicates directly compete with O. lurida for available space and as a result have decreased the size of O. lurida recruits by up to 50 percent in Tomalas Bay, CA. In Washington, indirect competition has been observed in areas where the Atlantic oyster, Crassostrea virginica was introduced for aquaculture. C. virginica grows more quickly than O. lurida forming dense beds and outcompeting the smaller oyster (Buhle and Ruesink 2009, Pritchard et al. 2015).

Predation from oyster drills, Urosalpinx cinerea and Ocebrina inornata also pose a threat to juvenile oysters. These invasive snails prefer to feed on smaller oysters reducing the size of the juvenile population (Pritchard et al. 2015).

Another detrimental effect of non-native species introductions is the potential for disease
transmission. The Pacific oyster, *Crassostrea gigas*, transplanted of the coast of Washington for aquaculture have been blamed for introducing the parasite *Microsytos mackini* to native oyster populations. The native *O. lurida* are more susceptible to this parasite than the non-native *C. gigas* resulting in increased mortalities (Bower et al. 1997; Pritchard et al. 2015).

*Ostrea lurida* are found in estuaries from the mid intertidal to shallow subtidal with the highest abundance around 0 m tidal height (Wasson et al. 2014). For settlement to occur, a hard intertidal to shallow subtidal substratum needs to be available (Heiman 2006). Large areas of hard substrate are necessary in sites with deep mud, whereas sites with little mud only need small substrate like gravel or snail shells (Wasson 2010; Wasson et al. 2014). Substrate size is of high importance to prevent oyster burial (Wasson et al. 2014). This will put them in close contact with non-natives like *F. enigmaticus* that are fast growing and occupy all the available hard substrate. Even though the adults have been resilient, the juveniles may not be able to compete with these additional challenges.

In Elkhorn Slough the introduction of anthropogenic derived hard substrate has increased with the addition of sea walls, docks and bridges in naturally occurring soft sediment environments (Heiman et al. 2008). The addition of water control devices including culverts has also increased the amount hard substrate available in Elkhorn Slough. Surveys conducted in Sydney Harbor, Australia showed non-native species were more abundant on anthropogenic hard substrates than native species, even though natives were twice as abundant in the surrounding system (Glasby et al. 2007). This suggests that addition of anthropogenic hard substrate may facilitate the success of non-native establishment (Glasby et al. 2007).

If the addition of hard substrate in a soft sediment system increases the establishment of
non-native species, this would further increase the potential for competition between non-native and native species at lower tidal heights. Eighty-four percent of cover on hard substrate in Elkhorn Slough is comprised of non-native sessile species (Wasson 2005). It has been suggested that non-native species compete for hard space, which ultimately limits oyster settlement (Heiman 2006). In Willapa Bay, Washington non-native fouling species have been shown to limit the growth and survival of Olympia oyster recruits in the low intertidal and shallow subtidal zone (Trimble et al. 2009; Wasson 2010). The Elkhorn Slough is a good model system to investigate effects of non-natives on two life stages of *O. lurida*. To further scientific understanding and to increase Olympia oyster restoration efforts, further study of the relationship between *F. enigmaticus* and *O. lurida* throughout multiple life stages is needed.

This study examined the effect of the *Ficopomatus enigmaticus* on growth of *Ostrea lurida* during the juvenile phase, at two tidal heights (-0.3 m and +0.3 m MLLW) in the Elkhorn Slough Estuary. I predicted that juvenile *O. lurida* would be overgrown by *F. enigmaticus*. I also predicted *F. enigmaticus* would have a greater competitive effect on juvenile *O. lurida* at -0.3 m than +0.3 m MLLW.

**METHODS**

*Study species*

Along the central coast of California, *O. lurida* spawn between the spring and fall when water temperatures are above 16°C (Coe 1931). *Ostrea lurida* are protandrous hermaphrodites, spawning first as a male and then alternating between male and female each reproductive cycle (Kimbro and Grosholz 2006). Males release sperm packets, which break apart in the water and
release spermatozoa that are taken into the female’s mantle cavity for fertilization of the eggs (Coe 1931). *O. lurida* is larviparous, with the fertilization of eggs and early development of larvae occurring in the female oyster’s gill cavity (Spencer 2002). The female broods the larvae for 10-12 days until they are in the pediveliger stage and approximately 170µm in length (Hopkins 1935), at which point they are released and free swimming until they settle as “spat” on a hard substratum in approximately two weeks time (Coe 1932). Once the larvae have settled they metamorphose into sessile juveniles (Hopkins 1935). They are mature at approximately one year of age (Coe 1932).

**Laboratory**

Thirty adult *Ostrea lurida* (ranging in size from 3 to 5 cm) attached to gravel substrate were collected from North Azevedo Pond in Elkhorn Slough to be used as broodstock. They were transported in 5-gallon buckets to Moss Landing Marine Laboratories, submerged in a freshwater bath for 30 minutes, and then cleaned using a soft brush to remove algae, and forceps to remove fouling organisms. The oysters were then distributed among four 38-liter tanks filled with unfiltered seawater. An additional 50 *O. lurida*, which had been removed from collected settlement plates were added to the broodstock.

The four tanks were located on a water table and each equipped with an air hose, a digital thermometer and an acrylic lid. Each tank of oysters was initially fed 5 ml of Shellfish diet 1800® every other day from May- August. After that time they were moved to the Monterey Bay Aquarium and fed live *Isochrysis sp.*, 5 days a week. On three occasions when *Isochrysis sp.* was unavailable live *Tetraselmis sp.* was substituted (Fig. 13).
To prepare the oysters for reproduction, the water temperature was raised from 12°C to 20°C over a two-week period. Methods for spawning oysters in the laboratory include keeping the broodstock at 20°C (Barnes et al. 2010). Two additional 38-liter tanks were used as sump tanks. They contained a heater, digital thermometer and air hose. Procedures included siphoning a quarter of the water from each of the oyster tanks prior to feeding. The replacement water was transferred from the sump tank and ranged between 18-20°C.

Prior to siphoning, the tanks were checked for signs of spawning by turning off overhead lights and shining a flashlight trough the side of the acrylic tank. When spawning occurred, the pediveligers were siphoned out of the tank and into a 120µm mesh filter, and gently transferred by rinsing a low flow of seawater over the filter carrying the larvae into a 38-liter tank with 20°C filtered seawater. Larvae were fed live *Isochrysis sp.* daily for 4 weeks. The veligers will remain in the water column for approximately two weeks (Imai et al. 1954). After the first two weeks, a plastic mesh was bent to fit inside the tank and zip tied together. Sixteen-10 cm x 10 cm ceramic settlement plates (with a hole drilled in the middle), were attached vertically to the plastic mesh.
using zip ties (Fig. 14a). After 28 days all the pediveligers had settled out on the plates. During the settlement phase, there was only air, no water flow and very minimal siphoning of the bottom of the tank to remove waste and to avoid loss of pediveligers.

Settled juveniles remained on the plates in the laboratory for 4 months, before the number per plate was reduced to avoid competition and to standardize the plates. The excess oysters were removed by scraping with an X-Acto knife until 20 remained per plate (Fig. 14b).

Figure 14. Juvenile *Ostrea lurida* settlement plates in the laboratory A) hanging vertically on plastic mesh B) with juvenile oysters ~1mm in length.

**Field Experiments**

A field experiment was deployed in March 2015 to test the effect of *Ficopomatus enigmaticus* on the growth and survival of juvenile *Ostrea lurida* settled onto plates as described above. A total of 16 settlement plates containing 20 oysters per plate were outplanted to racks in the field. The oysters ranged in length from 2-3 mm (Fig. 15a).

The settlement plates were initially left for two weeks to allow for recruitment of non-native species. Every two weeks during low tide, the plates were removed from the racks, cleaned, photographed and the oysters measured using calipers to determine length, width and
depth. Half of the plates were cleared of all non-native species using a brush and a forceps to establish the “F. enigmaticus removal” treatment, leaving only the oysters on the plates (Fig. 15b). On the second half of the plates, non-native species other than *Ficopomatus enigmaticus* were removed to establish the “F. enigmaticus present” treatment, using forceps, a soft brush, and scraper, leaving only the oysters and *F. enigmaticus*.

In addition to measuring oyster morphometrics in the field, ImageJ64 photo analysis software was used in all of the field experiments to determine the area (cm²) of each oyster. A line was drawn across each plate to standardize size (10 cm), and then a number was added on top of each juvenile oyster in each image. The area was measured by tracing the outside of the shell to determine area (cm²) and recorded for that individual oyster throughout the study. PhotoQuad software was used to determine percent cover of *F. enigmaticus* from the photographs taken every two weeks. PhotoQuad software was used to overlay a quadrat onto the images taken during each sampling event. A 100-point uniform quadrat was used to sample the percent cover of *F. enigmaticus* on the plates.

![Figure 15. Juvenile *Ostrea lurida* settlement plates in the field experiment A) outplanted oysters (March 2015) B) oysters on final sampling date (July 2015).](image)
Data analysis

A RM-ANOVA was used to examine the trajectories of changing percent cover of *F. enigmaticus* to establish that my treatments worked. Repeated Measures Analysis of Variance (RM-ANOVA) in JMP Pro 12 was used to examine relationships between percent cover of *F. enigmaticus* at two tidal heights over time and the growth rate of the oysters. The Greenhouse-Geisser (G-G) adjustments of degrees of freedom were used when the assumption of within-class sphericity was not met. This follows the recommendations of Von Ende (2001).

A two-way Analysis of Variance (ANOVA) in JMP Pro 12 was used to examine the relationship of height, treatment and height by treatment for the presence and removal experiments.

RESULTS

Effect of *Ficopomatus enigmaticus* on juvenile *Ostrea lurida*

*Ficopomatus enigmaticus* settled more quickly at -0.3 m below MLLW than at +0.3 m above MLLW and by the culmination of the study had covered a mean of >70% of the settlement plates compared to ~10% at +0.3 m MLLW (RM-ANOVA, Time: G-G ε = 0.324, F_{7,27} = 43.330, P < .0001; Fig. 16). A RM-ANOVA was used to test for time and treatment effects. The results indicated percent cover of *F. enigmaticus* increased between sampling dates and there was a significant difference between tidal height (Time*Treatment: G-G ε = 0.324, F_{7,27} = 30.272, P < .0001; Fig. 16).
At the lower tidal height of -0.3 m MLLW, juvenile *Ostrea lurida* grew most quickly during the first few months in the field regardless of treatment. A RM-ANOVA was used to test for time and treatment effects. The results indicated oyster size increased between sampling dates (RM-ANOVA, Time: GG $\varepsilon = 0.772$, $F_{4,9}=295.34$, $P=0.0003$) in both treatments. In addition, there was no significant difference in the size trajectories among the treatments, indicating that juvenile oysters grew at similar rates in the presence and absence of *Ficopomatus enigmaticus* (RM-ANOVA, Time*Treatment: GG $\varepsilon = F_{4,9} = 0.542$, $P = 0.721$; Fig. 17a).

At the upper tidal height of +0.3 m MLLW, juvenile *O. lurida* grew most quickly after the first month in the field. There was a significant effect of time on oyster size, indicating that oysters grew bigger throughout the course of the experiment (RM-ANOVA, Time: GG $\varepsilon = 0.424$, $F_{4,9} = 806.920$, $P < .0001$). Similarly to the findings at the lower tidal level, the
experimental treatments had no effect on the size trajectories of juvenile oysters, indicating that growth did not differ between the treatments with *F. enigmaticus* present or removed (RM-ANOVA: Time*Treatment, GG ε = 0.424, F_{4,9} = 0.170, P = 0.812; Fig. 17b).

![Graph](image)

Figure 17. Juvenile *Ostrea lurida* area (cm$^2$) in presence and absence of *Ficopomatus enigmaticus*. Measured as a mean of oyster size per plate and mean of all plates by date A) -0.3 m MLLW B) +0.3 m MLLW.

A total of 320 juvenile oysters were outplanted across 16 settlement plates (n=20 oysters per plate). The oysters remained in the Elkhorn Slough for five months during which time *Ficopomatus enigmaticus* was allowed to settle on half the plates at each tidal height (-0.3 m and +0.3 m MLLW). The remaining plates were cleaned of all sessile organisms except juvenile *Ostrea lurida*. Juvenile oyster growth rates were also calculated as the average percent change in area per plate across the two treatments. Using this metric (% change in area), a two-way ANOVA showed a significant effect of tidal height on oyster growth (F_{1,12} = 7.779, P = 0.016),
such that juvenile oysters consistently grew fastest at tidal heights below MLLW. However, I did not detect a significant effect of the Ficopomatus treatment on growth of juvenile oysters \((F_{1,12} = 0.166, P = 0.691)\), and there was no interaction of tidal height and non-native removal treatment \((F_{1,12} = 0.089, P = 0.772; \text{Fig} \ 18)\), suggesting that growth differences among the treatments were consistent at each tidal height.

The change in growth of juvenile oysters was re-analyzed after F. enigmaticus had settled and covered a mean of >25% of the plates at -0.3 m tidal height. This percentage was chosen because the total percent cover of F. enigmaticus never reached 50% cover at +0.3 m MLLW. A two-way ANOVA was conducted to analyze the results, finding a significant effect of tidal height \((F_{1,12} = 7.362, P = 0.019)\), with greater oyster growth observed at -0.3 m MLLW. There was no significant effect of treatment \((F_{1,12} = 0.061, P = 0.809)\), nor an interaction between height and treatment \((F_{1,12} = 0.681, P = 0.425; \text{Fig} \ 19)\).
DISCUSSION

This study found that settlement of *Ficopomatus enigmaticus* was directly affected by tidal height, with increased settlement below MLLW. The presence or absence of *F. enigmaticus* had no significant effect on juvenile oyster growth, unlike the previous adult study (Chapter 1). This result is supported by a previous study by Deck (2007), in San Francisco and Tomales Bays, which reported no effect of sessile species on growth of juvenile *O. lurida*.

Juveniles grew rapidly in the first few months in the Elkhorn Slough, with their growth stabilizing after four months in the field (at 7 months of age). The oysters at the lower tidal height (-0.3 m MLLW) were slightly larger than those at +0.3 m MLLW), regardless of treatment. This is similar to the study by Deck (2007), which found that subtidal oysters had increased growth versus those in the intertidal, due to food availability.
The second half of this study also demonstrated a significant effect of tidal height on oyster growth, though the pattern reversed with increased growth above MLLW. This result suggests an effect of *F. enigmaticus* settlement at -0.3 m MLLW. In addition, the oysters at -0.3 had completely covered the plate by the second half of the study causing density dependent competition to occur, where those at +0.3 had space available on the plates to continue growing. In a previous study, Wasson (2010) found that competition did not play a major role in the mortality of juvenile *O. lurida*.

The other difference between the adult and juvenile experiments was an artifact of the experimental design. The juvenile plates had 20 oysters per plate, versus the solitary adults. The presence of neighbors on the juvenile plates may have mitigated some of the physical factors, including desiccation or heat stress above MLLW. Facilitation by conspecifics or other sessile species is important during juvenile life stages of *O. lurida* (Deck 2007). Having neighbors results in neutral to positive effects during oyster’s early life stages (Miriti 2006; Schiffers and Tielborger 2006; Deck 2007).

In conclusion, if *O. lurida* are established before non-native species settle it is suggested that they will continue to grow with little impact from non-native species. The negative effects demonstrated in previous studies have been by competitors on *O. lurida* recruitment (Deck 2007). To fully understand how future populations of *O. lurida* will be affected, additional research needs to be done which includes continuing to examine the effect of known non-native species on larval oyster settlement and survival.

What we do know is that recruitment levels are currently low (Wasson et al. 2015) and strong recruitment events may only occur every few years (Deck 2007). For these reasons along
with the addition of multiple stressors altering the Elkhorn Slough Estuary, I recommend laboratory rearing and outplanting of juvenile *O. lurida*. This work has demonstrated the potential for outplanting laboratory reared juvenile *O. lurida*, 5 months of age or older, to aid in the recovery of the native oyster population in Elkhorn Slough.
LITERATURE CITED


Elton, C.S. 1958. The ecology of invasions by animals and plants. Methuen.


APPENDIX A

GENETIC ANALYSIS OF SPECIES

FROM ELKHORN SLOUGH
Table 3. Molecular identifications of organisms. Shows top result of BLAST query of Genbank with percent sequence similarity. Identities of 2 organisms recruited to settlement tiles were determined using molecular analysis. Six samples were successfully amplified by PCR and sent for Sanger sequencing. Of these samples all 6 sequences, showed strong quality sequences that could be used for analysis.

<table>
<thead>
<tr>
<th>Field Identification</th>
<th>Number of Samples</th>
<th>Molecular Identification</th>
<th>Accession Number</th>
<th>Percent Identical</th>
<th>Final Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Molgula sp.</em></td>
<td>5</td>
<td><em>Molgula manhattensis</em></td>
<td>JQ742953</td>
<td>99</td>
<td><em>Molgula manhattensis</em></td>
</tr>
<tr>
<td><em>Mytilus sp.</em></td>
<td>1</td>
<td><em>Mytilus trossulus</em></td>
<td>KF931968</td>
<td>99</td>
<td><em>Mytilus trossulus</em></td>
</tr>
</tbody>
</table>