

Interspecific Interactions in Oyster Reef Communities:  
The Effect of Established Fauna on Oyster Larval Recruitment

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Master of Science

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by  
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APPROVAL SHEET

This thesis is submitted in partial fulfillment of  
the requirements for the degree of  
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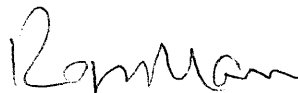


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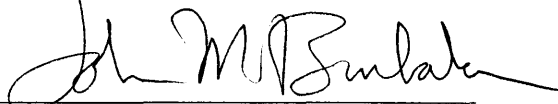
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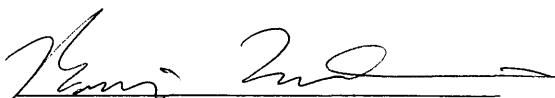
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## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABSTRACT.....	x
INTRODUCTION.....	2
AIMS AND OBJECTIVES.....	5
REVIEW OF RELEVANT LITERATURE.....	6
3.1. Crassostrea virginica larvae.....	6
3.2. Early investigations of larval recruitment.....	7
3.3. Adult-larval interactions.....	8
3.3.1. Interspecific interactions in benthic systems.....	8
3.3.2. Biofilms.....	10
3.3.3. Gregariousness in <i>Crassostrea virginica</i> .....	10
3.3.4. Interspecific interactions among common oyster reef epifauna.....	12
3.3.5. Methodological concerns in adult-larval interactions research.....	17
MATERIALS AND METHODS.....	20
4.1. Spawning and larval rearing.....	20
4.2. Settlement substrates.....	22
4.3. Experimental design.....	25
4.4. Larval additions.....	26
4.5. Post-settlement larval extractions.....	31
4.6.1. Effect of epifauna on settling oyster larvae.....	32
4.6.2. Effect of water soluble cues on settling oyster larvae.....	32
4.6.3. Effect of <i>Neanthes succinea</i> on settling oyster larvae.....	34
4.7. Analyses.....	36
4.8. Quantification of <i>Neanthes succinea</i> community characteristics.....	40

Table of Contents (continued)

	Page
RESULTS .....	41
5.1 Effect of epifauna on settling oyster larvae .....	41
5.1.1. Experiment 1 .....	44
5.1.2. Experiment 2 .....	49
5.1.3. Experiment 3 .....	49
5.1.4. Experiment 4 .....	49
5.1.5. Experiment 5 .....	56
5.1.6. Experiment 6 .....	56
5.1.7. Experiment 7 .....	61
5.1.8. Combined analyses on epifauna experiments .....	61
5.2 Effect of water soluble cues on settling oyster larvae .....	68
5.2.1. Effect of bathwaters from epifauna experiments .....	68
5.2.2. Initial investigations - Experiment 7 .....	71
5.2.3. Serial dilution of cues - Experiment 8 .....	71
5.2.4. Effect of ammonia concentration on oyster larvae .....	72
5.3 Predation on oyster larvae by <i>Neanthes succinea</i> .....	81
5.3.1. Initial investigations – Experiments 4 & 5 .....	81
5.3.2. Test of proximity hypothesis in time series – Experiment 7 .....	86
5.4 Quantification of <i>Neanthes succinea</i> population characteristics .....	86
DISCUSSION .....	91
6.1 Species-specific effects .....	91
6.1.1. <i>Cliona</i> sp. ....	91
6.1.2. <i>Membranipora tenuis</i> .....	93
6.1.3. <i>Balanus improvisus</i> .....	94
6.1.4. <i>Crassostrea</i> sp. ....	98
6.1.5. <i>Neanthes succinea</i> .....	99
6.2 General patterns and ecological significance .....	101
LITERATURE CITED .....	104
VITA .....	113

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## LIST OF TABLES

	Page
Table 1: Larval spawning and culture conditions .....	21
Table 2: Experimental treatments .....	28
Table 3: Summary of statistical analyses.....	39
Table 4: Summary of treatment effects on settlement rate .....	42
Table 5: Summary of significant treatment effects on mortality .....	43
Table 6: Summary of significant treatment differences of re-binned barnacle treatments from control settlement rate ( <i>C. virginica</i> ) .....	67
Table 7: Summary of ammonia concentrations and larval settlement rates .....	70

## LIST OF FIGURES

	Page
Figure 1: Map of study locations. Rappahannock River, Pungoteague Creek, Virginia ...	24
Figure 2: Schematic diagram of microcosm .....	29
Figure 3: Photographs of experimental setup .....	30
Figure 4: Average settlement rate of <i>C. virginica</i> larvae by treatment (Exp 1).....	45
Figure 5: Average mortality of <i>C. virginica</i> larvae by treatment (Exp 1) .....	46
Figure 6: Average settlement rate of <i>C. ariakensis</i> larvae by treatment (Exp 1).....	47
Figure 7: Average mortality of <i>C. ariakensis</i> larvae by treatment (Exp 1) .....	48
Figure 8: Average settlement rate of oyster larvae by treatment (Exp 2).....	50
Figure 9: Average mortality of larvae by treatment (Exp 2) .....	51
Figure 10: Average settlement rate of oyster larvae by treatment (Exp 3).....	52
Figure 11: Average mortality of larvae by treatment (Exp 3) .....	53
Figure 12: Average settlement rate of oyster larvae by treatment (Exp 4).....	54
Figure 13: Average mortality of larvae by treatment (Exp 4) .....	55
Figure 14: Average settlement rate of oyster larvae by treatment (Exp 5).....	57
Figure 15: Average mortality of larvae by treatment (Exp 5) .....	58
Figure 16: Average settlement rate of oyster larvae by treatment (Exp 6).....	59
Figure 17: Average mortality of larvae by treatment (Exp 6) .....	60
Figure 18: Average settlement rate of oyster larvae by treatment (Exp 7).....	63

List of Figures (continued)

	Page
Figure 19: Average mortality of larvae by treatment (Exp 7) .....	64
Figure 20: Mean settlement of larvae ( <i>C. ariakensis</i> ) in re-categorized barnacle cover treatments across experiments .....	65
Figure 21: Mean settlement rate of larvae ( <i>C. virginica</i> ) in re-categorized barnacle cover treatments across experiments .....	66
Figure 22: Average settlement rate of oyster larvae by bathwater treatment (Exp 8) .....	74
Figure 23: Average mortality of larvae by bathwater treatment (Exp 7).....	75
Figure 24: Average settlement rate of oyster larvae by bathwater treatment (Exp 8) .....	76
Figure 25: Average mortality of larvae by bathwater treatment (Exp 8).....	77
Figure 26: Average mortality of larvae in different bathwaters and with different exposure durations (Exp 8) .....	78
Figure 27: Effect of bathwater dilution on settlement of oyster larvae (Exp 8) .....	79
Figure 28: Effect of bathwater dilution on mortality of oyster larvae (Exp 8).....	80
Figure 29: Effects of clamworm density on the number of recovered larvae (Exp 4).....	82
Figure 30: Effects of clamworm density on the mortality of recovered larvae (Exp 4) ....	83
Figure 31: Effects of clamworm density on the number of recovered larvae (Exp 5).....	84
Figure 32: Effects of clamworm density on the mortality of recovered larvae (Exp 5) ....	85
Figure 33: Logarithmic relationship between the number of larvae found in clamworm treatments and the duration of larval exposure (Exp 7).....	88
Figure 34: Logarithmic relationship between the mortality of larvae remaining in clamworm treatments and duration of larval exposure (Exp 7).....	89
Figure 35: Size-Frequency histogram of clamworm populations at Pungoteague Creek site .....	90

## ABSTRACT

The behaviors of oyster larvae are difficult to monitor or experimentally manipulate, especially in field conditions. As a result, little is known of the fate of oysters in the larval portion of their life cycle, prior to recruitment. At the transition from pelagic larvae to benthic adults, larvae are likely to come into contact with many invertebrates resident on oyster reefs. Of these, fouling epifauna are generally believed to reduce the settlement of interspecific larvae through competitive exclusion and predation. Studies of these interactions, however, often utilize artificial settlement panels, which can exhibit different recruitment patterns to those observed on natural substrates. I therefore investigated the interactions between reef-associated fauna and settling oyster larvae on natural shell substrates.

Over a series of laboratory microcosm studies, native (*Crassostrea virginica*) and non-native (*Crassostrea ariakensis*) larvae were exposed to reef-collected shells, each supporting a single species of reef-associated fauna. The presence of adult bryozoans (*Membranipora tenuis*) had little effect on either larval settlement rate or mortality. The boring sponge (*Cliona* sp.) significantly decreased oyster larval settlement, and generally increased oyster mortality. Barnacles (*Balanus improvisus*) typically facilitated settlement. Barnacle molds and empty barnacle tests, intended to mimic the surface area and rugosity of live barnacles, did not significantly affect settlement. However, in some trials, adult barnacle bathwater enhanced settlement of both oyster species, implicating the role of waterborne cues. Such bathwaters were found to cause oyster larval mortality, as were bathwaters created by adult clamworms or even adult oysters. Predation by clamworms (*Neanthes succinea*), which were found at very high densities on field-collected oyster shells, caused significant oyster larval mortality in these experiments.

The combined roles of both positive and negative interactions between oyster larvae and reef fauna require enumeration under field conditions. The results from this study highlight the need for clarification of these roles in order to optimize shell supplementation restoration efforts, and to more thoroughly understand the settlement behaviors and mortality sources of recruiting oyster larvae.

Interspecific Interactions in Oyster Reef Communities:  
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## 1. INTRODUCTION

Much consideration has been given to the positive impacts of healthy oyster reefs on local biodiversity, supporting the need for oyster restoration efforts nationwide. The ecological benefits of oyster reefs have been well documented (e.g., Coen and Luckenbach, 2000) and have been positively correlated with oyster size and abundance (Luckenbach et al., 2005). As ecological engineers, oyster reefs can provide protection for motile species (Lenihan et al., 2001), while the relief provided by reefs can impact local ichthyofaunal diversity (Harding & Mann, 1999) and fisheries species (Breitburg et al., 2000). The recent decline in oyster populations in the Chesapeake Bay has led to a greatly reduced filtering capacity of oysters which is contributing to the diminished water quality seen in this region (Newell, 1988; Kemp et al., 2005; but see Pomeroy et al., 2006). Healthy oyster populations have the potential to improve conditions for seagrass beds (Newell and Koch, 2004), and thus the commercially important blue crab (Heck and Thoman, 1984). It has been proposed that these ecological benefits should be the focus and metrics of success of restoration efforts (Coen and Luckenbach, 2000; Breitburg et al., 2000).

The current plight of native oyster reefs, *Crassostrea virginica* in the mid Atlantic region, and in particular the Chesapeake Bay, is also well known. The combined effects of decades of unregulated harvests (Gross and Smyth, 1946), habitat degradation (Rothschild et al., 1994), reduced water quality (Seliger et al., 1985), infections by the endemic parasites *Perkinsus marinus* (Dermo), and *Haplosporidium nelsoni* (MSX) (Ford

and Tripp, 1996; Lenihan et al., 1999), and the interactions between these factors (Lenihan and Peterson, 1998) have led to the extreme depletion of this once-seemingly endless resource; current levels of population are 1% of those at the start of the last century (Newell, 1988). Limited success to date in native restoration has generated interest in alternative restoration proposals. One includes the introduction of an exotic oyster species, the Suminoe oyster, *Crassostrea ariakensis*, native to Asia, into the Chesapeake Bay to revitalize oyster populations to restore both the fishery resource and ecological function. Given the current interest in the potential of *C. ariakensis* to achieve such revitalization, this study incorporated both native (*C. virginica*) and non-native (*C. ariakensis*) oysters into experiments. This allowed comparisons of larval recruitment preferences and settlement behaviors between oyster species.

The removal of substrate through over-harvesting (and subsequent substrate limitation for oyster to settle upon) has led to the practice of adding hard substrate to coastal waters during the oyster spawning season in an attempt to increase oyster recruitment. The addition of shell is especially valuable at times when little natural shell growth is occurring, as oyster shells may only have a half-life on reefs of 2 to 10 years (Powell et al., 2006) before they dissolve or become buried into sediments. Efforts to increase available substrate for recruitment through shell plantings are nonetheless limited by the availability of shells for restoration projects.

Such shell additions are, however, typically done without consideration of how the colonization of these substrates by other organisms may affect oyster recruitment and survival. Interspecific adult-larval interactions among invertebrates have been studied extensively in a variety of environments, with larval recruitment inhibition by adults

often playing a key role in the composition of community assemblages (Young and Chia, 1981; see reviews by Scheltema, 1974; Woodin, 1983; Steinberg et al., 2001). There are, however, examples of predators attracting prey to settle (Hadfield and Pennington, 1990), and of invertebrate larvae being drawn to exudates from prey or host species (Lambert et al., 1997; see review by Pawlik, 1992; Hadfield and Paul, 2001). Amongst epifaunal invertebrates, there is evidence of facilitation of oyster larval recruitment by the presence of adult barnacles (Osman et al., 1989) with unknown mechanisms. Nearly all of the research involving epifauna, however, has been conducted using artificial plates as settlement substrates, which typically show differential recruitment patterns compared to natural substrates (Goddard et al., 1975; Harriott and Fisk, 1987; Tamburri et al., 2008). This study represents a change in emphasis from previous epifaunal interactions research utilizing artificial substrates to those using natural, reef-collected shells as settlement substrates.

The transition from pelagic larvae to benthic adults is difficult to monitor or experimentally manipulate, especially in field conditions. Once spawned, oyster larvae are effectively lost to researchers until they have recruited to the benthos. Further, surveys of oyster populations do not enumerate larval recruits until they have reached a length of several millimeters, due to practical constraints. As such, the processes affecting oyster recruitment are, regrettably, poorly understood. Overall, this research is intended to clarify understanding of the relationships between oyster larvae and reef dwelling invertebrates on natural settlement plates, thereby providing insight into basic ecological processes of recruitment, and secondarily, appropriate use of resources in restoration efforts.

## 2. AIMS AND OBJECTIVES

The primary objective of this study was to investigate the effects of the presence of epifaunal invertebrates on oyster larval settlement and recruitment, using natural substrates. Through a suite of microcosm experiments, native eastern oyster (*C. virginica*) and non-native Suminoe oyster (*C. ariakensis*) larvae were exposed to shells encrusted by fouling species at varying density levels. In this manner, the settlement and recruitment preferences and behaviors of oyster larvae of both species were quantified, with the goal of improving our understanding of the interactions between competent oyster larvae and sessile adult invertebrate communities. Further, investigations were designed to identify the underlying mechanisms and ecological significance of inhibitory and facilitatory oyster larvae recruitment responses to natural epifaunal assemblages.

During the course of investigation, it became clear that other factors were influencing oyster larvae within my experiments (and potentially in the field) which warranted investigation. As such, the objectives of the study were expanded to include investigating the effects of waterborne cues and *Neanthes succinea* on larval oyster settlement. Apart from clarifying the roles of epifauna on the ecology of settling oyster larvae, this study should serve as a resource to direct management decisions regarding where substrate additions are required, which are currently made without regard to the effects of fouling organisms to future oyster recruitment.

### 3. REVIEW OF RELEVANT LITERATURE

#### 3.1. *Crassostrea virginica* larvae

*C. virginica* are oviparous fertilizers, and larvae begin as trochophores of approximately 50 µm diameter. After 24-48 hours, the larvae develop into shelled veligers, feeding and swimming through the use of a ciliated velum (see Thompson et al., 1996). Swimming in the larval stage occurs in vertical helical pattern (see Kennedy, 1996). At the time of settlement, the larvae are approximately 300 µm in diameter (see Thompson et al., 1996). Thorson (1964) concluded that *Crassostrea virginica* larval behaviors, like many other benthic invertebrates, are likely photopositive during most of their larval cycle, although larval behaviors become photonegative near the time of settlement, bringing the larvae into contact with substrates to colonize.

Before describing larval behaviors at the transition to a benthic, sessile adult form, it is important to distinguish some of the pertinent terminology and how they will be used throughout this study (see Rodríguez et al., 1993). Settlement is a reversible, behavioral process; larvae reach the substrate and temporarily affix while searching for an appropriate colonization site. Once such a location has been found, oysters will undergo metamorphosis – a permanent morphological and physiological transition to the adult form. In *C. virginica*, metamorphosis includes loss of the velum, foot and eyespot, development of labial palps, gill proliferation, and organ revolution (Baker and Mann,

1994). Upon completion of metamorphosis, the juvenile oyster no longer retains any larval organs, and has thus completed recruitment. Juveniles are typically first seen approximately 48 hours post settlement (Baker and Mann, 1994). This study deals primarily with the recruitment preferences of oyster larvae. However, as full completion of metamorphosis could not be ensured at the termination of these experiments, the term settlement will be frequently used – in this case describing only larval settlement which leads, or has led to, recruitment.

### 3.2. *Early investigations of larval recruitment*

Some of the earliest work on epifaunal recruitment focused on simple observations of larval settlement patterns and behaviors in nature. Nelson (1924) was the first to publish accounts of direct observation of oyster (*Ostrea virginica*) settlement, and made note of larval settlement behavior immediately preceding recruitment. In particular, the larvae were seen to “move over an appreciable area of solid surface” as they “test” the surface with their foot for appropriate recruitment sites. He also made note of potential recruitment choices:

“I have recently shown (Nelson, '23) that oyster larvae will not attach to shells which are extensively pitted by the boring sponge, *Clione*, or which are badly corroded and which present surfaces that are microscopically rough.”

Visscher (1928) found that barnacle cyprids have a similar ability to “walk” around on the substrate, and “hunt” for their preferred recruitment location. These early studies established the ideology that larval settlement is not necessarily a passive process, but that substrate selection is a complex series of behaviors exhibited by the larval form.

Subsequently, oyster recruitment behavior has been correlated with physical parameters including temperature (Lutz et al., 1970), light (see Thorson, 1964), and currents (Bushek, 1988), as well as the interaction of several physical parameters (Hidu and Haskin, 1971). Many studies have shown that *C. virginica* larvae preferentially recruit subtidally, despite a primarily intertidal adult distribution (McDougall, 1942; Chestnut and Fahy, 1952; 1953; Nichy and Menzel, 1967; Roegner and Mann, 1990; 1995). These authors explain that this discrepancy between fundamental niche and realized niche is likely due to increased subtidal predation mortality (see Hutchenson, 1957). Crisp (1967) found that oysters preferentially recruit to the smooth interior of shells. More recently, oyster larvae have been shown to avoid sediment covered substrates, and have displayed preferences to natural over artificial substrates (Tamburri et al., 2008). Although physical parameters were largely held constant in the current investigation, these studies form the basis for many methodological decisions of the study intended to maximize overall recruitment.

### 3.3. *Adult-larval interactions*

#### 3.3.1. *Interspecific interactions in benthic systems*

Interspecific interactions have been studied extensively in a variety of benthic systems, with most of the work focused on inhibitory mechanisms: predation of larvae, space pre-emption, allelopathy, larval avoidance, substrate modification, or flow modification. Woodin (1976, 1978, 1983) summarizes the interactions between adults and larvae in dense infaunal communities, noting the mechanisms (e.g., predation, currents, space limitations) by which the common assemblages are maintained, again focusing on inhibition. Woodin (1976) proposed that:

“(The) preferential settlement of larvae in infaunal organisms seems to be due to the presence of particular microorganisms, not adults of the larval species. In contrast, the settlement preferences of larvae in epifaunal systems often seem to relate to the presence of adults of the same species”

Woodin et al. (1993) demonstrated that allelopathy can occur simultaneously in these systems. Koh and Sweatman (2000) similarly demonstrated that in coral reefs, the extracts of certain adult coral species were toxic to the larvae of the 11 competitive dominants tested. These same extracts had no effect on conspecific larvae. Working with coral reef epifaunal settlement on roughened Plexiglas plates, Breitburg (1985) found that prior residents, grazing, and temporal and spatial variability played important roles in community development, highlighting that grazing, and temporal and spatial variability indirectly affect recruitment by directly affecting the prior resident community composition. In contrast, predators can often detect chemical cues from interspecific prey and settle nearby in response (Lambert et al., 1997; see review by Pawlik, 1993;

Hadfield and Paul, 2001) Adult predators can also lure prey to settle (Hadfield and Pennington, 1990) using chemical cues. Steinberg et al. (2005) summarized that settlement inducers are generally water soluble, while settlement inhibitors are typically comparatively insoluble.

### 3.3.2. *Biofilms*

Bacterial biofilms were first mentioned by ZoBell and Allen (1935) at the Scripps Institute, California, USA, who found that bacteria became “permanently” attached to glass slides after being submerged for only 1-2 hours. They also found that slides with bacterial fouling were more likely to be settled by macroinvertebrates than sterile slides. The biofilms that are effective in enhancing *Crassostrea virginica* were characterized by Weiner et al. (1985) using *Alteromonas collwelliana* cultures taken from hatchery surfaces. Weiner et al. (1989) later showed *A. collwelliana* significantly increased the settlement of both *C. virginica* and *C. gigas* on a variety of artificial surfaces. Biofilms were most effective after 72 hr of bacterial growth, when the bacterial film had grown to several cells deep. Much of the biofilm research has been conducted in the context of the prevention of the biofouling of boat hulls and other submerged man-made substance, and has lead to the isolation of several compounds and biogenic exudates which preclude the settlement of invertebrate larvae (e.g. Dobretsov, 2005).

### 3.3.3. *Gregariousness in Crassostrea virginica*

Gregarious behavior has been identified in many epifaunal invertebrates, including barnacles (Crisp and Knight-Jones, 1953; Bushek, 1988; Raimondi, 1988;

Browne and Zimmer, 2001) holothurians (Young and Chia, 1982), scyphozoan polyps (Gröndahl, 1989), and tubeworms (Toonen and Pawlik, 2001). Gregariousness is often viewed as a method of increasing reproductive success (Denny and Shibata, 1989) and juvenile survival (Buss, 1981) through ensuring proximity to conspecifics. Studying *C. virginica*, Hidu (1969) found that larvae preferentially recruited to shells already set with 24 hour-old spat, and that the rate of recruitment increased proportionally with the abundance of other spat already established. A water-soluble cue to settlement had already been proposed for oyster larvae (*Ostrea edulis*) by Cole and Knight-Jones (1949). Hidu (1969) found that the presence of such a cue was likely to increase recruitment of *C. virginica* larvae, having observed significantly higher recruitment rates in a tank containing a plankton-net bag of 2 month-old spat than a control tank with no bag.

In attempting to identify the source of this cue, Keck et al. (1971) found that adult oyster feces and pseudofeces, as well as oyster shell liquor, increased larval recruitment compared to controls. Oyster liquor in their study included “fluid that drains from the shell cavity when valves were pried open as well as the juices released when oyster meats were chopped.” This trend, however, reversed when experiments were run for longer than 48 hours, possibly due to build-up of bacteria on treated plates. Hidu et al. (1978) established that this cue is effective in water, and thus does not require adhesion to shells in order to promote gregariousness. They also found that extrapallial fluid from *C. virginica* induces settlement in *O. edulis*, and vice versa. These authors concluded that the cue may cause the larval behaviors to become photonegative, thereby indirectly inducing larvae to settle (see Thorson, 1964).

Through a series of 4 bioassays, a settlement-inducing cue for *C. virginica*, identified by Zimmer-Faust and Tamburri (1994), was found to consist of low-molecular-weight peptides with arginine at the C-terminal. Flume studies by Turner et al. (1994) and Tamburri et al. (1996) showed that the cue remains effective in moving water, and induces downward swimming. Tamburri et al. (2007) conducted flume experiments which showed that downward swimming triggered by this cue was 3 times slower than that due to gravitational falling.

Pechenik (1999) reviewed the positive impacts of gregarious behavior, but also noted that gregariousness in filter-feeding invertebrates may in fact negatively affect the adult population through the cannibalism of conspecific larvae. Ertman and Jumars (1988), studying siphonal currents in sparse aggregations of the cockle, *Clinocardium nuttallii* and the soft shell clam, *Mya arenaria*, found that the inhalent siphon had no effect on currents beyond 3-4 cm laterally, and 1-2 cm vertically. The exhalent stream, however, could affect currents to 13 cm vertically in the water column, and had the potential to aggregate settlement downstream. Tamburri et al. (2007) also contradict the findings of Pechenik (1999) in their investigation of *C. gigas* reporting that although the capability for cannibalism exists in adults, it rarely occurs in moving water. Larvae needed to be within ~ 2 mm of the gape in order to be cannibalized, which accounts for only about 2% of the total surface area of the adult.

#### 3.3.4. *Interspecific interactions among common oyster reef epifauna*

Interspecific interactions between adults and larvae received scientific attention much later than that of gregariousness (see Scheltema, 1974), and much of the epifaunal

work in this field has involved barnacles as the test species (for this reason, barnacles are an obvious choice for test organism in this study). Butler (1955) suspended Plexiglas plates off Pensacola, Florida, and found that more oysters recruited to the upper side of the plates, which he correlated with fouling level by barnacles on the under side. Butler speculated that:

“Barnacles may set more quickly than oysters on newly exposed cultch and the sweeping action of their appendages in collecting food repels the larvae. On upper surfaces, this sweeping action does not interfere too much with larvae which drift down between the barnacles and set. On vertical and under surfaces, however, when an oyster larva comes into contact with this field of activity it closes and falls away from the surface. Relatively few barnacles can seriously interfere with the setting rates on vertical and under surfaces.”

Bushek (1988) found that plates already colonized by oysters had lower settlement of barnacle cyprids than control plates with no fouling, and also observed gregarious settlement responses in barnacles when comparing control plates to those already set with barnacle adults. The inhibition of cyprid settlement by adult oysters, however, was more significant than the gregarious behavior. Young and Gotelli (1988) found that colonial species were inhibited to a greater degree by live barnacles than dead barnacles, while solitary species showed no response to barnacle treatment. Living barnacles did not, overall, inhibit recruitment rates but rather altered the spatial

arrangement of recruits. Bros (1987), however, noted that the removal of barnacles decreased recruitment compared to unaltered control plates, and that the addition of barnacle moulds to clean plates increased recruitment. There was, however, no difference between plates on which barnacles had been specifically removed and plates on which fouling removal was random. Bros (1987) attributed this to high percentage cover of plates by barnacles (up to 60%) such that the treatments were essentially confounded.

Although barnacles are generally thought of as gregarious settlers (e.g., Crisp and Knight-Jones, 1953; Bushek, 1988; Raimondi, 1988; Browne and Zimmer, 2001), Navarrete and Wieters (2000) found that, under natural field conditions, the presence of a large barnacle species (*Semibalanus cariosus*) can decrease settlement by conspecifics and other barnacle species. The authors attribute this to predation by adult barnacles on larvae, as this reduction in recruitment was not observed in an extremely high recruitment year.

Other researchers have focused on fouling assemblages, monitoring epifaunal communities on natural and experimental rocks. Osman (1977) found that larval selectivity, biological interactions, substrate size, seasonality, and disturbance regime all played important roles in the development and maintenance of epifaunal communities, with physical disturbance being the most important. Osman (1977) also proposed that these factors are not independent (e.g., smaller substrates are more easily disturbed). Competition for space was the main biological driver (see “intermediate disturbance hypothesis” in Connell, 1978). Using a series of suspended asbestos-cement panels, Dean and Hurd (1980) observed that both inhibition and facilitation played roles in

species succession, although inhibition was more common and no species required facilitation for settlement. Pertinent to the current study, the presence of *Balanus improvisus* was found to slightly decrease settlement rates of the tunicate *Molgula manhattanensis*. Later, Dean (1981) created “mimics” (or moulds) of some of the common fouling organisms to examine the effects of structure alone, and was able to explain several of the trends from the previous study. The precise mechanisms were unclear, but may include alteration of flow, addition of surface area, refuge, or competition / mutualism.

Osman and Whitlatch (1995) studied recruitment onto plates fouled by a suite of organisms at differing percent coverage levels under natural conditions. Although they observed some significant interactions between adults and larvae, their main conclusion was that the main effect of sessile adults was to change “the quality of available space.” Bryozoans and ascidians were generally not settled upon; however, the areas surrounding these organisms were often more densely settled upon than controls, suggesting that larvae which landed on these adults simply moved to adjacent unfouled areas (also suggested for barnacles by Young and Gotelli, 1988). Osman and Whitlatch (1995) found little evidence of larval predation affecting settlement. Adult barnacles and oysters slightly increased overall settlement (except for *Botryllus*), perhaps due to the additional hard-surfaces or the physical structure created by these organisms (see also Bros, 1987). Intraspecific interactions seemed to be the strongest. Pineda and Caswell (1997) expanded on the “quality of available space” and coined the term “intensification effect.” Their model, supported by work on laboratory-controlled barnacle recruitment, predicts higher recruit densities as a function of decreasing available space and higher larval

retention. The model developed by Pineda and Caswell (1997), however, does not take account of the congregating effects of gregarious behaviors.

Cole and Knight-Jones (1949) published the first account of interactions between fouling species and oyster larvae (*Ostrea edulis*). They found a nearly 3-fold increase in oyster settlement on shells which had been fouled by “assemblages of filamentous algae, ascidians, polyzoans [bryozoans], diatom and bacteria patches, small mussels, and hydroids.” Larvae were more attracted to the clean sides of fouled shells than to control shells. Oyster larvae settlement behaviors in response to epifauna was later investigated by Osman et al. (1989), in a project that closely resembles the present study in both its methods and objectives. These researchers selectively removed species from naturally fouled PVC plates, leaving a single fouling species on each plate. Competent oyster larvae were then exposed to these plates (both in the lab and *in situ*), and larval preferences determined by their choice of settlement location. In their first experimental run, they found that oyster recruitment significantly increased in response to most fouling treatments, regardless of percent coverage. The two exceptions found were that high barnacle coverage did not affect total oyster recruitment compared to controls, and the presence of *Ciona intestinalis* decreased oyster settlement at any percent coverage. Upon replication of the barnacle experiment, the authors contrarily found that both low and high barnacle treatment levels attracted significantly higher recruitment levels than the controls, regardless of whether the barnacles were alive or dead. Tamburri (unpublished data) found that such relationships are likely driven, in part, by a settlement response on the part of oyster larvae to “low-grade” biological cues emitted by adult organisms.

The current study expands on the Osman et al. (1989) study in several ways, in order to more thoroughly answer some of the questions previously posed by these authors. Of greatest significance is my replacement of artificial substrate with reef-collected shells. There are potential differences in the both colonizing species and the degree of fouling that would occur on PVC plates submerged short-term compared to natural shells collected from a reef ecosystem (Sutherland, 1974; Rheinhardt and Mann, 1990). Also, oyster larvae prefer to recruit to natural oyster shells compared to PVC (Tamburri et al., 2008). Thus, the conclusion that areas already set with barnacles are preferred over unfouled PVC by oyster larvae reveals nothing about the relationship between oyster larvae and their natural habitat. Finally, there may be a serious omission in methods with respect to bacterial biofilms. Osman et al. (1989) make no mention of whether biofilms removed along with the non-target fouling organisms were allowed to re-develop. Coupling the above limitations with evidence that oyster larvae prefer to settle on shells with a bacterial biofilm (see above; Weiner et al., 1985), reveals that the proposed relationships between fouling level and larval settlement reported in Osman et al. (1989) requires more stringent investigation. In order to ameliorate some of the limitations of Osman et al. (1989)'s findings, the current study follows more closely the methods employed by Tamburri et al. (2008), conducting settlement preference investigations using similar wells, trays, physical conditions, and larval counts per well.

### *3.3.5. Methodological concerns in adult-larval interactions research*

There are a number of concerns when working with settlement plates, and the lessons from previous studies provided cautionary guidelines for the current study.

Sutherland (1974) gave examples from settlement plates (and other environments) suggesting stable, yet small scale variability in community structure (alternate stable states) is likely due to the prior history of predation and disturbance events. This can be seen as a cautionary tale for conducting research on newly submerged plates, as these may differ in composition compared to natural populations. There is also direct evidence of discrepancies between communities found on natural and artificial substrates (Goddard et al., 1975; Harriott and Fisk, 1987)

Working with interactions between ascidians and larvae, Young (1989) warned that “consumption of larvae in the laboratory cannot be used to assume significant inhibitory effects in the field.” Such caution was also given by Young and Gotelli (1988) studying the effects of barnacles on invertebrate larval recruitment. This is not to say that predation (and even cannibalism) of larvae does not occur in natural environments. For example, gut content analysis on *Mytilus edulis* by Lehane and Davenport (2004) established that adult mussels will consume settling bivalve larvae throughout the year, both under laboratory and field conditions.

Young (1990) specifically cited some of the problems with adult-larval interaction research; overestimation of the consumptive radius of an established adult (but see Tamburri et al., 2007) and overall small effect sizes resulting in low statistical power. Nevertheless, Young (1990) recognized the often impossibility of sample sizes large enough to achieve a high power. The issue of sample size is exemplified by the contrary findings of Grosberg (1981) and Bullard et al. (2004). Grosberg (1981) found that many settling invertebrates had the ability to detect and avoid the presence of dominant competitors, specifically the tunicate *Botryllus schlosseri*. When Bullard et al. (2004)

repeated this study with minor modifications and a larger sample size, they contrarily found no evidence of tunicate avoidance by settling invertebrate larvae. Overall, these concerns need to be considered when designing epifaunal interspecific interactions experiments, and were managed in this study through the use of natural, reef-collected shells, factoring of dead larvae, and adequate treatment replication.

## 4. MATERIALS AND METHODS

### 4.1. *Spawning and larval rearing*

*C. virginica* and / or *C. ariakensis* larvae, depending on availability of larvae and spawning schedules, were obtained from the Virginia Institute of Marine Science (VIMS) Eastern Shore Laboratory (ESL) Castagna Shellfish Hatchery for use in recruitment preference experiences. Multiple adults were used in each spawn to ensure at least some level of competency (i.e., development to the eyed larvae stage). Broodstock conditioned at 20 °C and 20 psu for at least 8 weeks were induced to spawn thermally and with the addition of frozen (and thawed) species-specific sperm. Larvae were reared at 25 °C (+/- 1 °C) and 20 psu, except in a few instances (to accommodate other researchers). Larvae were fed a daily mixture of *Isochrysis* sp. (C-Iso), *Chaetoceros* sp. (Chaet B), and *Tetraselmis suecica* (WTET), with water changes on alternate days. After 14 days of rearing, larvae were checked daily for competence, and were only used in experimental trials when the percent of larvae that was 'eyed' exceeded 90%. In some instances the competent larvae were stored on a nitex mesh in the refrigerator (5 °C) for no more than 24 hours prior to experimentation (Table 1).

**Table 1: Larval spawning and culture conditions**

Experiment	Species	Spawn Date	Males	Females	Experiment Start Date	Percent eyed larvae	Other information
1	<i>C. virginica</i>	9-Aug-07	9	12	25-Aug-07	91	Salinity reduced during culture
1	<i>C. ariakensis</i>	10-Aug-07	13	26	25-Aug-07	90	Salinity reduced during culture
2	<i>C. virginica</i>	21-Apr-08	19	6	5-May-08	90	
3	<i>C. ariakensis</i>	5-May-08	4	3	20-May-08	92	
4	<i>C. virginica</i>	20-May-08	4	5	3-Jun-08	99.6	Held on nitex in refrigerator overnight
5	<i>C. virginica</i>	16-Jun-08	12	4	30-Jun-08	97	
6	<i>C. ariakensis</i>	7-Jul-08	3	15	22-Jul-08	98	Salinity reduced during culture
7	<i>C. virginica</i>	5-Aug-08	12	12	20-Aug-08	91	Held on nitex in refrigerator overnight
8	<i>C. virginica</i>	21-Aug-08	8	18	5-Sep-08	95	

#### 4.2. *Settlement substrates*

Oyster shells (*C. virginica*) were collected from subtidal oyster reefs located in tributaries of the Chesapeake Bay (Rappahannock River and Pungoteague Creek; Figure 1). A variety of methods were employed in obtaining shells, including dredging, free-diving, and submerging clean air-dried shells within cages along a reef. Shells were selected or rejected for experimentation according to a number of criteria, in particular the dominant fouling species present. Shells with large holes or undulations were avoided, as they may have confounding effects on larval settlement. Test organisms included barnacle (*Balanus improvisus*), bryozoan (*Membranipora tenuis*) and boring sponge species (*Cliona* sp.).

Non-dominant fouling species (e.g., hydroids) were removed, creating shells housing single-species assemblages of epifauna. Shells were cut into approximately 3 cm x 3 cm squares in order to standardize settlement rates per unit area of experimental shell. Only one side of each shell was used as test substrate, thus the reverse side was cleared of all fouling organisms. As *Cliona* sp. cannot easily be removed from the substrate, shells containing boring sponges, either live or dead, were not considered for other experimental treatments. Shells were submerged in and rinsed copiously with fresh water in attempts to eradicate *Neanthes succinea* from the test shells. No alteration of the shells took place within the 2 days prior to experiments, thereby ensuring the adequate development of bacterial biofilms. In some instances (described below) air-dried shells were used directly in experiments after being allowed to acquire a bacterial biofilm. All experimental shells were maintained at the VIMS Eastern Shore Laboratory Castagna Shellfish Research Hatchery at 20 psu (1  $\mu\text{m}$  filtered seawater mixed with sand-filtered

tap water) and ambient temperature. Fouling organisms were fed daily on a mixed species diet of *Chaetoceros* sp. (ChaetB) and *Isochrysis* sp. (C-Iso) at concentrations of approximately 5,000 cells ml<sup>-1</sup>, with twice-weekly water changes.

**Figure 1: Map of study locations. Rappahannock River, Pungoteague Creek, Virginia**



### 4.3. *Experimental design*

The general experimental design for each trial consisted of 16 plastic trays each containing 8 individual wells (4.3 cm x 5.7 cm). Wells contained sufficient very fine sand (63 to 125  $\mu\text{m}$  grain size, combusted at 500 °C for at least 10 hours) to cover the cleaned underside of an experimental oyster shell, preventing (or at least reducing) settlement of larvae on this surface, while leaving the experimental surface exposed. Subsequently, 50 ml of adult oyster bathwater (approximately 10 filtering oysters in 10 L water for 12 hours) from the parental oyster stocks was added to each well. In experiments including barnacle cues, the barnacle cue consisted a 50:50 mixture of barnacle bathwater (approximately 500 filtering barnacles in 4 L water for 12 hours) and concentrated oyster bathwater (10 filtering oysters in 5 L water for 12 hours), while the remaining shells were submerged in a 50:50 dilution of the concentrated oyster bathwater with clean water. Subsamples of all bathwaters were retained prior to experimentation and frozen for ammonium analyses. Trays were partially submerged in a circulating water table in order to maintain conditions at 25 °C and elevate oyster larval recruitment rates equally across all treatments and experiments (Figure 2).

Once the wells were temperature acclimated, and any remaining suspended sediment grains settled, the experimental shells were added to the wells. Sixteen shells from each of 8 substrate treatments were used in each experimental trial. Substrate treatments varied with experimental trial according to the availability of suitable shells, as well as the stated experimental objective of a trial. Overall, treatments included the following: control, no shell (negative control, see below for details); control, shell with no fouling organisms (positive control); shells with high, medium, and low percent

coverage by the barnacle *Balanus improvisus*; shells encrusted by the bryozoan *Membranipora tenuis*; and shells inundated with the boring sponge *Cliona* sp.. Later experiments also utilized other treatments: barnacle moulds (Sculpey® impressions made from real barnacles and affixed to control shells using marine adhesive 5200); barnacle cues (control shells submerged in adult barnacle bathwater, described above); dead barnacles (barnacle treatments in which the flesh of the barnacle had been removed and replaced with marine adhesive 5200); and clamworm (*Neanthes succinea* added to air-dried control shells) treatments (Table 2). One replicate shell of each treatment level was placed in each tray, establishing a full factorial, repeated Latin squares design. Once the treatments were loaded, each well was photographed in order to calculate the available surface area of the shell (i.e. the area of the shell not covered by sand) using ImagePro<sup>®</sup> image analysis software (Figure 3).

#### 4.4. Larval additions

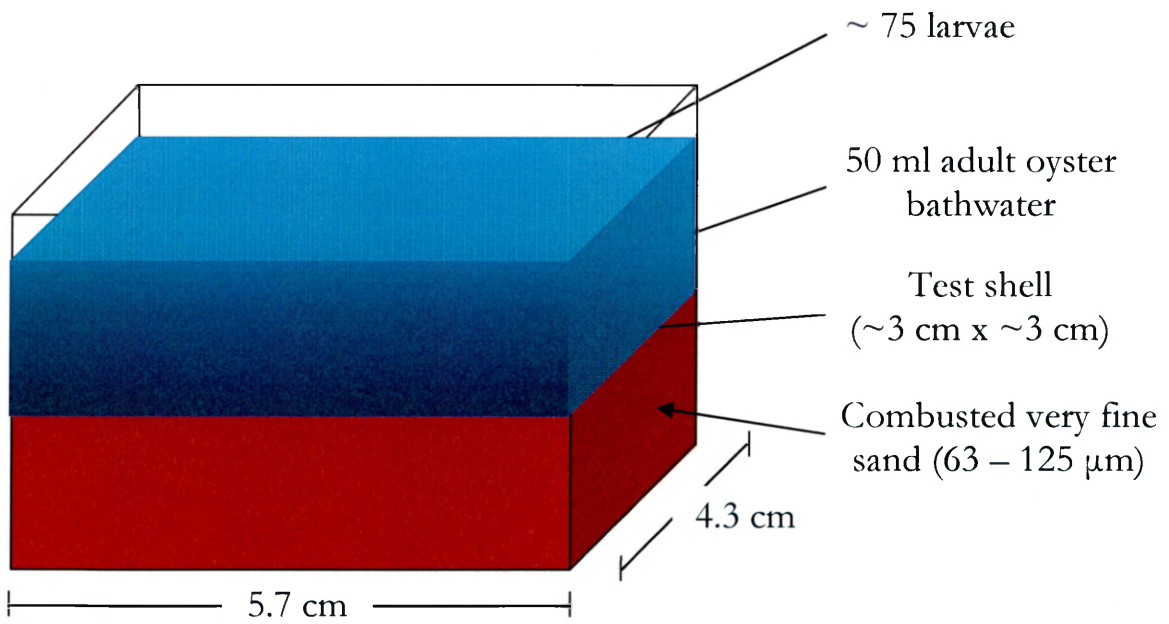
Approximately 75 larvae (determined volumetrically, less than 1 ml addition) were added to each well in a pre-determined, randomized order. The mean number of larvae added in these experiments (as determined from recovery in negative control treatments) ranged from 46.9 to 87.1 with a standard deviation range from 10.0 to 19.0. The addition sequence divided larval additions into 8 rounds. Each round of additions introduced larvae to one random well in each tray, with the tray order also having been randomized. This approach was intended to avoid trends in oyster larval recruitment related to addition order by treatment, well position, or tray number. In the case of experiment 1, both *C. ariakensis* and *C. virginica* larvae were available for

experimentation simultaneously. As such, half of the trays were randomly selected to receive *C. ariakensis* larvae, while the remaining trays were loaded with *C. virginica* larvae (i.e. each oyster species was tested in 8 replicates of each treatment).

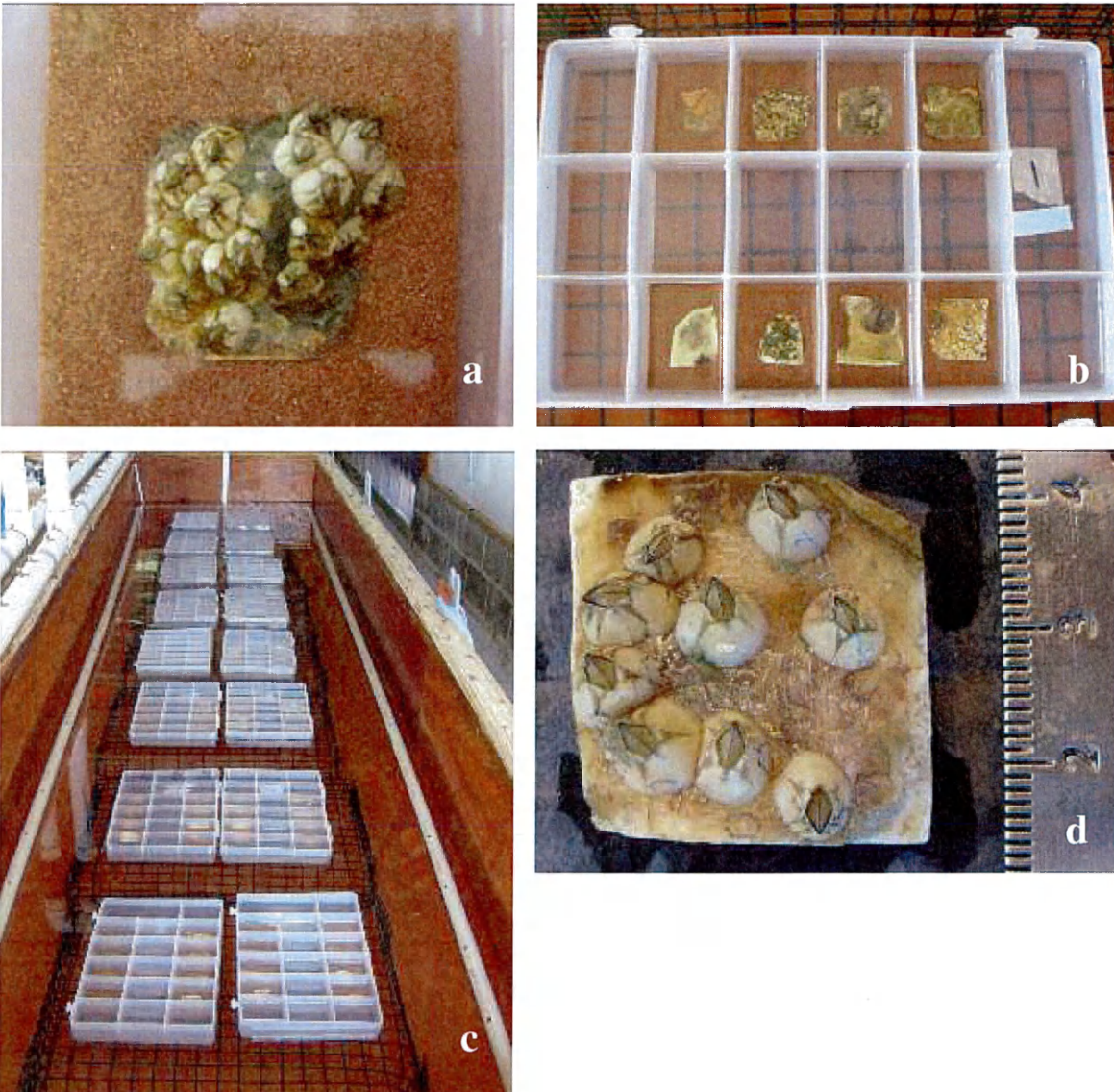
**Table 2: Experimental treatments - treatments garnered for each experiment**

Experiment	Experimental Treatments											
	Negative Control	Positive Control	Low Barnacle	Medium Barnacle	High Barnacle	Bryozoans	Cilona	Barnacle Mould	Dead Barnacle	Barnacle Cue	Worms	Cues
1		2X	X	X	X	X	2X					
2	X	X	X	X	X	X	2X					
3	X	X	X	X	X	X	2X					
4	X	X	X	X	X	X	X				X	
5	X	X	X	X	X			X	X		X	
6	X	X	X	X	X			X	X	X		
7	X	X	X	X	X			X	X	X	X	
8												X

**Figure 2: Schematic diagram of microcosm**



**Figure 3: Photographs of experimental setup:** a) shell immersed in microcosm (such photographs were used to determine available surface area); b) experimental tray containing 8 microcosms; c) typical layout of trays in heated, recirculating water bath; d) photograph of shell post experimentation (as would be used to calculate fouling surface area) with newly recruited oyster larvae circled.



#### 4.5. *Post-settlement larval extractions*

Seventy-two hours after the last larval addition, each experiment trial was terminated. Experimental shells were removed from each well in turn and lightly rinsed over their respective wells, capturing any larvae in each well that did not metamorphose during the 72 hr trial period. All trays were then refrigerated (5 °C) until they were processed. Shell substrates were analyzed by counting larvae in duplicate under a stereo dissecting microscope (at least 10 x magnification). Larvae attached to each shell were circled, and following the completion of the count, each shell was individually photographed using a digital camera mounted 20 cm above an illuminated photographic platform.

The water and sand from each well were siphoned into a 200 µm sieve, retaining any unsettled larvae on the screen, while allowing the sediment to pass through. Material retained on the screen was then examined under a stereo dissecting microscope. Live and dead larvae were counted separately, as were the larvae which had metamorphosed on the sand. The operational definition of dead larvae was the complete dissolution of the eyespot. Finally, counts of any larvae that metamorphosed on the well itself were recorded.

#### 4.6. *Experimental treatments and hypotheses*

As noted above, the primary objective of this study was to identify the effects of reef epifauna on settling oyster larvae. Experimentation lead to an evolving understanding of the factors involved – resulting in subsequent experiments designed to

investigate each new factor (Table 2). In some instances, this required slight modifications to the afore-mentioned methods (noted in detail below). A total of eight experiments were conducted, each of which concentrated on at least one (and up to three) of the following topics.

#### 4.6.1. *Effect of epifauna on settling oyster larvae*

In line with the primary objective, the first 4 experiments were designed to investigate the effects of established epifauna on the settlement and survival of oyster larvae. More specifically, these experiments investigated the relative effects of barnacles, bryozoans, and *Cliona* sp.

The next 3 experiments expanded on these early trials – attempting to identify the underlying mechanisms causing the trends seen. As such, the design focused on barnacles, and treatments included the barnacle mould, dead barnacle, and barnacle cue microcosms described above.

#### 4.6.2. *Effect of water soluble cues on settling oyster larvae*

The barnacle cue treatments prompted further investigations into the effects of water soluble cues emitted by established adults. Two experiments were conducted to accomplish this, starting with a series of microcosm experiments conducted starting on August 20<sup>th</sup>, 2008. Although similar in design to the epifaunal microcosm experiments detailed above, experiments offered larvae only the walls of the microcosm on which to settle (i.e. no sand or shells). Adult oysters (*C. virginica*; 14 adults in 7 L) and adult barnacles (*B. improvisus*; 506 adults in 4 L) were placed in 1 µm filtered seawater for 12

hours to create adult bathwaters for each species. Bathwaters were then passed through new 1  $\mu\text{m}$  cartridge filters in order to remove any particles. Four plastic wells were filled with 50 mL of water for each of the following treatments: control water (1  $\mu\text{m}$  filtered seawater), oyster bathwater, barnacle bathwater, and a 50:50 combination of the oyster and barnacle bathwaters (approximating conditions in live barnacle microcosms). Wells were immersed in a heated (25 °C) recirculating water bath and the microcosms were allowed to acclimate to temperature. Approximately 75 competent *Crassostrea virginica* larvae (volumetrically determined) were subsequently added to each well in a randomized order. Seventy two hours after the final larval addition, the wells were moved to the refrigerator (5 °C) to prevent further larval settlement, then processed as above.

Another similar microcosm experiment was conducted starting on September 5<sup>th</sup>, 2008. Bathwaters were created from filtering oysters (*C. virginica*; 8 adults in 6 L water), barnacles (*B. improvisus*; 303 adults in 2.5 L water) and clamworms (*N. succinea*; 63 worms in 3 L water). The bathwaters and control waters were passed through new 1  $\mu\text{m}$  cartridge filters after termination of the bathwaters. The oyster and barnacle bathwaters were then diluted with 1 $\mu\text{m}$  filtered seawater in order to create bathwaters with concentrations of 1x, 0.3x, 0.1x, 0.03x, and 0.01x, relative to full strength. Samples of each bathwater were retained for ammonium analysis. The adults used to generate the bathwater were dried at 100 °C and weighed, then combusted at 500 °C for 5 hours and re-weighed to calculate tissue biomass (ash free dry weight).

Six wells from each of 18 trays (same as above) were filled with 50 mL of one of the following: control water (1 $\mu\text{m}$  filtered seawater; 3 trays); oyster bathwater at 1x (3

trays), 0.3x, 0.1x, 0.03x, and 0.01x concentrations; barnacle bathwater at 1x (3 trays), 0.3x, 0.1x, 0.03x, and 0.01x concentrations; and clamworm bathwater. All trays were placed in a recirculating water bath at 25 °C. Approximately 75 competent *Crassostrea virginica* oyster larvae (as above) were added to these wells in a series of 6 additions in which the order of trays was randomized, but the order of wells was in sequence.

Twenty four hours after the final larval addition, one control tray, one full strength oyster bathwater tray, and one full strength barnacle bathwater tray were removed from the 25 °C water bath. The contents of each well were siphoned onto a 200 µm sieve, and the live and dead larvae retained on the sieve were enumerated. Larvae that had metamorphosed onto the walls of the well were separately counted. Forty eight hours after the final larval addition, the same procedure was applied to another batch of control and full strength oyster and barnacle bathwater trays. Seventy two hours after the final larval addition, the remaining trays were removed from the 25 °C water bath and placed in the refrigerator (5 °C), and subsequently processed as above.

#### 4.6.3. *Effect of Neanthes succinea on settling oyster larvae*

Very early in experimentation, it became clear that clamworms (*Neanthes succinea*) were impossible to eradicate from the microcosms. As a result experiments were conducted to determine the effect of clamworms on settling oyster larvae. The purpose of these experiments was also to create estimates of the effect of clamworms in microcosms – allowing the post hoc identification of microcosms likely inhabited by clamworms.

Preliminary investigations on this front were integrated into the matrix of epifaunal treatments. The 16 wells were each filled with an air dried (and thus clamworm free) shell. Haphazardly selected clamworms were then added to some of these wells. In experiment 4, four randomly selected microcosms received 2 clamworms, four received 4 clamworms, and four acted as controls with no clamworms (the other four wells were “blanks”). For experiment 5, five wells received 1 clamworm, five received 2 clamworms, and 6 acted as controls. In all other respects, these microcosms were treated in the exact same manner as the other treatments in the experiments (i.e. same larvae, larval enumeration procedure, etc.).

A final iteration of the afore-detailed microcosm experiments was conducted on August 20<sup>th</sup>, 2008 (Experiment 7). Similar to the cue experiments detailed above, no sand substrate was placed in the microcosms. Filtered (1  $\mu$ m) seawater was placed into 15 wells (50 mL; as above) and 15 plastic 1 L beakers (300 mL). The different container sizes were intended to test the possibility that clamworm predation on oyster larvae in other microcosms was predicated on proximity. One air-dried shell and one haphazardly selected clamworm were then placed into each container. Finally, approximately 75 competent *Crassostrea virginica* oyster larvae (as above) were added to each well in sequence.

The microcosms were terminated by removing the test shell and clamworm from the container and rinsing the shell gently over the container, capturing any unmetamorphosed larvae within the microcosm. Larvae which had metamorphosed on the shell were enumerated immediately, as were any dead larvae that could be found within the mucous trail of the clamworm, or within the clamworm itself. Three

microcosms of each size container, randomly selected, were terminated after each time period: 2 hours, 6 hours, 12 hours, 24 hours, and 72 hours. After 72 hours, water remaining in each of the microcosms was processed as above.

#### 4.7. Analyses

Larval counts were used to generate the following parameters for each well; 1) total number of oyster larvae found; 2) larval mortality (the number of dead larvae found / the total number of larvae found); and 3) larval settlement rate (expressed as the total number of larvae settled anywhere in microcosm / the total number of larvae found). From the photographs of individual shells taken after each trial (Figure 3), total shell areas and percents of shell surfaces covered by the fouling organism were determined using ImagePro<sup>®</sup> image analysis software.

The *Neanthes succinea* microcosms indicated that clamworms removed larvae from the system. In order to remove this effect of high clamworm predation on overall trends in all other treatments, any well in which fewer larvae than expected (as defined by 2 standard deviations from the average number of larvae found in negative control treatments) were excluded from epifaunal analysis.

Data collected on mortality and settlement rate were transformed using arcsine transformations ( $p' = \arcsine \sqrt{p}$ ) before statistical analysis, as the dependent variables in this study are percentages (see Zar, 1984). In some cases, the arcsine transformation did not generate normally distributed data, and a Box-Cox procedure was conducted on the original dataset (Sokal and Rohlf, 1981). If there were zeros in the dataset, one (1) was added to each data point in order to meet the requirement of non-zero values for the Box-

Cox procedure. The lambda value resulting from the Box-Cox procedure was applied to the data points in a power transformation, and normality was assessed again. In one instance, there was a marginal failure to meet the assumption of normality, but the robustness of the ANOVA procedure nevertheless deemed the parametric analysis to be appropriate.

ANOVAs were performed using Minitab<sup>®</sup> statistical software on each individual experiment. The response variable of the ANOVA models was the transformed settlement rate or mortality, while the factors included measured parameters i.e., treatment, shell orientation (which face of the shell was exposed, interior vs. exterior), shell size and others, as well as their interactions, as appropriate (Table 3). Oyster species (*C. virginica* and *C. ariakensis*) were analyzed similarly, but independently. Non-significant individual factors and interactions were removed from the model, and the ANOVA procedure repeated on the reduced model. Tukey's pairwise comparisons were conducted between the positive control treatment and the other test treatments.

To analyze for the effects of barnacle cover across experiments 1-7, the live barnacle treatments were re-categorized according to their actual percent cover into bins of low ( $0\% < x \leq 25\%$ ), medium ( $25\% < x \leq 50\%$ ) and high ( $50\% < x$ ) percent cover of available shell, using the data gathered from the photography of each individual shell. In this analysis (multiple experiments analyzed simultaneously) experiment number was included in an ANOVA model, with the *a priori* prediction that differences in competence between larval batches affect overall recruitment rates without changing the nature of differences between treatment levels. In one instance, the probabilities from

several one-way ANOVAs were combined via the formula  $\chi^2 = -2 \sum (\ln (P))$  to more robustly assess the effects of barnacle cover (Sokal and Rohlf, 1981).

As the experiments evolved, some slightly different analyses were required. For the bathwater cue time series trials (experiment 8), the control, oyster, and barnacle bathwater time series were analyzed at each time point (i.e. termination time – 24, 48, or 72 hours) using an ANOVA model relating mortality to bathwater type. In this way, the time point at which divergences in mortality between treatments could be discerned. Further, the settlement rate and mortality in the oyster and barnacle bathwater dilutions were compared using a parametric regression. For the time series worm trials within experiment 7, ordinal logistic regressions were performed on each of the parameters using the container size and the termination time as factors. As container size was a non-significant factor, regressions were conducted to directly compare the parameter to the continuous termination time variable.

**Table 3: Summary of statistical analyses.**

Experiment	Overall Objective	Secondary Objective	Analysis Type	Control	Dependent Variables*	Non-significant Factors in Full Model**	Factors in Final Model
1	Effect of epifauna		ANOVA	Positive control (by species)	M, S	SA, O	treatment
2	Effect of epifauna		ANOVA	Positive control	M, S	SA, O	treatment
3	Effect of epifauna		ANOVA	Positive control	M, S	SA, O	treatment
4	Effect of epifauna		ANOVA	Positive control	M, S	SA, O	treatment
4	Clamworm predation	Confounding of clamworms	ANOVA	No worm treatment	M, LC	SA	clamworm treatment
4	Effect of waterborne cues	Microcosm ammonia conc	ANOVA	Negative, Positive control	NH <sub>3</sub> conc	-	treatment
5	Effect of epifauna	Barnacle mechanisms	ANOVA	Positive control	M, S	SA, O	treatment
5	Clamworm predation	Confounding of clamworms	ANOVA	No worm treatment	M, LC	SA	clamworm treatment
6	Effect of epifauna	Barnacle mechanisms	ANOVA	Positive control	M, S	SA, O	treatment
7	Effect of epifauna	Barnacle mechanisms	ANOVA	Positive control	M, S	SA, O	treatment
7	Clamworm predation	Clamworm predation rate	Regression	-	M, LC	Container size	time
7	Effect of waterborne cues		ANOVA	Clean water treatment	M, S	-	bathwater type
8	Effect of waterborne cues		ANOVA	Clean water treatment	M, S	Position in tray	bathwater type
8	Effect of waterborne cues	Rate of bathwater effect	ANOVA	Clean water at time	M	-	bathwater type
8	Effect of waterborne cues	Bathwater dilution	Regression	-	M, S	-	bathwater concentration
1-7	Effect of waterborne cues	Epifauna expt bathwaters	Regression	-	M, S	Experiment #	-
1-7	Effect of epifauna	Effect of barnacles	ANOVA	Positive control	S	-	expt #, barnacle density
1-7	Effect of epifauna	Effect of barnacles	ANOVA	Positive control	S	CaCO <sub>3</sub> SA	expt #
Field	Clamworm community		T-test	-	CN, CS, CM	-	location (crest or slope)

\* M = mortality; S = settlement rate; LC = larval count; CN = number of clamworms; CS = size of clamworms (4th setiger width); CM = mass of clamworms

\*\* SA = available shell surface area; O = shell orientation

#### 4.8. *Quantification of Neanthes succinea community characteristics*

Reef samples were obtained from ten randomly selected sites on a subtidal reef at the mouth of Pungoteague Creek, Virginia on July 30<sup>th</sup>, 2008. Five sites were located on the crest of the reef, while five were on the slope. At each site, a .25 x .25 meter quadrat was haphazardly placed, and all material within (to a depth of 6 - 10 cm) was removed by hand and placed in a submerged basket lined with 1mm mesh.

Samples were rinsed and dried at least 3 times (approximately 30 minutes total) over a 5 mm sieve, and rinse spoils were captured on a 1 mm sieve. Sieve contents were removed quickly to minimize loss of clamworms through the mesh. Shells retained on the 5 mm sieve were crushed (by hand or hammer), and any clamworms which remained in the shells were removed. Rinse spoils and clamworms removed from the shells were placed in 40% buffered formalin, Rose Bengal solution.

After fixing, organic materials were picked from the samples without magnification and placed in 70% Ethanol. Samples were then analyzed under a stereo dissecting scope (at 10x magnification), and identifiable clamworm heads were enumerated. Whole clamworms were removed, and the width of their 4<sup>th</sup> setiger was measured. The whole clamworms were then dried at 100 °C and combusted at 500 °C for 5 hours to calculate biomass (ash free dry weight).

Basic descriptive statistics were reported for each parameter measured. Analyses comparing differences between the crest and slope of the reef were compared using two-sample Student's T-tests.

## 5. RESULTS

### 5.1 *Effect of epifauna on settling oyster larvae*

Each individual experimental trial was analyzed independently, due to the differences in treatments between experimental trials (Table 2). These individual experiment analyses were performed using the original designations of high, medium and low barnacle treatment level (e.g. the ‘high barnacle’ treatment in each experiment included the 16 shells with the most dense barnacle assemblages, as determined by visual approximation prior to experimentation). As discussed above, transformations were performed on non-normal data prior to analysis. Finally, microcosms in which the total larvae (live and dead) retrieved from a microcosm at the end of the experiment was fewer than 2 standard deviations below the mean in the negative control (presumably due to *N. succinea* predation) were excluded from the analyses. The negative control treatments themselves were also excluded from analysis due to the high number of zero data points for the ‘percent total set’ parameter. In all, seven experiments were conducted to investigate the effect of adult epifauna on settling oyster larvae, each described in detail below. Table 4 summarizes the significant treatment differences from control settlement rates in each experiment as determined using Tukey’s pairwise comparisons. Table 5 describes the same for mortality.

**Table 4: Summary of treatment effects on settlement rate.** Symbol (+ or -) indicates direction of deviation from positive controls, p-values from Tukey's pairwise comparisons to positive control. Empty cells indicate no significant difference from positive control treatments. N/A indicates treatments not included in each experiment.

Experiment	Species	Low Barnacles	Medium Barnacles	High Barnacles	Bryozoans	Cilona	Dead Barnacles	Barnacle Moulds	Barnacle Cue
1	<i>C. virginica</i>	+ p = 0.0254		+ p = 0.0184			N/A	N/A	N/A
	<i>C. ariakensis</i>			+ p = 0.0093			N/A	N/A	N/A
2	<i>C. virginica</i>					- p = 0.0282	N/A	N/A	N/A
3	<i>C. ariakensis</i>						N/A	N/A	N/A
4	<i>C. virginica</i>					- p = 0.0003	N/A	N/A	N/A
5	<i>C. virginica</i>	+ p = 0.0001	+ p < 0.0001	+ p = 0.0001	N/A	N/A			N/A
6	<i>C. ariakensis</i>			+ p = 0.0034	N/A	N/A		- p = 0.0412	
7	<i>C. virginica</i>		+ p = 0.0147		N/A	N/A			

**Table 5: Summary of significant treatment effects on mortality.** Symbol (+ or -) indicates direction of deviation from positive controls, p-values from Tukey's pairwise comparisons to positive control. Empty cells indicate no significant difference from positive control treatments. N/A indicates treatments not included in each experiment

Experiment	Species	Low Barnacles	Medium Barnacles	High Barnacles	Bryozoans	Cliona	Dead Barnacles	Barnacle Moulds	Barnacle Cue
1	<i>C. virginica</i>					+ p = 0.0066	N/A	N/A	N/A
	<i>C. ariakensis</i>						N/A	N/A	N/A
2	<i>C. virginica</i>					+ p = 0.0195	N/A	N/A	N/A
3	<i>C. ariakensis</i>					+ p = 0.0012	N/A	N/A	N/A
4	<i>C. virginica</i>					+ p = 0.0001	N/A	N/A	N/A
5	<i>C. virginica</i>				N/A	N/A			N/A
6	<i>C. ariakensis</i>				N/A	N/A			+ p = 0.0001
7	<i>C. virginica</i>	+ p = 0.0047	+ p < 0.0001	+ p < 0.0001	N/A	N/A			+ p < 0.0001

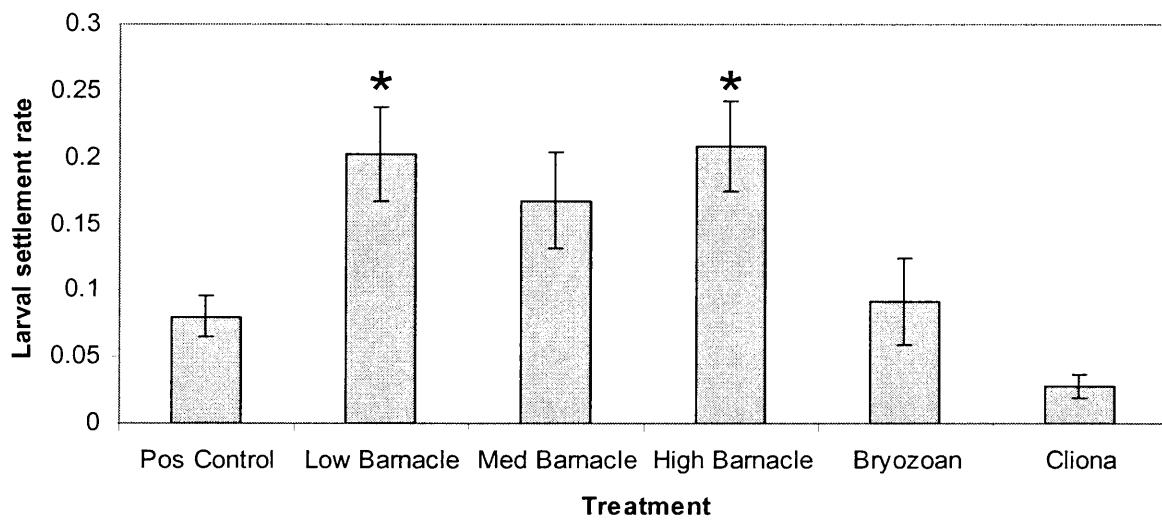
### 5.1.1. Experiment 1

As experiment 1 included both *C. virginica* and *C. ariakensis* oyster larvae, each oyster species was tested in only 8 replicates of each treatment (as opposed to 16 in all other experiments). Due to the lack of a negative control treatment, microcosms with fewer than 2 standard deviations below the average total found in the positive control treatment for each species were excluded from analysis in an attempt to remove what is presumed to have been replicated with high predation by clamworms.

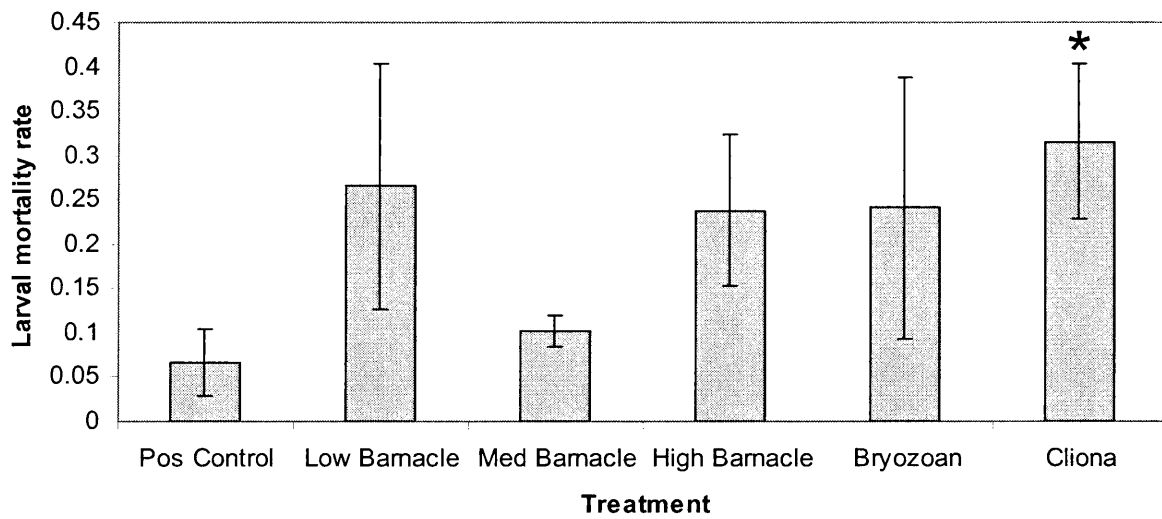
One-way ANOVAs were conducted for each oyster species independently using the fouling treatment as a fixed factor and the transformed settlement rate as the response. Tukey's pairwise comparisons were applied to treatment differences relative to positive controls only. For *C. virginica* trials, larvae showed significant settlement preferences between treatments ( $F = 9.09$ ;  $p < 0.001$ ; Figure 4). Pairwise comparisons showed that oyster settlement in the high barnacle ( $p = 0.0184$ ) and low barnacle ( $p = 0.0254$ ) treatments was higher than settlement in the positive control treatments. Larval mortality was also different between treatments ( $F = 3.29$ ;  $p = 0.012$ ; Figure 5). Mortality in the *Cliona* treatment was significantly higher than that in the control treatment ( $p = 0.0066$ ).

*Crassostrea ariakensis* larvae also settled at different rates according to treatment ( $F = 4.46$ ;  $p = 0.002$ ; Figure 6). Settlement in high barnacle treatments was significantly higher than in positive control treatments ( $p = 0.0093$ ). Mortality also differed between treatments ( $F = 3.01$ ;  $p = 0.019$ ; Figure 7). The difference in mortality in the control and *Cliona* treatments was marginally not significant ( $p = 0.0508$ ).

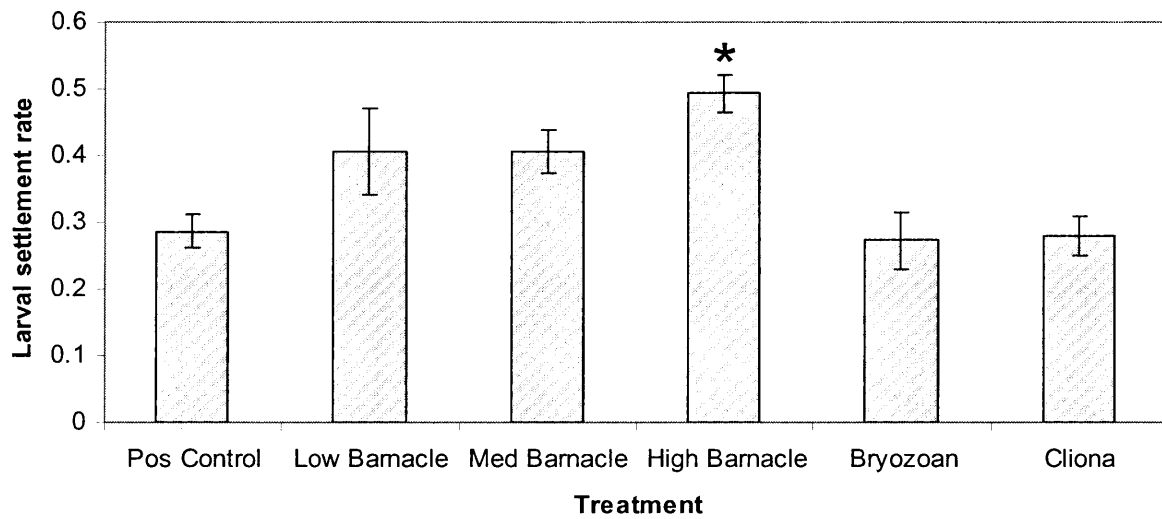
**Figure 4: Average settlement rate of *C. virginica* larvae by treatment (Experiment 1).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



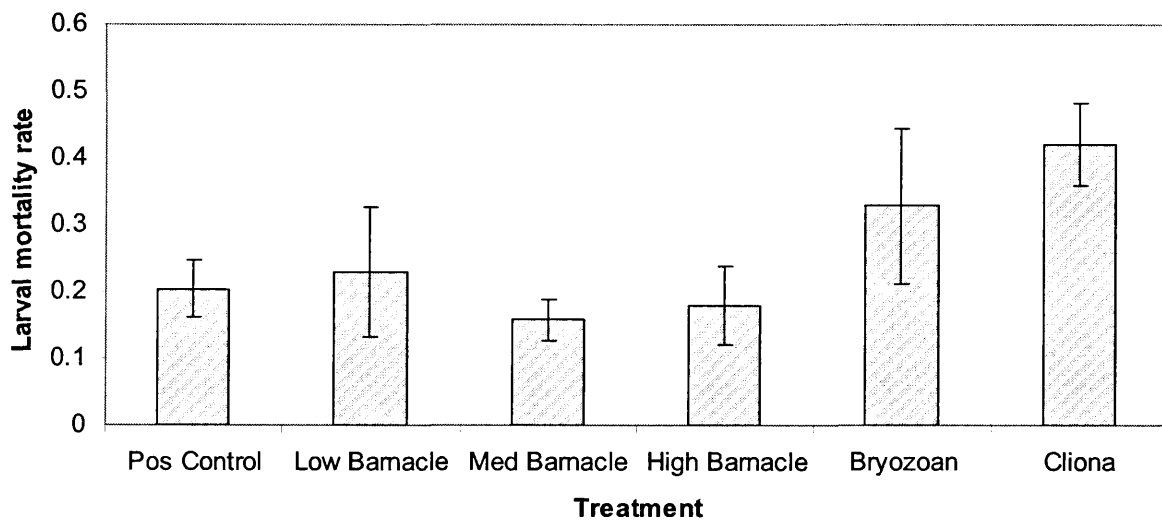
**Figure 5: Average mortality of *C. virginica* larvae by treatment (Experiment 1).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 6: Average settlement rate of *C. ariakensis* larvae by treatment (Experiment 1).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 7: Average mortality of *C. ariakensis* larvae by treatment (Experiment 1).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. No treatments were significantly different from controls.



### 5.1.2. Experiment 2

*C. virginica* larvae showed significant preferences in settlement rate between the treatments studies ( $F = 7.18$ ;  $p < 0.001$ ; Figure 8). Tukey's pairwise comparisons showed that settlement in *Cliona* sp. wells was significantly lower than in positive control treatments ( $p = 0.0282$ ). Larval mortality rate also differed between treatments ( $F = 3.78$ ;  $p = 0.003$ ; Figure 9). Again, mortality was higher in *Cliona* treatment than in control treatments ( $p = 0.0195$ ).

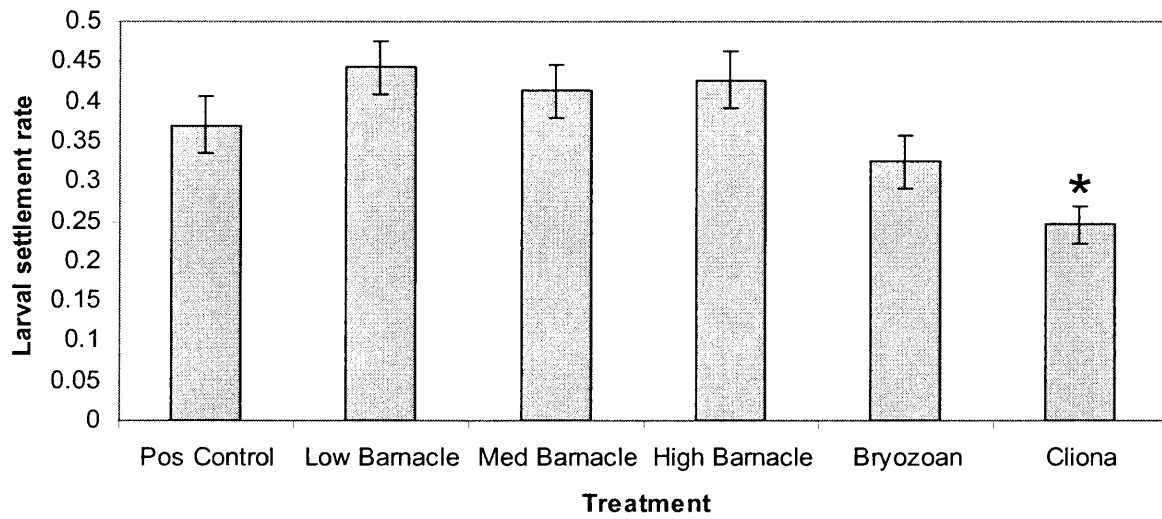
### 5.1.3. Experiment 3

*Crassostrea ariakensis* larvae settled at different rates depending on the treatment ( $F = 3.85$ ;  $p = 0.003$ ; Figure 10). Settlement rate did not significantly differ, however, between the positive control treatment and any of the other treatments. Mortality differed between treatments ( $F = 4.87$ ;  $p = 0.001$ ; Figure 11), with the *Cliona* treatment showing higher mortality than control treatments ( $p = 0.0012$ ).

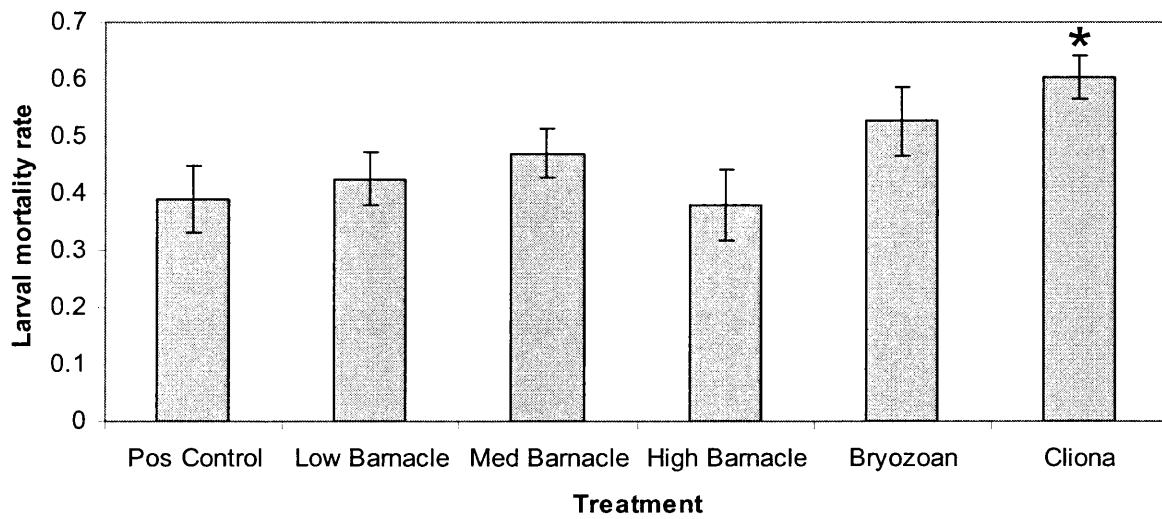
### 5.1.4. Experiment 4

Larval (*C. virginica*) settlement differed between treatments in the ANOVA model ( $F = 6.08$ ;  $p < 0.001$ ; Figure 12). Pairwise comparisons indicated that settlement in *Cliona* sp. treatments was significantly less than that in positive control treatments ( $p = 0.0003$ ). Mortality also showed significant differences between treatments ( $F = 5.14$ ;  $p = 0.001$ ; Figure 13). The *Cliona* treatment had significantly higher mortality than control treatments ( $p = 0.0001$ ).

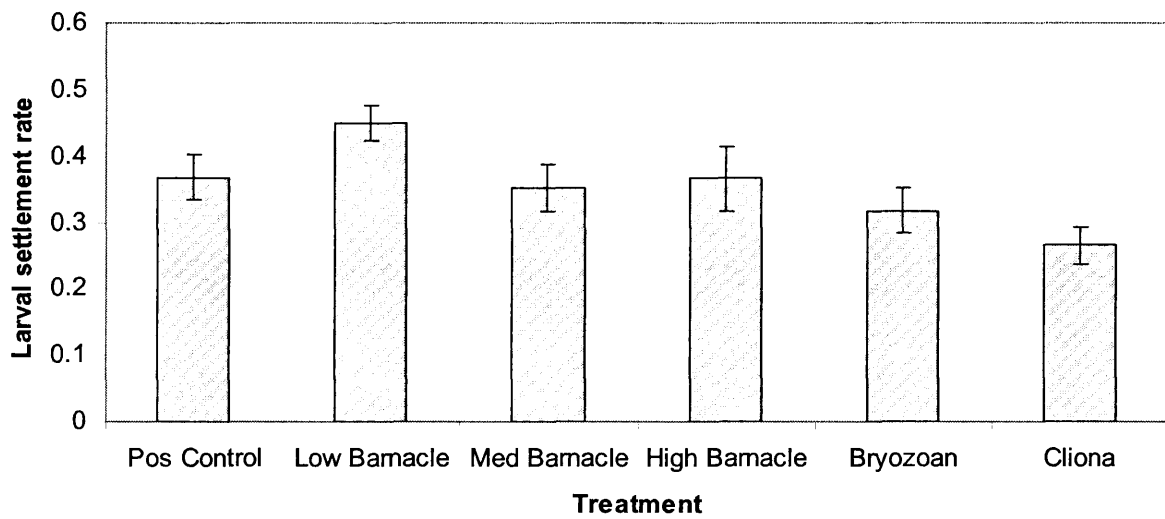
**Figure 8: Average settlement rate of oyster larvae by treatment (Experiment 2).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



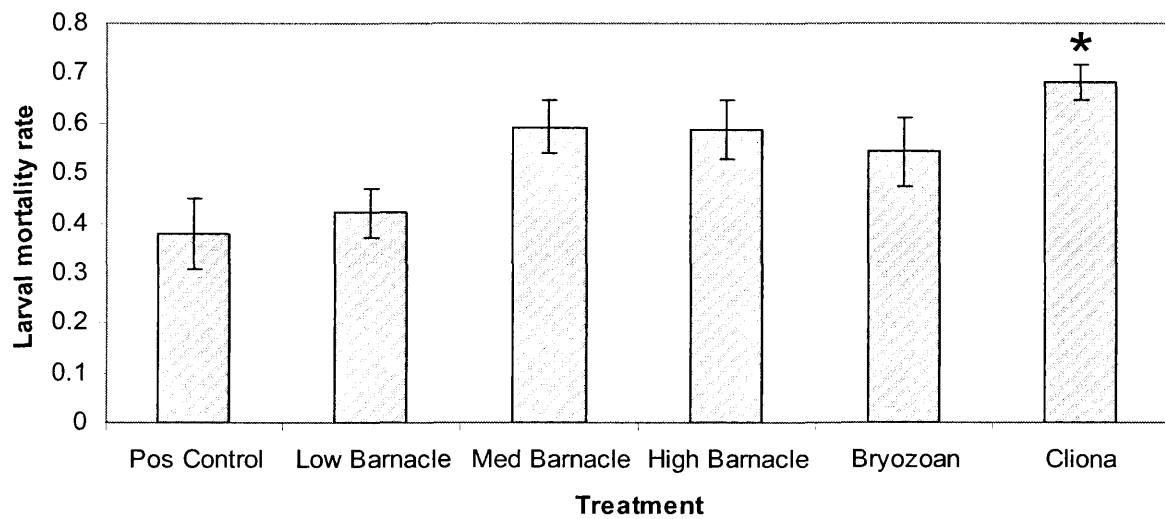
**Figure 9: Average mortality of larvae by treatment (Experiment 2).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



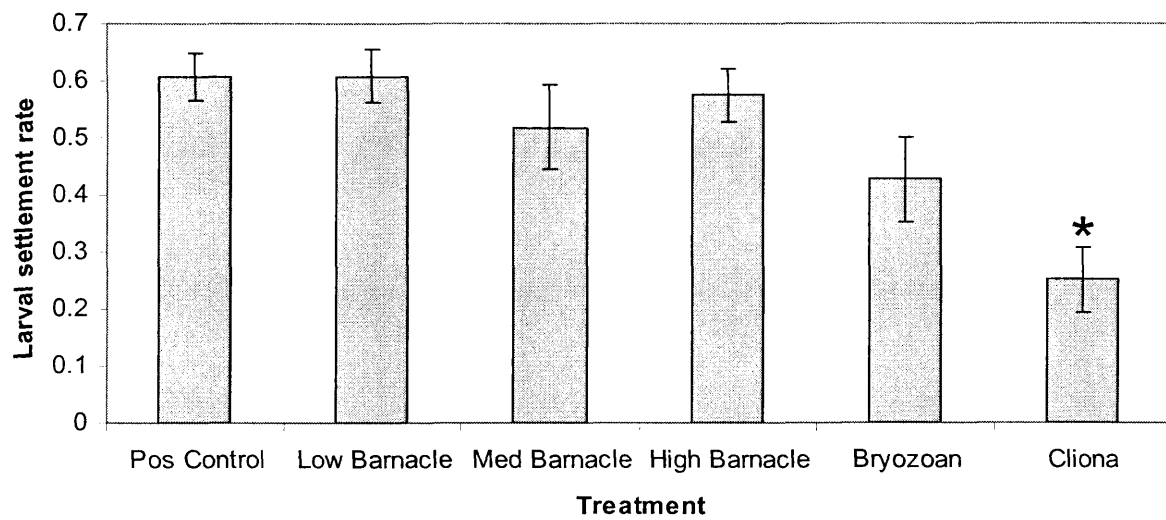
**Figure 10: Average settlement rate of oyster larvae by treatment (Experiment 3).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. No treatments were significantly different from controls.



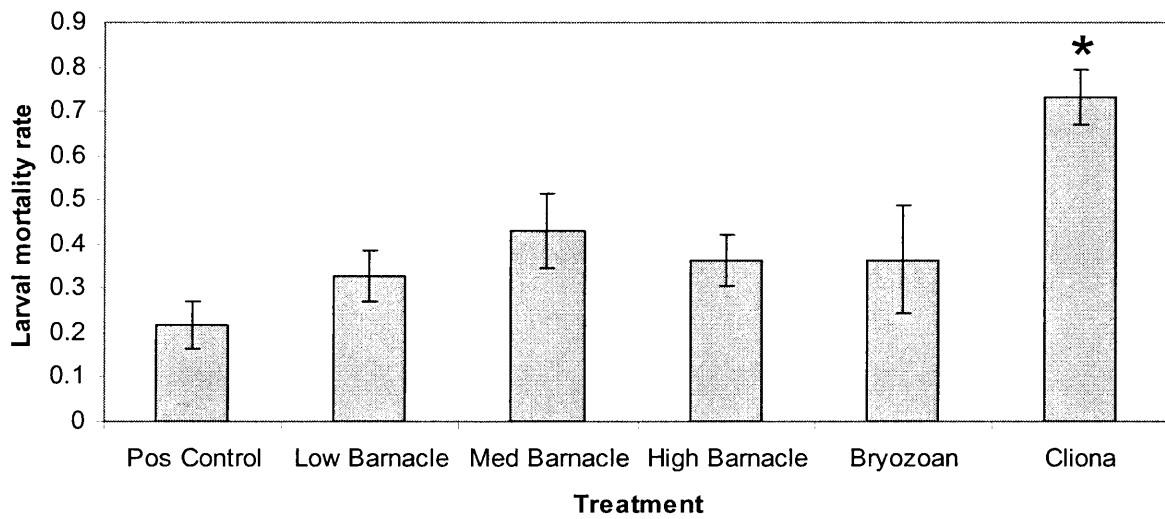
**Figure 11: Average mortality of larvae by treatment (Experiment 3).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 12: Average settlement rate of oyster larvae by treatment (Experiment 4).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 13: Average mortality of larvae by treatment (Experiment 4).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



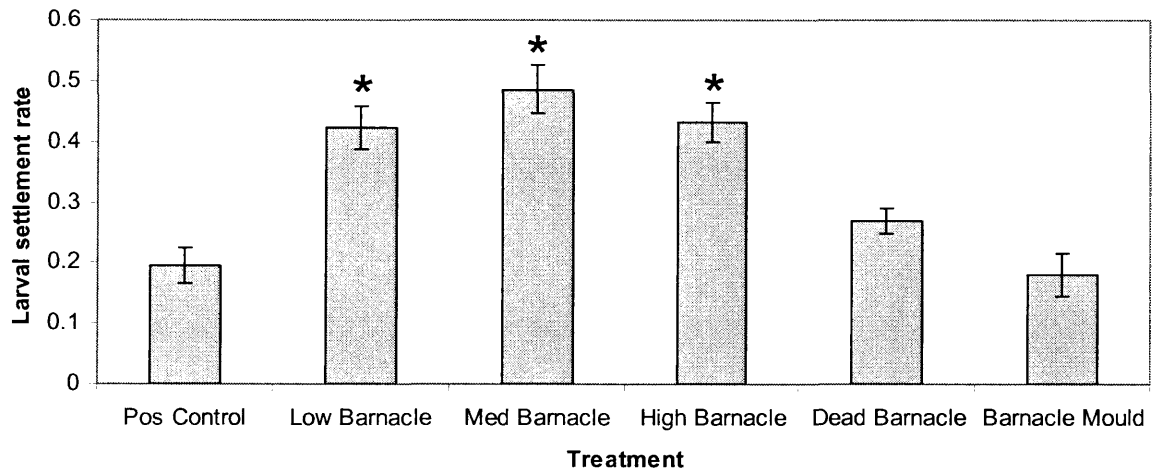
#### 5.1.5. Experiment 5

*C. virginica* larvae showed significant differences in settlement rate between the treatments studies ( $F = 16.44$ ;  $p < 0.001$ ; Figure 14). Larval settlement rates in the high ( $p = 0.0001$ ), medium ( $p < 0.0001$ ), and low ( $p = 0.0001$ ) barnacle treatments were all significantly greater than in the positive control. Mortality differed between treatments ( $F = 5.70$ ;  $p < 0.001$ ; Figure 15); however, no treatment differed significantly from controls.

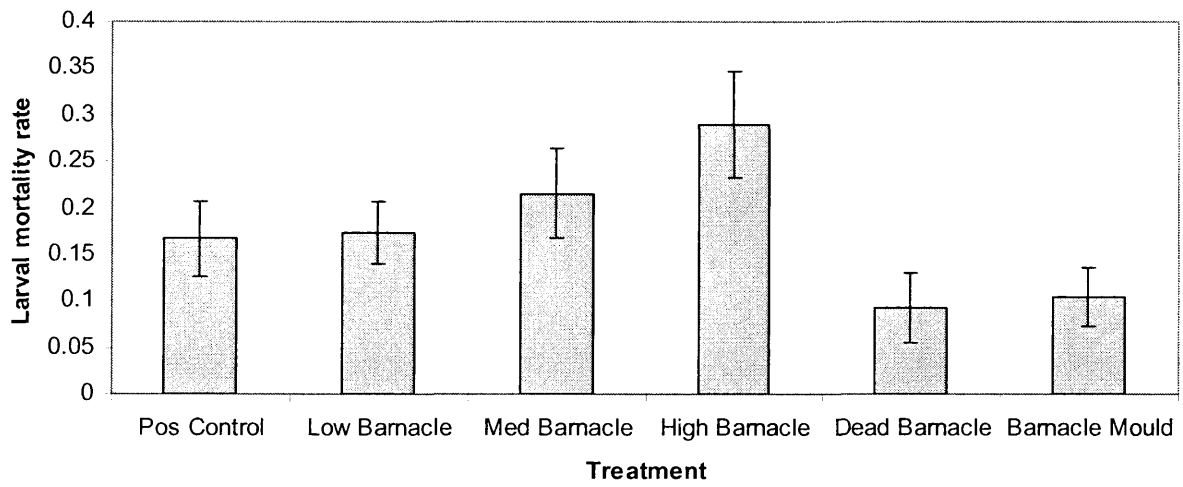
#### 5.1.6. Experiment 6

*C. ariakensis* larvae showed significant preferences in settlement rate between the treatments studies ( $F = 10.58$ ;  $p < 0.001$ ; Figure 16). Settlement rate was significantly higher in the high barnacle treatment than in the positive control treatment ( $p = 0.0034$ ). The barnacle mould treatment, however, showed significantly depressed settlement from the positive control treatment ( $p = 0.0412$ ). Larval mortality showed differences between treatments ( $F = 4.49$ ;  $p < 0.001$ ; Figure 17). Pairwise comparisons indicated higher mortality in the barnacle cue treatment than in the positive control treatment ( $p = 0.0001$ ).

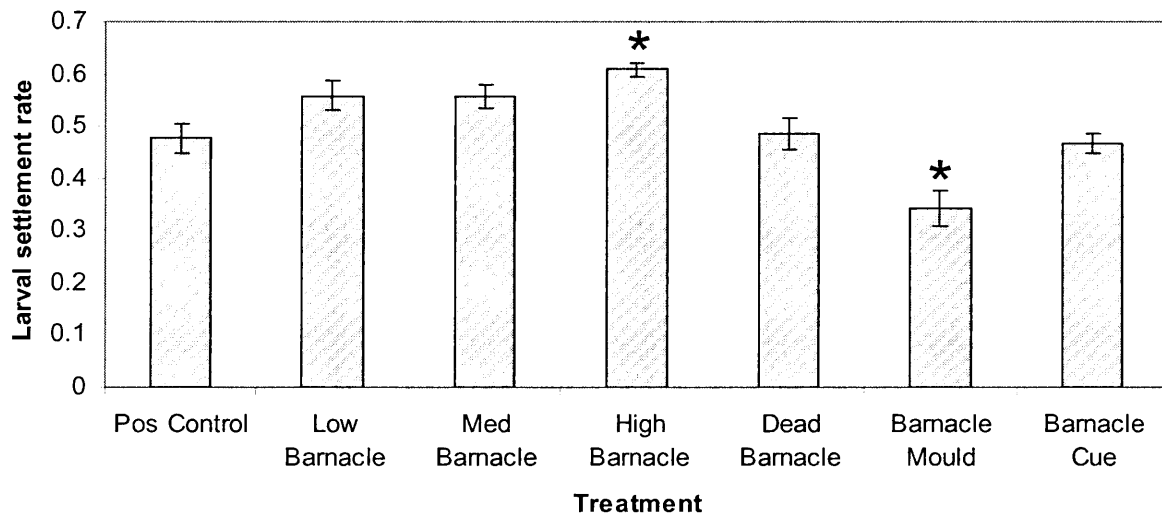
**Figure 14: Average settlement rate of oyster larvae by treatment (Experiment 5).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



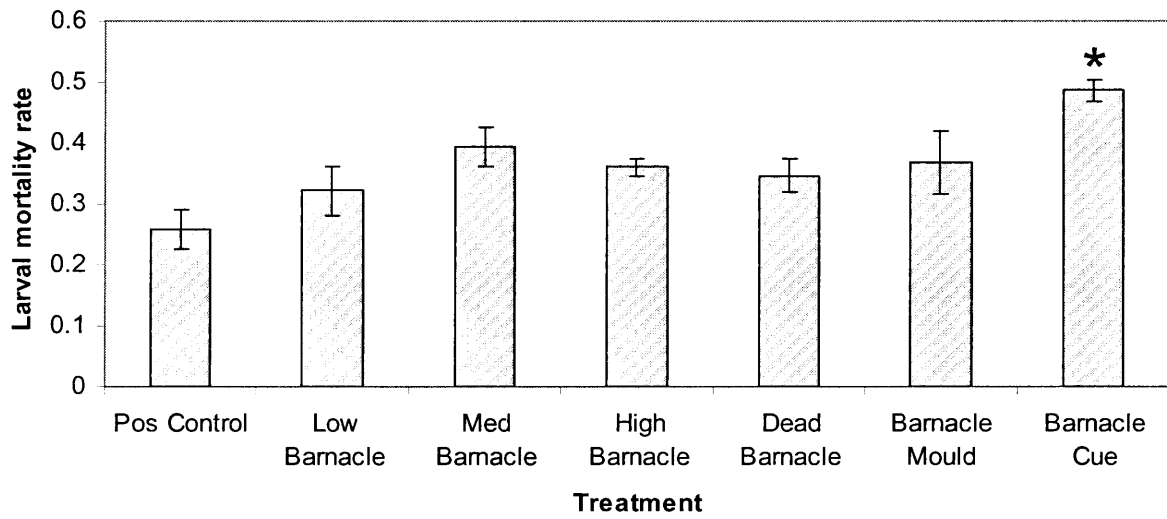
**Figure 15: Average mortality of larvae by treatment (Experiment 5).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. No treatments were significantly different from controls.



**Figure 16: Average settlement rate of oyster larvae by treatment (Experiment 6).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 17: Average mortality of larvae by treatment (Experiment 6).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



### 5.1.7. Experiment 7

*C. virginica* larvae in this experiment again settled at different rates in the different treatments ( $F = 4.38$ ;  $p = 0.001$ ; Figure 18). Tukey's pairwise comparisons indicated that settlement rate in the medium barnacle treatment was greater than that in the positive control treatment ( $p = 0.0147$ ). ANOVA on the larval mortality showed differences between treatments ( $F = 17.56$ ;  $p < 0.001$ ; Figure 19). Several treatments showed higher mortalities than controls: barnacle cue ( $p < 0.0001$ ), as well as high ( $p < 0.0001$ ), medium ( $p < 0.0001$ ), and low ( $p = 0.0047$ ) live barnacle treatments.

### 5.1.8. Combined analyses on epifauna experiments

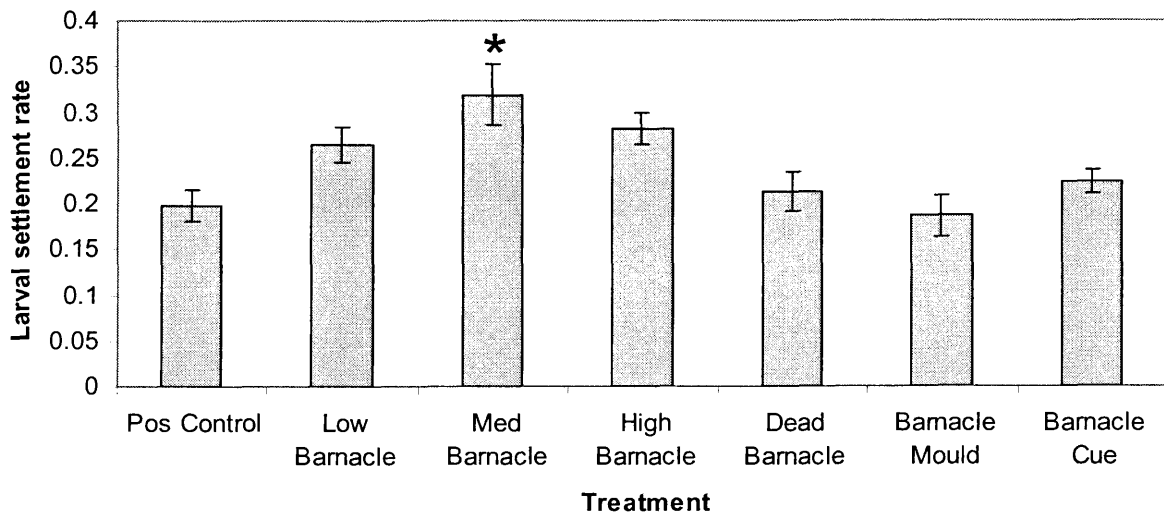
To further evaluate larval settlement preferences, analyses were conducted which compared barnacle treatments to controls across all experiments. Prior to these analyses, the barnacle treatments were re-binned into actual percent cover (discussed above) based on the percentage of available surface area covered by barnacles. As such, analysis spanning experiments was appropriate because these *post hoc* treatments are defined by the shell characteristics, not arbitrary treatment designations.

Two-way ANOVAs were conducted comparing the transformed settlement rate to the barnacle cover level and the experiment number. In this manner, *Crassostrea ariakensis* larvae showed significant differences in larval settlement rate between both treatments ( $F = 5.82$ ;  $p = 0.001$ ; Figure 20) and experiments ( $F = 35.28$ ;  $p < 0.001$ ), but not their interaction ( $F = 2.02$ ;  $p = 0.067$ ). Tukey's pairwise comparisons indicated that, overall, larvae settlement rate was lower on positive control shells than on high barnacle ( $p = 0.0115$ ), medium barnacle ( $p = 0.0422$ ), and low barnacle ( $p = 0.0028$ ) shells.

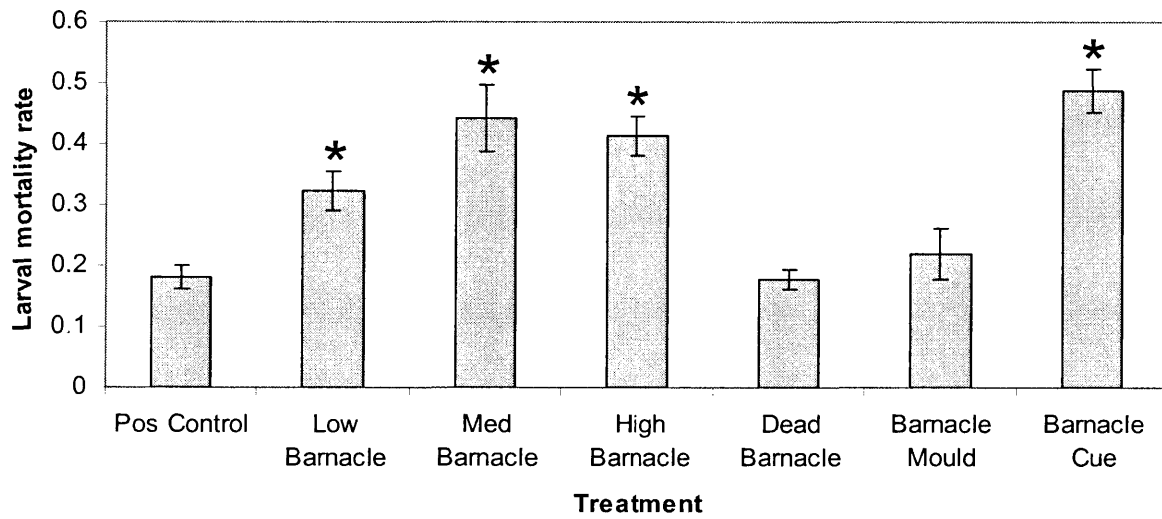
Similar analysis was conducted for *Crassostrea virginica* larvae across experiments. The ANOVA model indicated significance differences in transformed settlement rate between experiments ( $F = 62.25$ ;  $p < 0.001$ ), treatments ( $F = 11.00$ ;  $p < 0.001$ ), and their interaction ( $F = 2.59$ ;  $p = 0.003$ ; Figure 21). As a result of the significant interaction, individual analyses were conducted for each experiment comparing settlement rate on positive control shells and re-binned barnacle treatments (see Table 6). One-way ANOVA on experiment 1 indicated that there were significant differences in settlement rate among treatments ( $F = 4.59$ ;  $p = 0.008$ ), and Tukey's pairwise comparisons indicated that settlement rate in the control treatment was less than that in the medium barnacle treatment ( $p = 0.0214$ ). Similarly, the treatment effect was significant in Experiment 5 ( $F = 14.69$ ;  $p < 0.001$ ), with the control shells showing lower settlement rates than the high ( $p = 0.0006$ ), medium ( $p = 0.0001$ ), and low ( $p < 0.0001$ ) barnacle shells. Finally, ANOVA indicated a significant effect of treatment in experiment 7 ( $F = 3.9$ ;  $p = 0.013$ ), and pairwise comparisons showed a significant difference between control and high barnacle treatments ( $p = 0.0108$ ). Experiment 2 ( $F = 0.9$ ;  $p = 0.447$ ) and experiment 4 ( $F = 0.55$ ;  $p = 0.649$ ), however, did not show significant differences between treatments in this analysis.

The probabilities from these one-way ANOVAs were combined via the formula  $\chi^2 = -2 \sum (\ln(P))$ , which results in a value of 34.6 (Sokal and Rohlf, 1981). In a chi-squared distribution with 10 degrees of freedom, this value indicates significant differences in *C. virginica* settlement between barnacle and control treatments across experiments ( $p = 0.0001$ ).

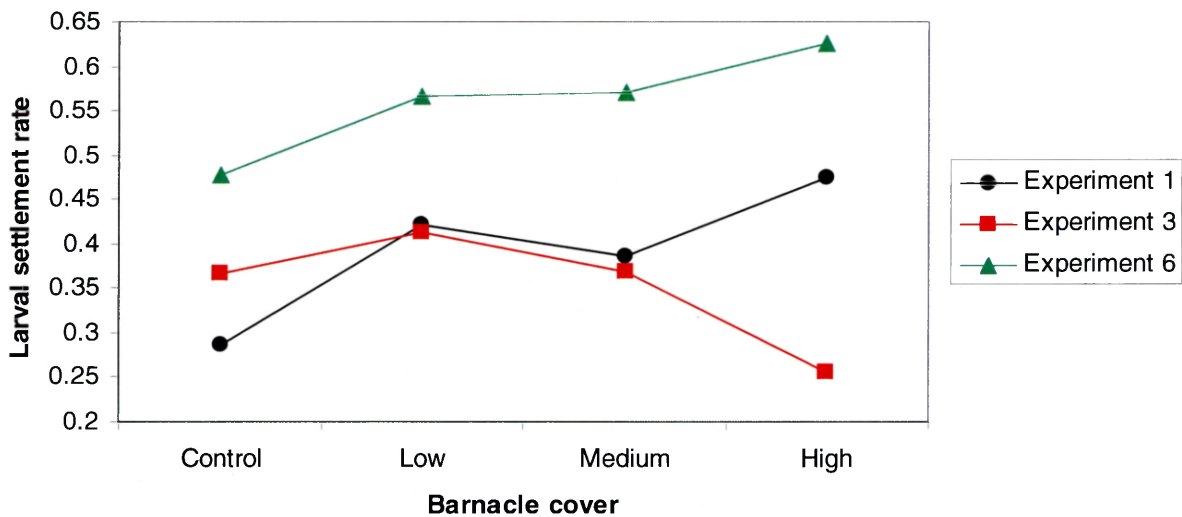
**Figure 18: Average settlement rate of oyster larvae by treatment (Experiment 7).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



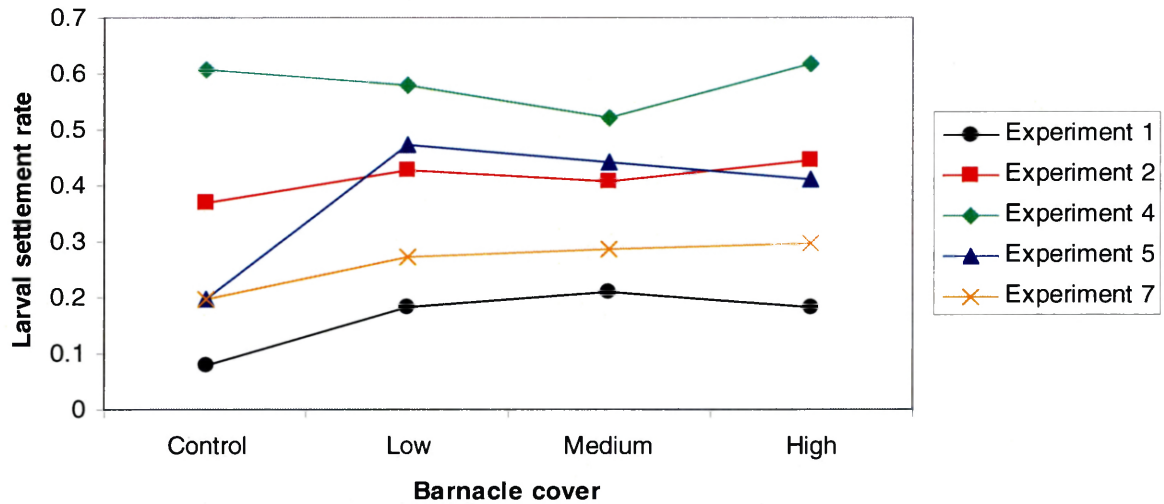
**Figure 19: Average mortality of larvae by treatment (Experiment 7).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from positive control treatment.



**Figure 20: Mean settlement of larvae (*C. ariakensis*) in re-categorized barnacle cover treatments across experiments.** Proportional settlement rate is over 72 hours. Controls have 0 % barnacle cover, low ( $0\% < x \leq 25\%$ ), medium ( $25\% < x \leq 50\%$ ) and high ( $50\% < x$ ) percent coverage. As such, the high barnacle treatment in Experiment 3 represents only 2 data points.



**Figure 21: Mean settlement rate of larvae (*C. virginica*) in re-categorized barnacle cover treatments across experiments.** Proportional settlement rate is over 72 hours. Controls have 0 % barnacle cover, low ( $0\% < x \leq 25\%$ ), medium ( $25\% < x \leq 50\%$ ) and high ( $50\% < x$ ) percent coverage.



**Table 6: Summary of significant treatment differences of re-binned barnacle treatments from control settlement rate (*C. virginica*).** Symbol (+ or -) indicates direction of deviation from positive controls, p-values from Tukey's pairwise comparisons to positive control.

Experiment	Barnacle Percent Cover		
	Low ( $x \leq 25\%$ )	Medium ( $25\% < x \leq 50\%$ )	High ( $50\% < x$ )
1		+	
		( $p = 0.0214$ )	
2			
4			
5	+	+	+
	( $p = 0.0006$ )	( $p = 0.0001$ )	( $p < 0.0001$ )
7			+
			( $p = 0.0108$ )

## 5.2. *Effect of water soluble cues on settling oyster larvae*

### 5.2.1. *Effect of bathwaters from epifauna experiments*

As detailed above, the epifauna experiment microcosms were all conducted using adult bathwaters, subsamples of which were retained for ammonia analysis (Table 7). Ammonia concentrations were considered a proxy for biomass in producing a soluble cue. The concentrations of ammonia were then compared to the settlement rate and mortality of larvae in the positive control microcosms via regression analysis. These analyses showed that the concentration of ammonia in oyster bathwaters was not a significant predictor across experiments of either settlement rate ( $T = 0.19$ ;  $p = 0.856$ ;  $R^2 = 0.6$ ) or mortality ( $T = 1.79$ ;  $p = 0.124$ ;  $R^2 = 34.8$ ) of oyster larvae.

Ammonia analysis was also conducted on the water in microcosms post-experimentation in experiment four (Table 7). Three samples were randomly selected for this extra analysis from each of the following treatments: negative control, positive control, medium barnacle, *Cliona*, bryozoan, and 2 worm. The negative control water taken from the microcosms post-experimentation showed no difference in ammonia concentration from that of the bathwater taken prior to experimentation (i.e. time of experimentation, larvae, and processing did not effect ammonia concentration).

ANOVA of this water chemistry data indicated that the concentration of ammonia in the water differed between treatments ( $F = 11.8$ ;  $p < 0.001$ ). The positive control bathwaters showed a slight, insignificant increase in ammonia concentration compared to the negative controls (i.e. the addition of a shell into the microcosm had little effect on ammonia concentration). Likely reflecting biological activity, ammonia concentrations

were significantly higher in the barnacle ( $p = 0.0061$ ), bryozoan ( $p = 0.0103$ ), and *Cliona* ( $p = 0.0054$ ) treatments than in positive control treatments. The concentration in the 2 worm treatment was also higher, yet the difference was marginally nonsignificant ( $p = 0.0811$ ).

**Table 7: Summary of ammonia concentrations and larval settlement rates.** Values from bathwaters used during experiments as well as select microcosms post-experimentation: (A) across experiments and (B) within selected replicates from Exp 4.

A.

Experiment	Bathwaters				
	Bathwater Species	Number of Adults	Water Volume (L)	Ammonia concentration (mg/L)	Larval settlement rate
1	<i>C. virginica</i>	10	10	0.2585	0.080331918
1	<i>C. ariakensis</i>	10	10	0.3074	0.286604398
2	<i>C. virginica</i>	10	10	0.2753	0.370036938
3	<i>C. ariakensis</i>	8	12	0.4267	0.367016336
4	<i>C. virginica</i>	8	12	0.2249	0.607020038
5	<i>C. virginica</i>	10	12	0.2349	0.195469135
6	<i>C. ariakensis</i>	10	6*	0.3044	0.477033198
7	<i>C. virginica</i>	14	7*	0.2684	0.198063312
8	<i>C. virginica</i>	6	6	0.5048	ND
6	<i>B. improvisus</i>	869	3	0.3405	ND
7	<i>B. improvisus</i>	506	4	0.2498	ND
8	<i>B. improvisus</i>	303	2.5	0.4457	ND
8	<i>N. succinea</i>	63	3	0.2441	ND

B.

Experiment	Microcosms				
	Treatment	# of Fouling Organisms	Water volume (mL)	Ammonia concentration (mg/L)	Larval settlement rate
4	Neg Control	0	50	0.1952	0.459459459
4	Neg Control	0	50	0.2307	0.042253521
4	Neg Control	0	50	0.2239	0.095890411
4	Pos Control	0	50	0.3501	0.493975904
4	Pos Control	0	50	0.2423	0.555555556
4	Pos Control	0	50	0.1965	0.640625
4	Med Barn	11	50	0.8637	0.583333333
4	Med Barn	14	50	1.02	0.549295775
4	Med Barn	16	50	3.8061	0.528301887
4	Bryozoan	ND	50	2.378	0.666666667
4	Bryozoan	ND	50	0.4245	0.526315789
4	Bryozoan	ND	50	2.784	0.582089552
4	Cliona	ND	50	2.484	0.4
4	Cliona	ND	50	2.438	0.181818182
4	Cliona	ND	50	0.6671	0.156862745
4	2 worms	2	50	0.7041	0.111111111
4	2 worms	2	50	0.8223	0.772727273
4	2 worms	2	50	0.4033	0.416666667

\* Bathwater diluted with equal amount of clean water prior to experimentation and ammonia analysis  
 ND = No Data – larval settlement not measured, or number of foulers immeasurable

### 5.2.2. *Initial investigations - Experiment 7*

In the experiment investigating the effect of bathwaters from different species, ANOVA indicated that the settlement rate of oyster (*C. virginica*) larvae differed based on the type of water used in the microcosm ( $F = 7.67$ ;  $p = 0.004$ ; Figure 22). Tukey's Pairwise comparisons showed that settlement rate was significantly lower for larvae immersed in control water than for those in barnacle bathwater ( $p = 0.0149$ ), oyster bathwater ( $p = 0.0057$ ), or a 50:50 combination of the two ( $p = 0.0057$ ). Similarly, the mortality of oyster larvae differed depending on the type of water used in the microcosm ( $F = 115.09$ ;  $p < 0.001$ ; Figure 23). Mortality was lower in control water microcosms than in barnacle bathwater ( $p < 0.0001$ ), oyster bathwater ( $p < 0.0001$ ), and their combination ( $p < 0.0001$ ). Further, oyster larvae mortality was lower in oyster bathwater treatments than in barnacle bathwater ( $p = 0.0001$ ) and the combination treatment ( $p = 0.0002$ ).

### 5.2.3. *Serial dilution of cues - Experiment 8*

Larval settlement rate in the full strength cue treatments differed (ANOVA:  $F = 4.61$ ;  $p = 0.013$ ) after 72 hours when analyzing all bathwater types (Figure 24). This difference was not, however, manifested in any statistically significant deviations from control water microcosms. Similar analysis on larval mortality indicated that the bathwater type again caused significant differences ( $F = 54.49$ ;  $p < 0.001$ ; Figure 25). Mortality was lowest in the clean water treatments, and significantly higher in the barnacle ( $p < 0.0001$ ), oyster ( $p = 0.0002$ ), and clamworm ( $p = 0.0247$ ) bathwater treatments.

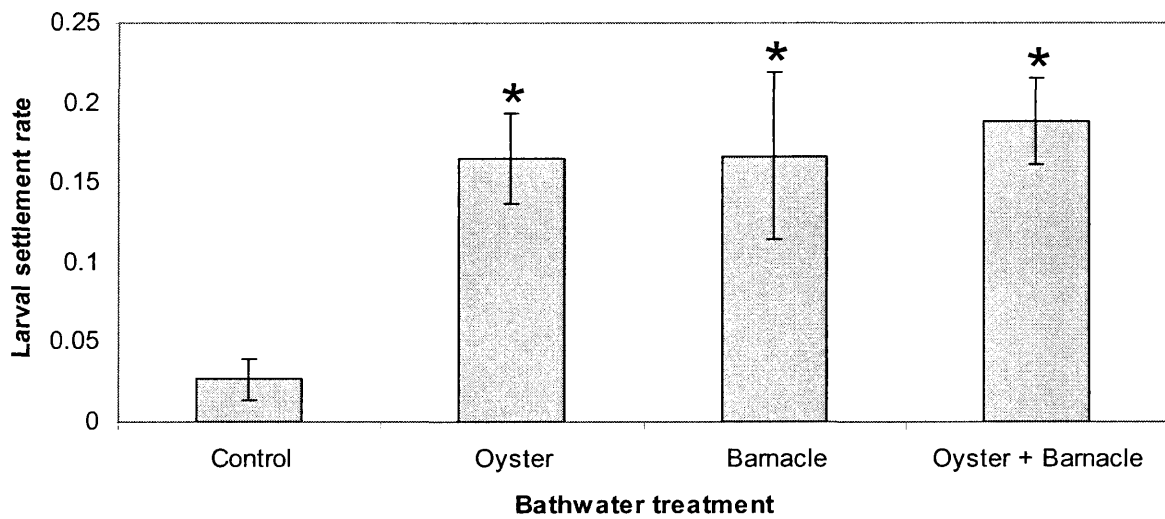
At each termination time in the time series analyses (24, 48, and 72 analysis), one tray of each of the control, barnacle bathwater and control bathwater treatments was terminated. ANOVAs were conducted using data from each of these time points were conducted to compare mortalities between the treatments (Figure 26). At 24 hours, no differences in mortality between treatments were detected ( $F = 1.11$ ;  $p = 0.355$ ). At 48 hours, differences between treatments began to emerge ( $F = 19.2$ ;  $p < 0.001$ ), with the barnacle bathwater causing significantly greater mortalities than control water ( $p = 0.0001$ ). Finally, at 72 hours, there continued to be differences in larval mortality depending on the type of bathwater in a microcosm ( $F = 68.19$ ;  $p < 0.001$ ). Both barnacle ( $p < 0.0001$ ) and oyster ( $p < 0.0001$ ) bathwater treatments showed higher mortalities than that of control waters.

The serial dilutions of oyster bathwater and the clean water controls were analyzed using regression, with the concentration of bathwater as the predictor, and the larval settlement or mortality as the response. These analyses revealed significant positive relationships between concentration of oyster bathwater and larval settlement rates ( $T = 2.97$ ;  $p = 0.005$ ;  $R^2 = 20.6$ ; Figure 27), and larval mortalities ( $T = 6.63$ ;  $p < 0.001$ ;  $R^2 = 56.4$ ; Figure 28). Similar analyses were conducted using the barnacle bathwater dilutions and control treatment. The relationship between barnacle bathwater concentration and larval settlement was not significant ( $T = 0.35$ ;  $p = 0.728$ ;  $R^2 = 0.4$ ; Figure 27), while a significant positive relationship was observed between barnacle bathwater concentration and larval mortality ( $T = 15.94$ ;  $p < 0.001$ ;  $R^2 = 88.2$ ; Figure 28).

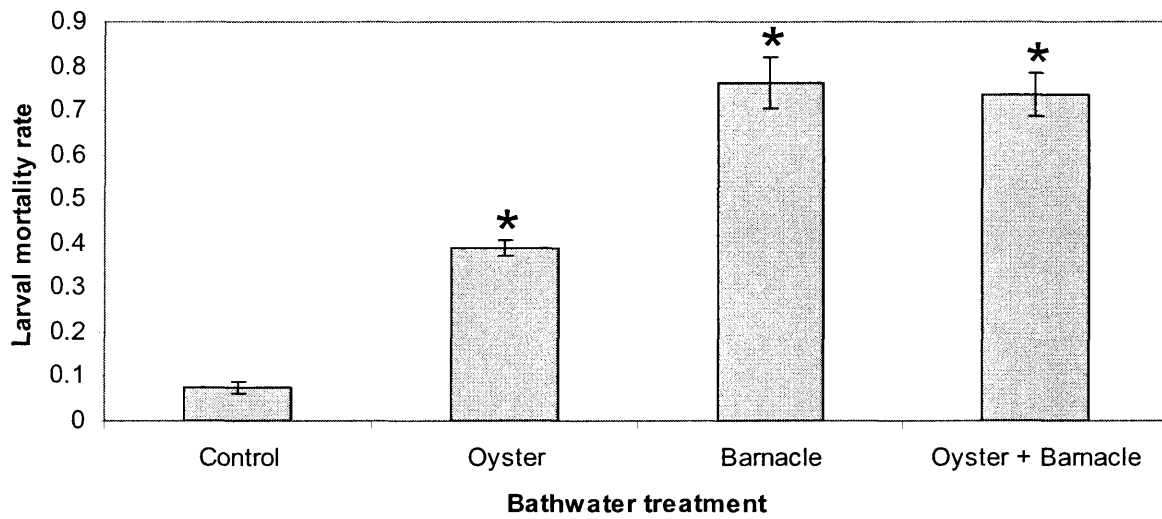
#### 5.2.4. *Effect of ammonia concentration on oyster larvae*

The bathwaters used for the previous two cue experiments were also retained for analysis of ammonia concentrations. These concentration values were then compared to the average settlement rate and mortality of oyster larvae after 72 hours of submersion in the bathwater. ACNOVA was conducted using the settlement rate or mortality as the response, and the experiment number and ammonia concentration (as the covariate) as predictors. Settlement rate varied significantly with ammonia concentration ( $F = 10.82$ ;  $p = 0.022$ ) and experiment number ( $F = 18.27$ ;  $p = 0.008$ ), but neither factor varied consistently with mortality.

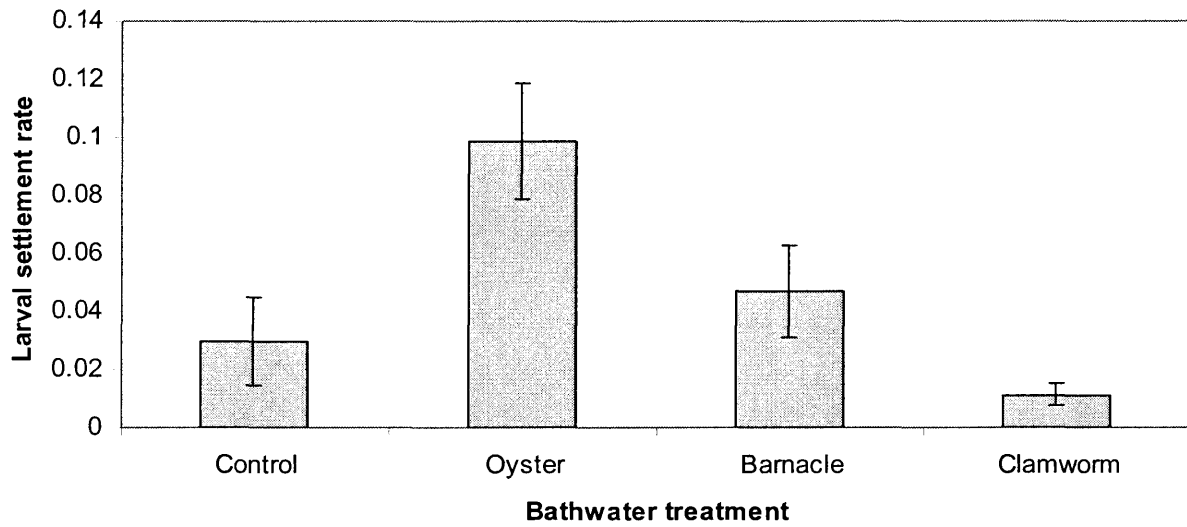
**Figure 22: Average settlement rate of oyster larvae by bathwater treatment (Experiment 8).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



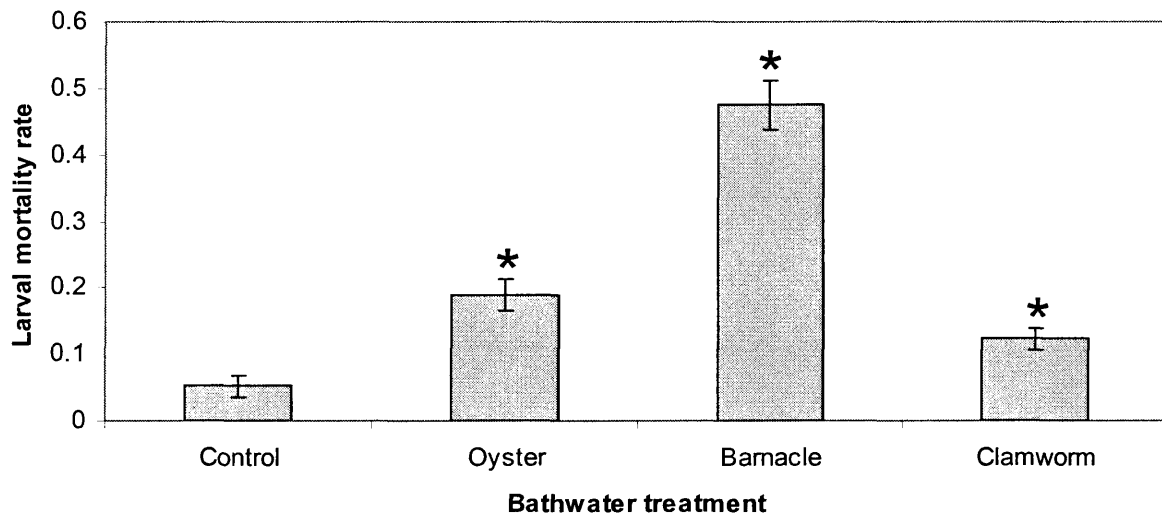
**Figure 23: Average mortality of larvae by bathwater treatment (Experiment 7).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



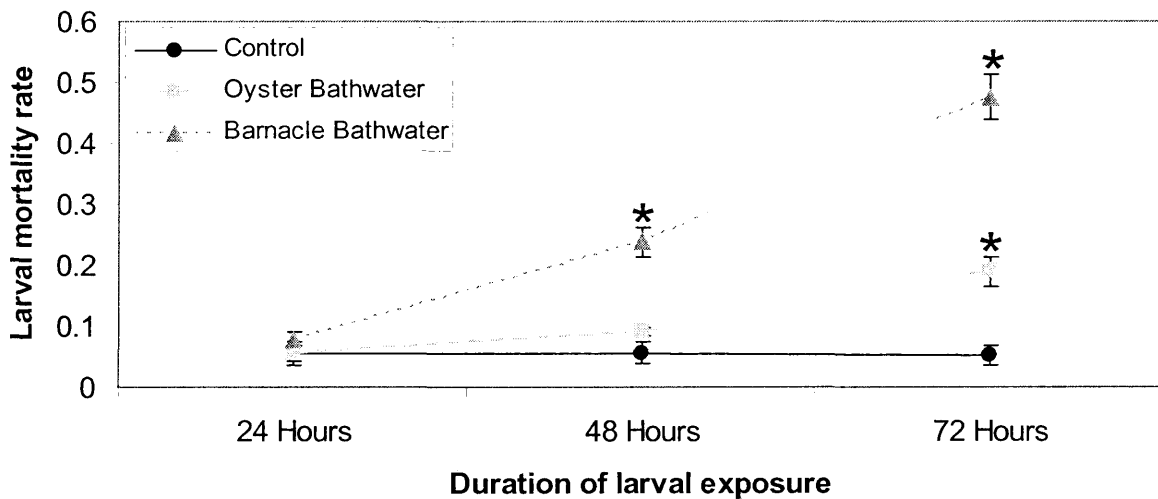
**Figure 24: Average settlement rate of oyster larvae by bathwater treatment (Experiment 8).** Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae in each treatment after 72 hours. No treatments differed significantly from control bathwater.



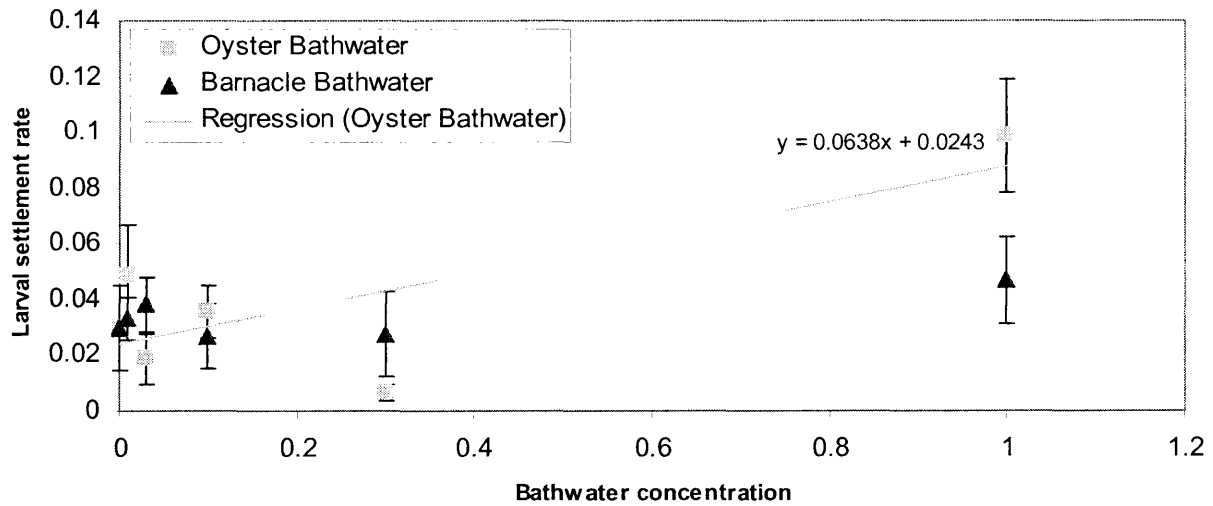
**Figure 25: Average mortality of larvae by bathwater treatment (Experiment 8).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



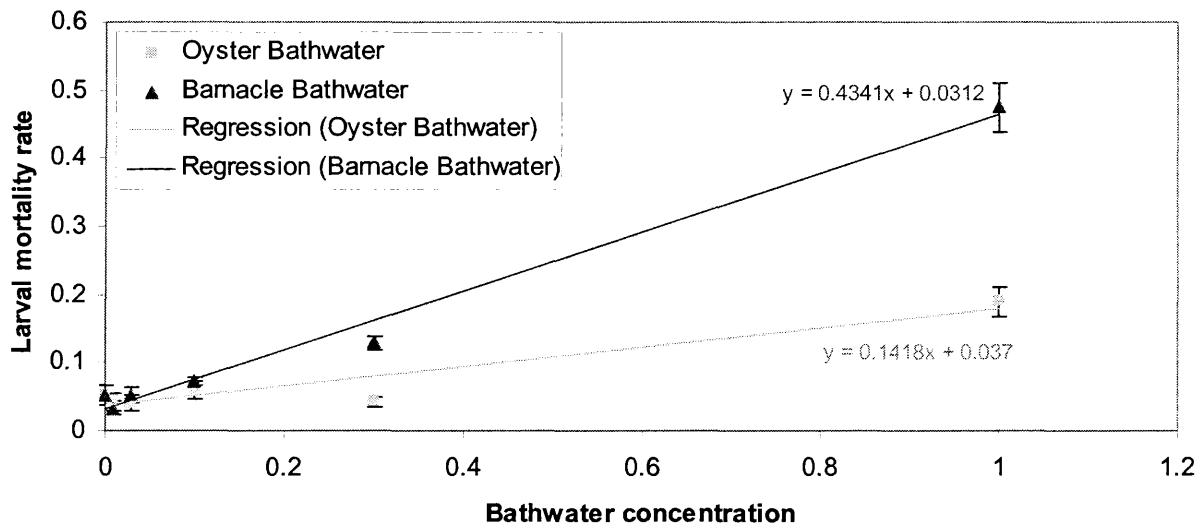
**Figure 26: Average mortality of larvae in different bathwaters and with different exposure durations (Experiment 8).** Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment at each time interval. Asterisks denote significant differences from control bathwater treatment at each time interval. After 72 hours of larval exposure, larvae in both oyster and barnacle bathwaters showed higher mortality than those in control water.



**Figure 27: Effect of bathwater dilution on settlement of oyster larvae (Experiment 8).** Linear regression indicated a positive relationship between settlement rate and bathwater concentration in both oyster and barnacle bathwater treatments. Values are means  $\pm$  1 Standard Error of the proportional settlement rate of larvae from each treatment after 72 hours.



**Figure 28: Effect of bathwater dilution on mortality of oyster larvae (Experiment 8).** Linear regression indicated a positive relationship between mortality and bathwater concentration in both oyster and barnacle bathwater treatments. Values are means  $\pm$  1 Standard Error of the proportional mortality of larvae recovered from each treatment after 72 hours.



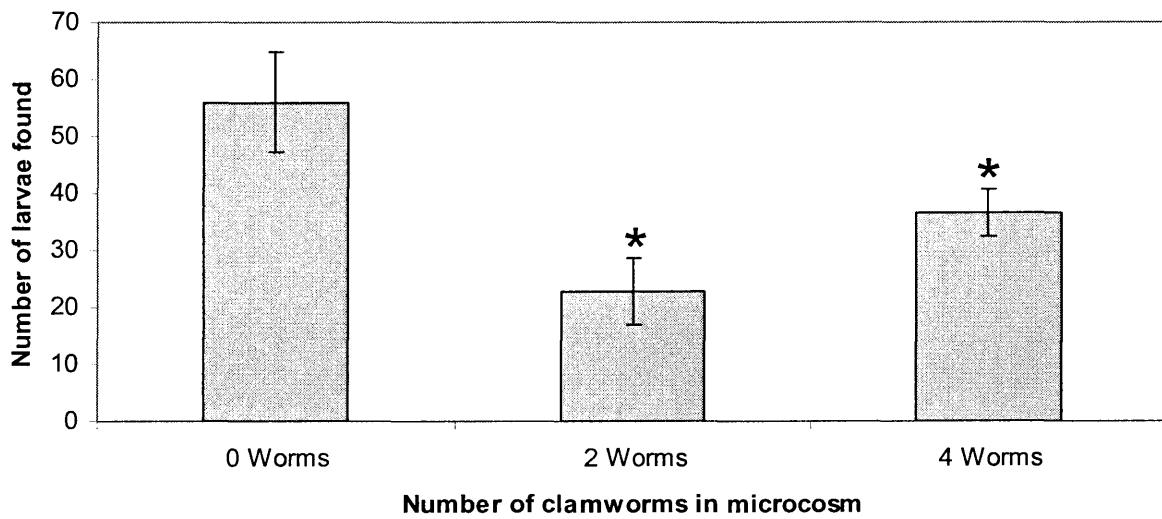
### 5.3. Predation on oyster larvae by Neanthes succinea

#### 5.3.1. Initial investigations – Experiments 4 & 5

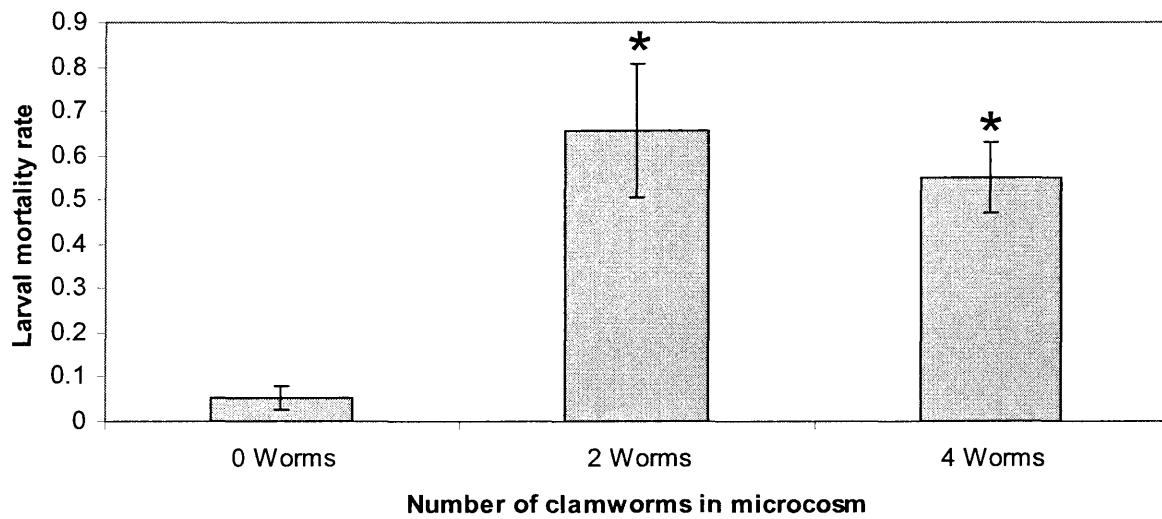
Worm treatments were incorporated into two of the above described epifauna experiments. ANOVA was conducted on transformed total larvae counts and mortalities to determine any differences due to worm presence or abundance. In experiment 4, this model indicated that total larvae found differed between treatments ( $F = 30.19$ ;  $p < 0.001$  Figure 29). Tukey's pairwise comparisons showed that the number of larvae found in the microcosms was significantly lower in the 2 worm ( $p = 0.0001$ ) and 4 worm ( $p = 0.0011$ ) treatments, relative to controls treatments with no worms. Mortality of the remaining larvae also differed between treatments ( $F = 15.97$ ;  $p = 0.001$ ; Figure 30), with the controls showing significantly lower mortality than the 2 worm ( $p = 0.0014$ ) and 4 worm ( $p = 0.0041$ ) treatments.

Similar analyses were also conducted on the clamworm experiments from experiment 5. Total number of larvae found again differed between treatments ( $F = 14.3$ ;  $p = 0.001$ ; Figure 31). Fewer larvae were found in control (no clamworm) treatments than in the 1 worm ( $p = 0.0027$ ) and 2 worm ( $p = 0.0008$ ) treatments. Analysis of the larval mortality also indicated a significant effect of treatment ( $F = 20.35$ ;  $p < 0.001$ ; Figure 32). Larval mortality was lower in the control treatments than in the microcosms containing either 1 worm ( $p = 0.0011$ ) or 2 worm ( $p = 0.0001$ ).

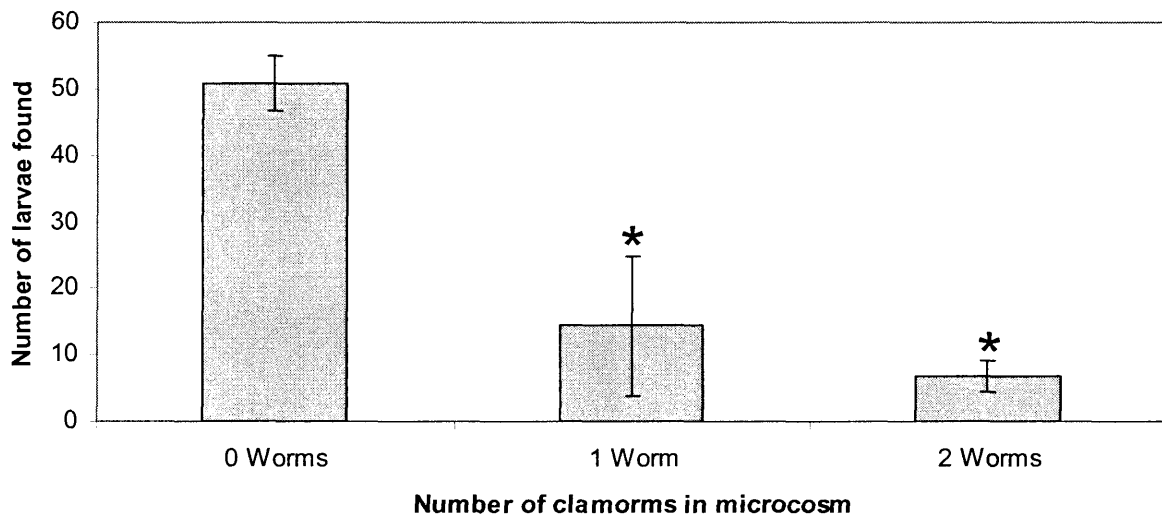
**Figure 29: Effects of clamworm density on the number of recovered larvae (Experiment 4).** Values are means  $\pm$  1 Standard Error of the number of live larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



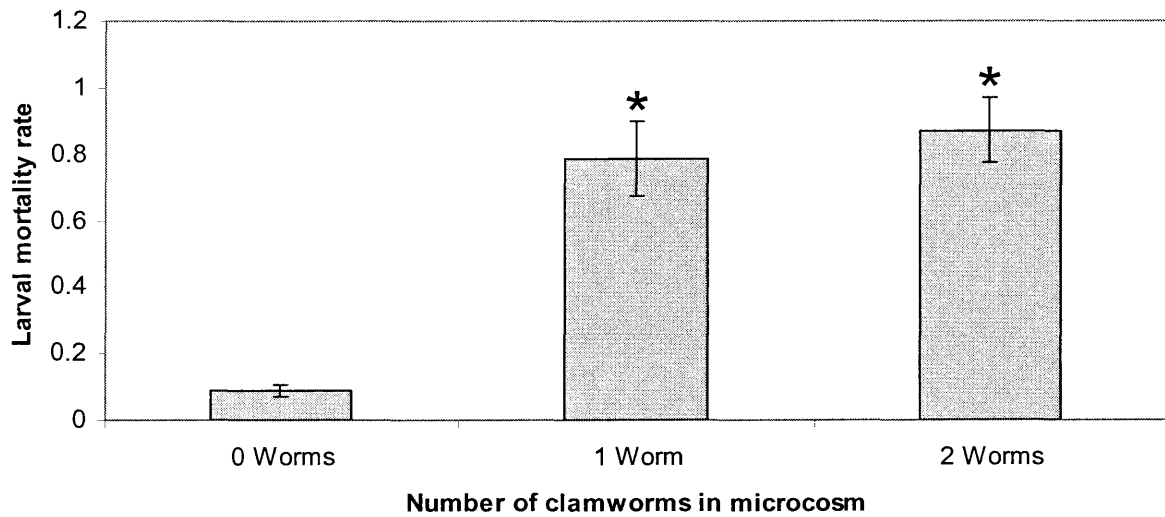
**Figure 30: Effects of clamworm density on the mortality of recovered larvae (Experiment 4).** Values are means  $\pm$  1 Standard Error of the mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



**Figure 31: Effects of clamworm density on the number of recovered larvae (Experiment 5).** Values are means  $\pm$  1 Standard Error of the number of live larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



**Figure 32: Effects of clamworm density on the mortality of recovered larvae (Experiment 5).** Values are means  $\pm$  1 Standard Error of the mortality of larvae recovered from each treatment after 72 hours. Asterisks denote significant differences from control bathwater treatment.



### 5.3.2. *Test of proximity hypothesis in time series – Experiment 7*

This series of experiments expanded on the previous clamworm trials by employing large microcosms (300 mL) in addition to the typical small (50 mL) microcosms. These microcosms were terminated in a time-series. Ordinal logistic regressions were conducted using container size and termination time in the model. The dependent variables analyzed were total larvae found and larval mortality.

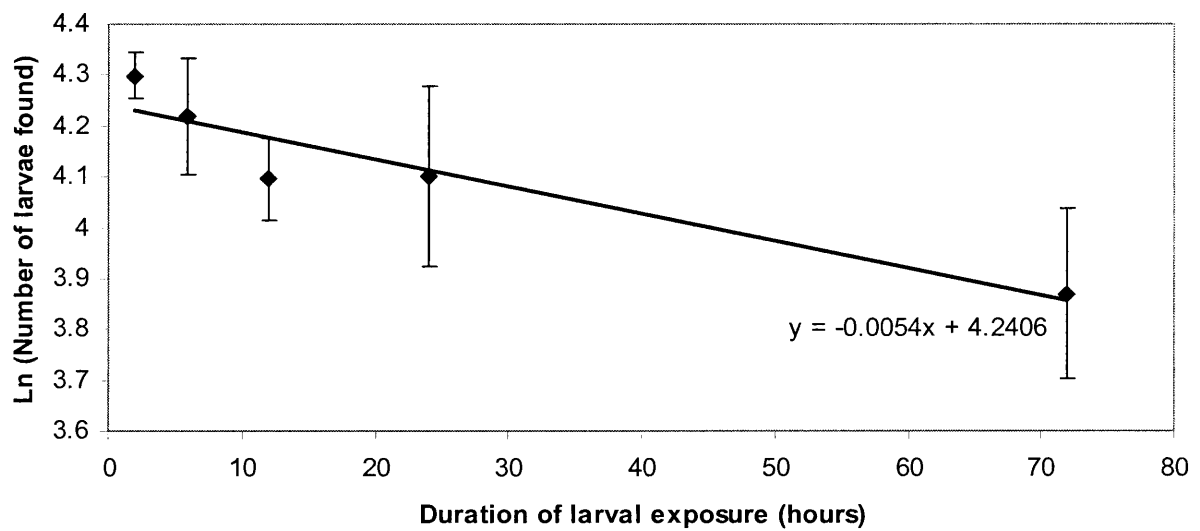
The ordinal logistic regressions indicated that container size was not a significant predictor of either total larvae ( $Z = 1.36$ ;  $p = 0.174$ ) or mortality in the microcosms ( $Z = 0.53$ ;  $p = 0.596$ ). The dependent variables were then log transformed (mortality values first required a  $y' = y + 1$  transformation), and microcosm size was no longer considered as a predictor. Linear regression subsequently showed a negative relationship between termination time and total number of larvae found ( $T = -2.5$ ;  $p = 0.019$ ;  $R^2 = 18.2$ ; Figure 33). Mortality of the remaining larvae was positively related to termination time ( $T = 4.05$ ;  $p < 0.001$ ;  $R^2 = 36.9$ ; Figure 34).

### 5.4. *Quantification of Neanthes succinea population characteristics*

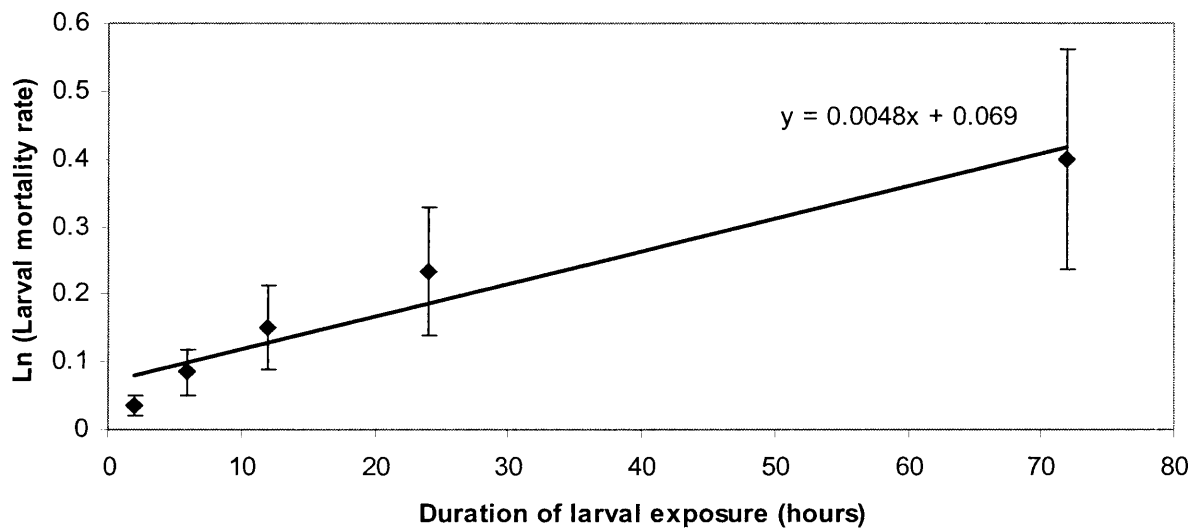
Overall, an average of 9776 clamworms (S.D. = 3624;  $n = 10$ ) were found  $m^2$  of oyster reef. There were no differences in the number of clamworms found on the reef crest and the reef slope ( $T = -1.12$ ;  $p = 0.306$ ). The average width of the 4<sup>th</sup> setiger was 0.9064 mm ( $n = 218$ ; S.D. = 0.3414; range = 2.1 mm; see Figure 35 for size-frequency histogram). No differences in 4<sup>th</sup> setiger width between clamworms found on the reef crest and the reef slope ( $T = 0.4$ ;  $p = 0.688$ ). Mean biomass for an individual clamworm

was 0.5 mg (S.D. = 0.2 mg, n = 148). Insufficient sample size precluded statistical comparison of sites with respect to tissue weights.

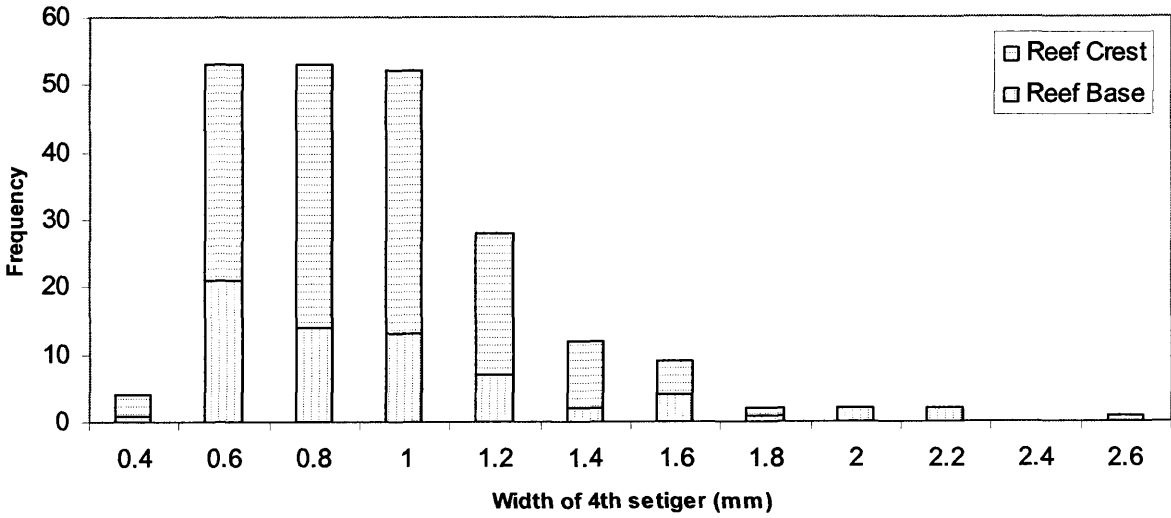
**Figure 33: Logarithmic relationship between the number of larvae found in clamworm treatments and the duration of larval exposure (Experiment 7).** Values are means  $\pm$  1 Standard Error of the natural logarithm of the number of larvae recovered.



**Figure 34: Logarithmic relationship between the mortality of larvae remaining in clamworm treatments and duration of larval exposure (Experiment 7). Values are means  $\pm$  1 Standard Error of the natural logarithm of the proportional mortality of recovered larvae.**



**Figure 35: Size-Frequency histogram of clamworm populations at Pungoteague Creek site.**



## 6. DISCUSSION

This study reveals several effects macrofaunal reef residents on the settlement and survival of *Crassostrea* larvae. Interactions with some established macrofauna increased settlement of oyster larvae, others decreased settlement and some had no evident effects. The reef-resident polychaetes worm *Neanthes succinea* affected oyster survival through direct predation, while barnacles (*Balanus improvises*) and perhaps the boring sponge *Cliona* sp) cause some mortality through waterborne chemicals. In most cases, my experiments do not definitively identify the mechanisms responsible for the observed patterns. They do, however, shed light on a variety of interactions between settling oyster larvae and resident organisms on oyster reefs.

### 6.1. *Species-specific effects*

#### 6.1.1. *Cliona* sp.

This study indicated that the presence of *Cliona* sp. in shells clearly hinders, but does not preclude, settlement by oyster larvae (contrary to the observations of Nelson, 1928). *Cliona* sp. treatments always had a lower mean percent total settlement rate than did positive control treatments. This was significantly different from the controls in 2 out of 3 *Crassostrea virginica* experiments, but in neither *C. ariakensis* experiment. The *C.*

*ariakensis* larvae should recognize the *Cliona* sp. infested shells as unsuitable settlement substrates, as the geographic range of *Cliona celata* (the boring sponge species most likely found at my sites) or one of its congeners likely overlaps the native range of *Crassostrea ariakensis* (see Calcinai et al., 2006; Zhang et al., 2005). There is, nevertheless, a potential difference in specific habitat between these two species in their native range, possibly leading to the inability of *C. ariakensis* larvae to detect shells inundated with boring sponges as unsuitable habitats. Further research is required on this front to more adequately address the mechanisms behind this phenomenon.

*Cliona* sp. treatments nearly always showed significantly higher mortality (%) than positive control treatments. Clearly, the size of the sponge's ostia precludes direct predation on oyster larvae. I anecdotally observed higher occurrences of clamworms in *Cliona* treatments, possibly due to the increased refuge for clamworms in the dissolved shells. As a result, many *Cliona* microcosms were removed from statistical analysis due to insufficient number of total larvae found. Clamworms, as discussed above, cause oyster larvae mortality through direct consumption, while larval mortality was higher in clamworm bathwater treatments than in control water treatments. Of these, only the latter is reflected in my measures of mortality. As a result, I was unable to directly discern if the increased mortality of oyster larvae in the *Cliona* microcosms is affected in any way by water soluble cues, or other exudates, emitted by adult sponges. The water chemistry analysis from samples in Experiment 4, however, indicated higher (albeit non-significantly) ammonia concentrations in *Cliona* microcosms than in 2 worm microcosms. Although never explicitly tested, I can thus infer a potential link between adult boring sponge bathwater and oyster larval mortality.

### 6.1.2 Membranipora tenuis

Experiments with *Membranipora tenuis* as test organisms indicated that although bryozoans may have a slight negative effect on overall larval settlement rate, it is not significantly different from control treatments. Only in a few instances were oyster larvae seen actually settling on the bryozoans, yet overall settlement rates never differed significantly from controls. This finding is consistent with the “quality of available space” paradigm proposed by Osman and Whitlatch, 1985 (see section 3.3.4.). It is conceivable that  $\beta$ -stage bryozoan colonies (100% cover) could completely preclude oyster recruitment, yet such conditions were rare in the samples obtained from either study site. The lack of a decrease in overall settlement rates in the bryozoan treatments, despite the rarity of larvae settled on bryozoans, suggests that waterborne cues or exudates emitted by adult bryozoan colonies are not affecting the settlement of oyster larvae.

There was some indication of an increase (although not significant) in larval mortality in the bryozoan microcosms compared to in positive control microcosms. The size and structure of the lophophore of *Membranipora tenuis* precludes it from direct predation on oyster larvae. Water chemistry analysis from experiment 4 indicated a significant difference in ammonia levels between bryozoan and control microcosms. It is possible that a waterborne cue or a byproduct of bryozoan metabolism is emitted by adult bryozoans which has an effect on oyster larvae mortality. Contrarily, increased

mortality of oyster larvae in bryozoan treatments may again be an unintended artifact of experimentation through unbalanced clamworm presence.

### 6.1.3. Balanus improvisus

#### 6.1.3.1. *Effects on oyster larval settlement*

Experiments using *Balanus improvisus* as test species indicated that adult barnacles likely facilitate settlement of oyster larvae. This increase in settlement rate is not a result of increased surface area, but an increase in density of newly recruited oyster larvae. Within the treatments using live barnacles, the reasons for this assertion are twofold. First, the positive control shells were clearly not space limited; the average density of larvae settled was approximately 3.2 larvae per square centimeter of available shell surface area (S.D. = 3.0). Much higher settlement densities are often found in such experimental conditions (pers. observation). Second, there was no relationship between the number of larvae settled on the test shell and total calcium carbonate substrate surface area (ANOVA with covariate;  $F = 0.20$ ;  $p = 0.653$ ;  $r^2 = 34.43$ ), with experiment number and species as significant ( $p < 0.001$ ) factors. This surface area was estimated by multiplying the area of the shells covered by barnacles by the coefficient 1.4 and adding the resulting value to the surface area of unfouled shell. This calculation required assumptions that 1) barnacle shells are perfectly cone-shaped, 2) the top radius of the cone is one-third that of the bottom radius, and 3) the angle transcribed by the cone is  $45^\circ$ .

Further evidence that the increase in settlement of oyster larvae in the presence of adult barnacles is not due to the increase in surface area was found in the experiments using barnacle mould and dead barnacle treatments. Although these treatments were created to mimic low-to-medium density barnacle treatments, none of them caused any significant increase in oyster larval settlement. These latter tests also suggest that the barnacle shell protein matrix is not the signal for increased oyster larval settlement in the presence of adult barnacles, presuming that dead barnacles retain the shell protein matrix of live barnacles.

One of the barnacle mould treatments actually showed statistically lower larval settlement than controls (experiment 6). A potential explanation for this is an effect of the materials used to create these treatments. Only a few larvae were ever recorded actually setting on the Sculpey® structures, and none were ever observed to set on exposed 5200 marine adhesive. This may have effectively lowered the available surface area available for oyster settlement in the barnacle mould treatments. Such an effect is not likely for the dead barnacle treatments, as the surface area covered by the marine adhesive was very small.

It is also possible that the small scale hydrodynamic currents created by feeding barnacles could have some effect on settling oyster larvae (similar to that proposed by Butler, 1955; see section 3.3.4.). To assess this, small scale video recordings of barnacle interactions with oyster larvae were taken which allowed visually evaluation of these interactions. These video recordings clearly showed that oyster larvae can, and often do, become entrained in small scale eddies created by feeding barnacles. In some cases, oyster larvae were observed being pulled into the shell of the barnacles, only to be

egested seconds later. The larvae would subsequently re-enter the water column in an apparently normal fashion. On a theoretical basis, if water currents created by barnacle feeding were forcing settlement of oyster larvae, then it would be expected that oyster larvae would always settle on one side of the barnacles (the power stroke of barnacle feeding structures is always in a consistent direction). Though no data on specific settlement patterns are reported here, I did not observe settlement patterns that would support this hypothesis in any of these experiments.

It is worth noting that only two experiments showed any deviation from the predicted increase in larval settlement in the presence of adult barnacles: experiments 3 and 4. These experiments (one using *C. virginica*, one *C. ariakensis*) were the only two which analyzed shells collected from the Rappahannock River, Virginia. It is possible that the fouling community at this location is somehow different from that found at the Pungoteague Creek site, or that the handling of the test substrates was different given the proximity of the two sites to the holding tanks. However, various methods were used for collection in the experiments using Pungoteague Creek shells (including the submersion of air dried shells, dredging, and hand collection through free diving; also spanning more than one calendar year). These different techniques did not result in differential settlement patterns, thus the apparent differences between sites was likely not an effect of the shells or fouling communities. Potential culprits could be the oyster bathwater used for these treatments (discussed below), differences in larval batches, or water chemistry characteristics of the seawater used in the experiments (these two experiments were performed in succession).

Finally, water soluble cues (or other byproducts of metabolism) emitted by adult barnacles likely have some effect on the settlement of oyster larvae. Bathwater experiments with no shell substrates clearly showed that barnacle bathwater can cause significant increases in settlement of oyster larvae, but experiments including shell substrates failed to give further evidence to this finding. This is a confusing result, as both types of bathwater microcosms (shell and no-shell) were conducted during experiments 6 and 7, and thus used the exact same bathwaters and oyster larval batches.

Overall, despite numerous experiments, the precise mechanisms causing increased oyster recruitment in the presence of adult barnacles remains elusive. Barnacle bathwaters clearly have an effect on oyster larvae, but their effect on settlement rate was not seen in the microcosms with shell substrates. It is possible that two (or more) of the mechanisms discussed, in concert, are actually driving enhanced oyster recruitment. Such potentialities were not directly investigated, and would require further study in order to be properly evaluated.

#### *6.1.3.2. Effects on larval oyster mortality*

Barnacle bathwater alone seems to be toxic to settling oyster larvae – causing significantly increased mortality in microcosms with and without shell. Live barnacle treatments, however, only had increased mortality relative to controls in one experiment (experiment 7). The barnacle bathwaters were created using the same barnacles as were used in the live barnacle treatments. Potentially, the bathwaters were created at too high concentrations; diluting barnacle bathwater in a ratio of 1:10 with water (but not 3:10) was enough to eliminate the significant increases in mortality from controls. This seems

unlikely, given that the ammonia levels in the experiment 4 microcosms were much higher than those of the barnacle bathwater experiments. Barnacles were never dissected for gut content analysis; however video investigations never revealed barnacle predation on oyster larvae (see above).

The materials used to create the barnacle mould and dead barnacle treatments may have had the unintended effect of altering the behavior of settling oyster larvae (e.g. preliminary investigations showed a potential toxicity of unbaked Sculpey® to 7 day old oyster larvae). Although none of the dead barnacle and barnacle mould microcosm experiments showed significantly increased mean mortality in oyster larvae compared to positive controls, some effect of these materials on the recruitment of larvae cannot be definitely determined.

#### 6.1.4. Crassostrea sp.

Given the differences in settlement between the positive and negative control treatments, the presence of *Crassostrea virginica* shells had the strongest effect (by far) among all treatments in these experiments on oyster larval settlement – to the point that inclusion of negative controls in statistical analysis always resulted in a non-normally distributed dataset. This was also true for mortality, indicating that simply the inclusion of an oyster shell in a microcosm results in elevated mortality. This is likely the effect of small organisms living in or on the shells which were not removed during processing, as opposed to a direct effect of oyster shells on oyster larvae.

It has been previously shown that water soluble cues emitted by conspecific (Hidu et al., 1978) and congeneric (Tamburri et al., 2008) adults result in increased settlement of oyster larvae (see section 3.3.3). This experiment affirms such findings, but also indicates that adult oyster bathwater may be toxic to settling oyster larvae. This effect is noticeable (i.e. significantly different from control water treatments) as soon as 72 hours after immersion. Bathwaters were created using exactly the same protocols as were used in the Tamburri et al. (2008) study.

Within oyster bathwaters, there was no correlation between ammonia concentration and either settlement rate or mortality on positive control treatments (i.e. in the epifauna experiments). This is likely because of the many differences between the larval cohorts and conditions between experiments. This study did not investigate how larvae of a single spawn would react to different oyster bathwaters, thus it is impossible to affirm whether the ammonia concentration of an oyster bathwater is affecting the settlement rate or mortality of larvae within it (see Zimmer-Faust and Tamburri, 1994). Still, it is inappropriate to infer from this study that ammonia concentration can serve as a surrogate estimate for the amount of waterborne cue attractive to oyster larvae.

#### 6.1.5. Neanthes succinea

Clamworms were shown to have significant effects on mortality of larval oysters. The average number of larvae found in clamworm treatments was always significantly lower than control treatments without worms, indicating that clamworms are removing oyster larvae from the system. A cue or metabolic exudate from the clamworms is also

likely toxic to the oyster larvae, as evidenced by the increased mortality of oyster larvae in clamworm bathwater treatments versus clean water. This bathwater driven mortality, however, is not likely to account for all of the increased mortality of larvae found in the microcosm experiments involving worms; in the bathwater water trials, larval mortality was approximately 7 % higher in clamworm bathwater than in control water, yet in the clamworms microcosms, larval mortality was on average 65% higher than controls (S.D. = 13%). It is thus likely that the oyster larvae removed from the system by clamworms account for only part of the total number of larvae killed by predation. This phenomenon was witnessed during one investigation under a microscope – a clamworm repeatedly came out of his burrow and expelled dead oyster larvae. This observation also helps explain where the oyster larvae are, once “removed from the system” in microcosms with clamworms.

Combining all of the clamworm experiments allowed for an estimation of the predation rate on oyster larvae by *Neanthes succinea*. To accomplish this, the number of live larvae in worm treatments was compared to the average number of live larvae found in the controls for that experiment. This method accounts for all mortality of larvae due to clamworms, but discounts natural larval mortality. Clamworm microcosm replicates had an average mortality of 50 larvae (S.D. = 17) killed in 72 hours, which corresponds to an 83% (S.D. = 26%) mortality rate directly attributable to the clamworms. Each individual clamworm killed an average of 34 oyster larvae (S.D. = 21) in 72 hours, although higher rates were seen in some cases (max = 33 larvae in 12 hours). This average rate was likely deflated, as inactivity by some clamworms was apparent.

At an average density of just under 10,000 clamworms  $\text{m}^{-2}$  of reef and an average predation rate of 11 larvae  $\text{day}^{-1}$  clamworm $^{-1}$ , a first-order estimate of the potential oyster mortality attributable to the *Neanthes succinea* population on the Pungoteage reef is over 100,000 settling larvae per square meter of reef per day. It is likely that the predation rates estimated in these microcosm studies are overestimates of those found in the field, given that oyster larvae were the only potential food source for the experimental clamworms. On the other hand, some of the clamworms in these microcosms did not seem to affect the oyster larvae (neither total larvae found nor mortality significantly differed from control treatments). The proximity between the oyster larvae and clams in the microcosms does not seem to affect this predation rate. This proximity seems reasonable in lieu of interstitial spaces on oyster reefs. As such, *Neanthes succinea* could pose a substantial sink for settling oyster larvae in the field, although direct measurement of these rates *in situ* would be very difficult.

## 6.2. *General patterns and ecological significance*

Larval behaviors are hard to model, especially in the field. Even immediately after spawning and fertilization, it is very difficult to track larval movements and mortality rates. At the time oyster larvae settle, it is nearly impossible to determine their home location, age or true abundances. As a result, many studies of larval behavior focus mainly on larval recruits, largely ignoring the process by which the larvae arrived in a certain location, and the perils of those who did not. This study aimed to fill a portion of that gap.

Reef-associated organisms can clearly affect larvae as they attempt to settle and recruit. Directly extrapolating field rates of settlement and mortality from laboratory experiments should be done with caution. Laboratory derived rates fail to account for the presence of multiple causes, alternate predators and prey, and a variety of other biological and physical factors. Nevertheless, from these experiments, it seems clear that clamworms can have major impacts on the survival of oyster larvae (ranging anywhere from 0 to 100%) through both predation (approximately 65%) and bathwaters (approximately 7%). Bryozoans are likely neutral with respect to oyster larval mortality and total settlement rates, but they certainly are not attractive settlement substrates. Estimates indicate that boring sponge presence causes an approximate 35 percent decrease in larval settlement (average decrease of 11 percentage points from controls) and a 2.5 fold increase in larval mortality (27 percentage points) compared to positive controls. Finally, with all other factors removed, barnacles likely increase larval settlement by approximately 50 percent (9 percentage points), while causing a similar increase in mortality.

I devoted much attention to the increases in both settlement rate and mortality in the presence of cues from adult oysters and barnacles. The increased mortality in oyster bathwater treatments is especially perplexing, perhaps explained by one of the following three hypotheses. First, metamorphosis is a very demanding process, and mortality during metamorphosis of oyster larvae is quite common (see Haws et al., 1997). Perhaps larvae are drawn to settle and metamorphose by the adult cues in the water (manifested in higher settlement rates in my microcosms) which leads to increased chances of larval mortality. Further, it is possible that the increase in swimming behaviors associated with

oyster cues (Tamburri et al., 1996) combined with a lack of preferable substrate (no shells were included in bathwater experiments) may have created conditions in which the oyster larvae exhausted their energetic reserves and thus perished. None of the experiments in this study conclusively indicated otherwise. Second, the communities of microorganisms were likely altered by the process of creating bathwaters. Increased organic matter in the bathwaters may have provided substrate for greater bacterial growth, which in turn caused increased mortality of oyster larvae. Finally, there may be some toxicological effect of the bathwaters on oyster larvae, either from a potential cue or from other biological exudates. True assays of the toxicity of adult cues would need to be assessed on pre-competent oyster larvae in order to remove the potential confounding effect of mortality during metamorphosis.

Overall, these investigations reiterate that oyster larval settlement is a complex and elusive topic, even in controlled laboratory conditions. Such studies are nevertheless valuable as they can help shape paradigms of community interactions and larval behaviors, and even management procedures. This investigation has shown that oyster larvae are clearly impacted by resident fauna as they attempt to settle, and that these interactions are not uniform across epifaunal taxa or easily predictable. Nevertheless, these interactions are likely important in describing the ecological processes of oyster larval settlement.

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