SETTLEMENT AND GROWTH OF THE MARINE BRYOZOAN *SCHIZOPORELLA JAPONICA,* AND EPIFAUNAL DEVELOPMENT IN THE SOUTH SLOUGH ESTUARY

by

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THESIS ABSTRACT

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Title: Settlement and Growth of the Marine Bryozoan *Schizoporella japonica,* and Epifaunal Development in the South Slough Estuary

The pre-metamorphic behaviors and settlement preferences of larvae can have significant effects on the success of adult invertebrates. This study describes various aspects of pre-metamorphic and post-metamorphic life stages of the bryozoan *Schizoporella japonica* in laboratory and field. The latency period of larval release had no effect on number of larvae released or settlement success of larvae in the laboratory. Larval size, ancestrula size and early colony size are positively correlated, and in this species, larval size effects persisted for up to 42 days, suggesting that larger larvae may have competitive advantages over smaller larvae in established, space limited fouling communities. We used a combination of water quality measurements, year-long settlement plates, and a month-long outplant of nonindigenous species along the estuarine gradient in South Slough to describe the structure and development of fouling communities with a particular focus on the settlement and survival of non-indigenous species.

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This thesis is dedicated to my family, my mother who instilled in me a love for the sea, my father who taught me to question all that surrounds me, and my sister who is a never-ending source of inspiration and unwavering support.

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CHAPTER I

GENERAL INTRODUCTION

Many marine invertebrates spend the first part of their life as a larva aloft in the plankton, weakly swimming though ocean currents until they encounter a hard substratum on which they metamorphose into an adult. This complex life-history pattern has evolved as an elaborate dispersal mechanism for many benthic organisms (Strathmann, 1993). Often overlooked in favor of adult experiences, the experience of the larva, including swimming behaviors, feeding or parental provisioning of energy, and pre-settlement behavior, plays a major role in structuring the populations of marine invertebrates. In particular, the larval settlement location is a crucial determinant of the survival of sessile adults, which are often unable to relocate after metamorphosis.

Bryozoans are fascinating colonial organisms, found in aquatic systems worldwide. There is broad appeal in studying bryozoans, from observing aspects of coloniality and the unique and highly evolved division of labor among polymorphic zooids to investigating pre-settlement behaviors and the drastic metamorphosis from motile larva to sessile ancestrula. The larvae of bryozoans are easily obtained and manipulated, and as a result, serve as excellent model systems in which to test questions about larval pre-metamorphic behaviors and metamorphosis, often resulting in conclusions that may have broader applications to other invertebrate larvae. In this thesis, I use the bryozoan *Schizoporella japonica* Ortmann 1890 as a model system to test questions about the larval experience, including and settlement and early colony growth.

The bryozoan *Schizoporella japonica* belongs to the order Cheilostomata, exclusively marine colonial bryozoans with calcium carbonate skeletons (Woollacott and Zimmer, 1977). On the west coast of North America, *S. japonica* is considered to be a non-indigenous species, introduced to the Pacific Northwest region after 1907 on the shells of oysters from their native region of northeastern Japan for use in the mariculture industry (Ross, 1976, Rumrill, 2007). Colonies of *S. japonica* are commonly found encrusting hard substrata in the inner boat basin of Charleston, OR. The exact introduced range of *S. japonica* is currently unknown, as it is commonly misidentified in scientific literature as *Schizoporella unicornis* or *S. errata*.

The larvae of *S. japonica* are non-feeding coronate larvae approximately 250 microns in diameter (Ross, 1976) that develop in the ovicell of the parent zooid until they are released into the water column (McCain 1972, Treibergs personal observation). They have a ciliated corona that is used in locomotion, an apical disc and pyriform complex that are used in searching substrata for an appropriate settlement location, and an internal sac that is involved in the drastic metamorphosis from larva to ancestrula (Zimmer & Woollacott, 1977).

Although *Schizoporella japonica* and other *Schizoporella* species are common members of fouling communities worldwide, their ecology has not been studied in great detail. Removal experiments demonstrate that a species of *Schizoporella* actively excludes other species from overgrowing itself. However, it is a poor invader of previously occupied space (Sutherland, 1978). Encrusting bryozoans may become impeded by adjacent conspecifics, colonial ascidians, sponges, invertebrate egg masses and tubicolous polychaetes, as well as by high silt levels and exposure to UV (Gordon,

1972). Besides being major competitors for substratum space in shallow subtidal regions, bryozoans are ecologically important consumers of primary production as well as food sources for numerous invertebrates such as pycgnogonids, echinoids, asteroids, ophiuroids, chitons, and opisthobranchs (Gordon, 1972). I have not observed any instances of macropredation on *S. japonica* in the inner boat basin of Charleston, OR, but I have observed multiple instances of predation on embryos or larvae in ovicells by a turbellarian (Treibergs, personal observation).

The genus *Schizoporella* is cosmopolitan, with over fifty species worldwide (Tompsett et al. 2009). As a result, species have been used as model organisms in numerous areas of study including larval settlement behavior, competition for substratum space, colony growth, environmentally mediated colony morphologies, epifaunal associations, and ecology of non-indigenous fouling organisms (Tompsett et al. 2009). My objectives in this thesis are to describe some aspects of the biology of *S. japonica* that have not yet been observed in Oregon populations, with a focus on the pre-settlement phase, metamorphosis, and early colony growth. Chapter II of this thesis describes the effects of surface texture, timing of larval release, and delay of metamorphosis on the settlement success of *S. japonica*. Chapter III investigates the carry-over effects of the size of *S. japonica* larvae on post-settlement colony success in the field. Chapter IV describes the presence and relative abundance of *S. japonica* and other fouling community invertebrates in the South Slough estuary for the year 2011, as well as the survival of outplants of *S. japonica* and the ascidian *Botrylloides violaceus* to sites arranged along a salinity gradient in the South Slough estuary. This thesis examines laboratory settlement of *S. japonica*, and is the first to show the effects of delaying the

cue for larval release on settlement success, as well as adds evidence to an increasing body of knowledge about the positively correlated effects of larval size on early colony growth in marine invertebrates. In addition, I have examined seasonal trends in fouling community composition and recruitment of non-indigenous species in South Slough.

CHAPTER II

LARVAL SETTLEMENT OF *SCHIZOPORELLA JAPONICA*

Introduction

Larval supply, settlement success and post-settlement survival are the three main factors that structure populations of benthic marine invertebrates (McKinney & McKinney 1993). The pre-settlement behavior, or the actions of a larva before it settles, in sessile colonial invertebrates affects larval recruitment and subsequent colony viability by influencing the dispersal of larvae as well as the chance that larvae will encounter suitable settlement substrata (Raimondi & Keough 1990; Rodriguez & Ojeda 1993). As a result, this behavior has significant effects on the distribution of sessile invertebrate species (Walters et al.1999; Miron et al. 2000; Burgess et al. 2009). This study is the first to investigate the settlement preferences and laboratory settlement dynamics of the cheilostome bryozoan *Schizoporella japonica* Ortmann, 1890.

Pre-settlement behavior determines how a larva explores its available substratum. For organisms with a sessile adult stage, this selective behavior becomes one of the most important determinants of successful recruitment and colony survival (Walters 1992; Burgess et al. 2009). Factors influencing the pre-settlement behavior of ciliated larvae in the field are numerous and variable, both between and within species, however they fall within two categories of exogenous and endogenous factors(Burgess et al 2009). Exogenous factors are well-studied, and include substratum type (Connell 1985; Yagunova & Ostrovsky 2010), chemical cues (Ryland 1974; Pawlik 1992), biofilms (Wisely 1958; Crisp & Ryland 1960; Brancato & Woollacott 1982; Maki et al. 1989;

Wieczorek & Todd 1997), presence or absence of nearby conspecifics (Keough 1984; Waldman 1988), competitors (Grosberg 1981; Young & Chia 1981), or predators (Johnson & Strathmann 1989; Young & Gotelli 1981), surface orientation (McKinney & McKinney 1993), micro-topography (Walters 1992), light (Burgess et al. 2009), flow (McKinney & McKinney 1993), surface texture (Ryland 1974) and surface material (Wisely 1958; Hurlbut 1991). Studies investigating how endogenous factors, such as larval choice (Keough & Downes 1982) and settlement behaviors (McKinney & McKinney 2002; Burgess et al. Marshall 2009), larval exploration of substratum (Walters 1992; Walters et al. 1999), larval age (Lucas et al. 1979; Woollacott et al. 1989; Miron et al. 2000; Gribben et al. 2006) and larval size (Marshall & Keough 2003; Burgess et al. 2009; Kosman & Pernet 2009) affect settlement are more difficult to undertake, and therefore are less commonly discussed in scientific literature (Burgess et al. 2009).

Bryozoans are excellent model organisms for studying pre-settlement behaviors and settlement preferences in the laboratory because larvae are easily obtained, partial parentage can be controlled, most are lecithotrophic and have a short pelagic larval duration on the scale of hours (McCain 1972), they metamorphose relatively quickly (Woollacott & Zimmer 1971), and high percentages of settlement can be obtained in some species (Keough 1984; Woollacott et al. 1989). A common pre-settlement behavior in bryozoan larvae is the active exploration of the substratum on a small scale (mm to cm), rather than settling immediately upon encountering a surface (Walters 1992). Benefits of such a behavior include finding the most secure location for attachment, often within pits or in corners, and finding a location with the highest food availability (strongest flow) without getting dislodged. Risks of this exploratory behavior include

getting knocked off, expending limited energy reserves, getting eaten, or getting carried away from the substratum (Walters 1992; Walters et al. 1999). Also associated with pre-settlement behaviors is the ability of larvae to delay metamorphosis. Despite its risks, the delay of metamorphosis in *S. japonica* may have ecological advantages (Young & Chia 1981; Burgess et al. 2009). Larvae that can assess environmental conditions and delay metamorphosis in favor of finding more suitable settlement locations will have increased fitness and be favored by natural selection (Walters 1992; Burgess et al. 2009). Bryozoan larvae whose settlement behaviors are dependent on age and size have fitness benefits compared to larvae that do not change behaviors over time (Raimondi & Keough 1990; Burgess et al. 2009). However, delaying metamorphosis comes at a cost(Pechenik et al.1998). Colonies of *B. stolonifera* whose larval metamorphosis was delayed by 4 hours or longer had significantly lower laboratory growth rates when compared to colonies whose larval metamorphosis was not delayed (Woollacott et al. 1989), and colonies of *B. neritina* whose larval metamorphosis was delayed had reduced field growth and fecundity (Wendt 1998).

Many studies of larval settlement focus on trends in behavior, highlighting the preferences of most larvae, and often overlooking or understating any variation in behaviors (Raimondi & Keough 1990). There can be significant variability in pre-settlement behavior within and between populations among most larvae that settle preferentially in response to a stimulus (Raimondi & Keough 1990). Sources of this variability include genetic variation, effects of varying parental environment, responses to varying physical processes or other environmental cues, larval age, larval size, and geographic location (Raimondi & Keough 1990). Settlement behavior varies between

geographically distinct populations of *B. neritina,* particularly in response to light, presence of conspecifics, time to settlement, and orientation at settlement (Raimondi & Keough 1990). Variability in larval settlement behaviors may have significant adaptive advantages to populations, for example, variable barnacle gregariousness. A species with most individuals exhibiting gregarious settlement but with some individuals settling independently from conspecifics will be able to create more patches of barnacles, and subsequently increase probability of population survival in cases of mass mortality from physical disturbances (Raimondi & Keough 1990).

In this study we tested the effects of surface texture, timing of larval release and delay of metamorphosis on the settlement success or the marine bryozoan *S. japonica* by collecting larvae and introducing them to various settlement surfaces and treatments through a series of four experiments. The purpose of this study was to describe the pre-settlement and settlement behaviors of S. *japonica* in the laboratory to make inferences about larval field behaviors as well as for its future use in experimental procedures both in the laboratory and the field.

It is not yet possible to observe pre-settlement behaviors of ciliated larvae in the field (Young 1995), and laboratory studies are often used to infer field settlement behavior. Despite major differences between the two environments, some laboratory studies of bryozoan settlement have found that settlement trends observed in the laboratory correlate with trends observed in the field (McKinney & McKinney 1993). One disadvantage to laboratory studies is that they often focus on a single species isolated from its community. As a result, any interspecies interactions that affect settlement are

not observed, and settlement patterns observed in a single species in the lab may not be representative of those observed of a single species within a community (Hurlbut 1991).

The bryozoan *S. japonica* is an attractive candidate for the study of larval behavior, larval settlement, colony growth, and non-indigenous fouling community ecology due to its ability to produce and release larvae year-round (McCain 1972, pers. obs.) as well as accessibility and high numbers in Charleston, OR. The genus *Schizoporella* Hinks, 1877 is presumed to be composed of over 50 living species that can be found in marine environments worldwide (Hayward & McKinney 2002; Tompsett et al.). As noted by Tompsett et al. (2009), species of *Schizoporella* have been used in various studies of larval settlement behavior ((Hurlbut 1991; McKinney & McKinney 2002), substratum competition (Turner & Todd 1993) and studies of fouling organism invasions (Relini et al. 1998; Koçak 2008). Morphological differences among *Schizoporella* species are subtle, and often difficult to detect without analysis with scanning electron microscopy (SEM), often resulting in the misidentification of species in ecological literature (Tompsett et al. 2009).

Materials and Methods

Study species, location, and collection methods

Schizoporella japonica is a cheilostome bryozoan that broods embryos and releases non-feeding coronate larvae that are competent to settle upon release. Larvae of *S. japonica* typically have a short pelagic larval duration, and will settle and metamorphose on a suitable surface within minutes to hours after being released from the

adult colony (McCain 1972). Colonies of *S. japonica* are commonly found encrusting hard substrata of the inner boat basin in Charleston, OR.

Laboratory experiments were conducted at the Oregon Institute of Marine Biology (OIMB), in Charleston Oregon, USA (43°20.730' N, 124°19.610' W). We collected live mussels and mussel shells (*Mytilus trossulus*) encrusted with reproductive colonies of *S. japonica* from the sides of docks in the inner boat basin of Charleston Harbor, OR. To reduce the degree of relatedness between colonies (Keough 1984), colonies were collected from multiple sites within the boat basin that were separated by a distance of over 20m. Mussels were transported in seawater for approximately 10 minutes, gently cleaned of all encrusting organisms excepting *S. japonica*. Colonies ranged from 3-10 cm in diameter, and were ascertained to be reproductive due to the presence of orange embryos within ovicells. Live mussels over 10 cm in length supporting bryozoans were pried open and scraped clean, to prevent the strong mussel feeding currents from taking in larvae. Colonies were then placed in dark aquaria of ambient temperature seawater and with minimal aeration. After a minimum of 72 hours (72 hours was determined to be an appropriate amount of time in darkness for the reliable collection of larvae in previous studies) in darkness, flow and aeration were stopped, and colonies were immediately exposed to bright light from five, 100 watt spotlights suspended 30 cm above the tank and oriented downward. Larvae are positively phototactic upon release, and were collected from the water's surface, first appearing within 30 minutes of light exposure. Collection of larvae continued each half hour until larvae were no longer released six hours after exposure to light. We collected new colonies for each experiment.

Effects of surface characteristics on settlement success

To test the effects of surface structure on larval settlement, 250 larvae of *S. japonica* were collected from 70 adult bryozoan colonies with the methods described above. Larvae were randomly introduced to 50, five cm diameter polystyrene petri dishes containing filtered seawater. Each dish had one of five surface treatments, with 10 dishes per treatment (n=10), and five larvae per dish. Surface treatments included 'smooth' (unmanipulated), 'grooved' (crosshatched scratches approximately 500 μm wide, one to three mm apart), 'sanded' (by hand for 10 seconds with an ISO grit designation of P60 and an average particle diameter of 269 μm), 'sanded with biofilm' (sanded plates that were submerged in a flow-through sea table for one week) and 'sanded with grooves' (grooved plates that were subsequently sanded). Petri dishes were maintained in darkness at ambient seawater temperature. After 24 hours, plates were analyzed for settlement with a dissecting microscope, and the average number of larvae settled per dish was calculated and compared among treatments with a one-way ANOVA. Plates were assayed for additional settlement after 48 hours.

Short-term settlement dynamics

We monitored the settlement state of larvae in the laboratory by introducing eight larvae of *S. japonica* (collected from 50 adult colonies) to each of 17 petri dishes that were roughened with sand paper and allowed to accumulate biofilm for 48 hours while immersed in a sea table. Larvae were monitored every eight hours with a dissecting scope, for an initial period of 32 hours, and then were monitored every 24 hours for 96

hours in total. 'Settlement' was calculated to be the average number of larvae settled per dish and was defined by the formation of an ancestrula. 'Swimming' was determined to be the average number of unattached larvae swimming per dish, and was defined by the visible beating of larval cilia.

Effect of cue timing on larval release and short-term settlement dynamics

Larval release was delayed to investigate the effects of timing of larval release on larval number and settlement success. Eighty mussel shells ranging from three to six cm in length containing colonies of *S. japonica* were collected before sundown on October 31, 2011. Colonies were randomly assigned to eight covered, opaque, two gallon tanks, with flow and aeration. At sunrise the next day (0800 on November 1, 2011) four of the eight tanks (natural light cycle treatment) were exposed to bright lights, and all larvae were collected and counted each hour for a total of five hours. Larvae were added in groups of eight to roughened petri dishes that had been allowed to accumulate biofilm for 48 hours. Dishes were then placed in a darkened sea table at ambient seawater temperature. Twenty-four hours after the introduction of larvae, dishes were analyzed to determine the number of swimming, settled, dead, or missing larvae. Plates were monitored again after an additional 24 hours (48 hours after the introduction of larvae) to document any additional changes in settlement. The remaining four tanks (delayed light cycle treatment) were kept in darkness with flow and aeration for 48 more hours, for a total of 72 hours in the dark. Colonies were then exposed to bright light at sunrise (0800 on November 3, 2011), and larvae were collected each hour and distributed among the

dishes, which were analyzed after 24 hours and 48 hours in the same manner as the natural light cycle treatments.

Larval count data were log transformed with a 'log(x+1)' function to decrease sample variance, and larval settlement percentage data were arcsin transformed. Treatment effects on mean larval abundance and larval settlement were compared with a one-way ANOVA.

Long-term settlement dynamics

In previous studies, half to three-quarters of larvae continued swimming in petri dishes after 48 hours, without settling. To further investigate the fate of such larvae, unsettled larvae were observed for a longer time. Nineteen larvae of *S. japonica* were obtained by the previously described methods. Each larva was maintained in an individual five-cm diameter petri dish with a sanded surface that had been immersed in a sea table for 48 hours to establish a biofilm. Larvae were monitored with a dissecting microscope over 13 days, to gauge settlement. 'Settled' larvae were defined by the formation of a complete ancestrula, 'swimming' larvae were defined by the presence of moving cilia or body movement, and 'failed' larvae were defined as a form notably different from that of swimming or settled larvae (Figure 1). Failed larvae included partially settled ancestrulae that did not possess a true ancestrula shape, orange smears on the surface of the plate, or orange unmoving masses that were not affixed to the substratum. In certain cases, larvae went missing, and these larvae were also counted among those that 'failed'.

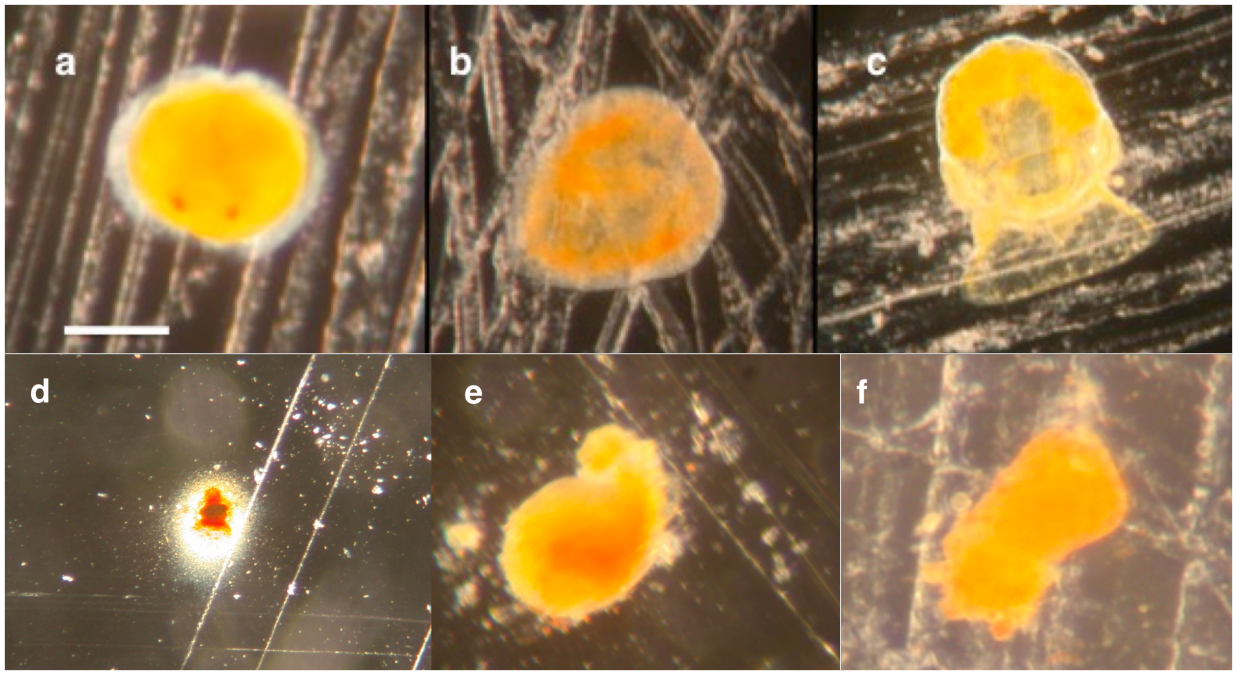

Figure 1. Stages of metamorphosis of *Schizoporella japonica*, a free-swimming larva (a), 24-hour ancestrula (b), 48-hour ancestrula budding next colony zooid (c), and evidence of failed metamorphosis: orange smear on substratum (d), and irregularly formed ancestrulae (e, f). Scale bar is 200 microns.

Results

General observations of larval behavior

After larvae were introduced to petri dishes, we observed an alternation between two main behaviors, swimming and exploring. Swimming behavior involved rapid movement in one direction in the water column in a tight spiraling pattern, and was commonly followed by exploring behavior, a pause on the surface of the petri dish wherein larvae expand and contract their body from a round shape to an elliptical shape, testing the substratum with their sensory plume and occasionally exhibiting crawling behavior. No interactions were observed among multiple larvae within a dish.

Effects of surface characteristics on settlement success

After 24 hours, an average of 2.3 $\pm$0.3 to 3.7$\pm$1.2 (1 s.e.) larvae settled per dish (out of 10) and no additional settlement was observed when dishes were monitored 24 hours later (Figure 2). There was a significant effect of surface treatment on settlement [$F(4, 45)=2.658$, $p= 0.045$]. Post-hoc comparisons with the Tukey-Kramer HSD test indicated that the mean settlement did not differ significantly between any pairwise comparisons. (The mean settlement for the 'sanded with biofilm treatment' differed the most from the 'grooved treatment' with a p value of 0.067.)

In the grooved surface treatment many ancestrulae were located in the grooves. In contrast, settlement on the roughened surface treatments occurred throughout the bottom of the dish. In all treatments, larvae occasionally settled on the sides of the petri dish, or where the sides and bottom meet to form a right angle. Larvae that settled on the sides of dishes were described as 'settled' even though the surface treatment was not applied to the sides of dishes.

Short-term settlement dynamics

After the monitoring period of 96 hours, an average of 4.4 larvae out of eight per dish settled, and we observed no additional settlement after the first 24 hours (Figure 3). An average of 1.7 larvae per dish continued swimming for the duration of the study, however we observed a decline in larval motility over time. This decline in motility included observations of slower swimming speeds, fewer muscular contractions and a decline in the time spent swimming as opposed to resting on the substratum. An average of 1.8 larvae per dish suffered mortality before the end of the experiment, sometimes through unsuccessful settlement attempts or by becoming entrapped in surface tension.

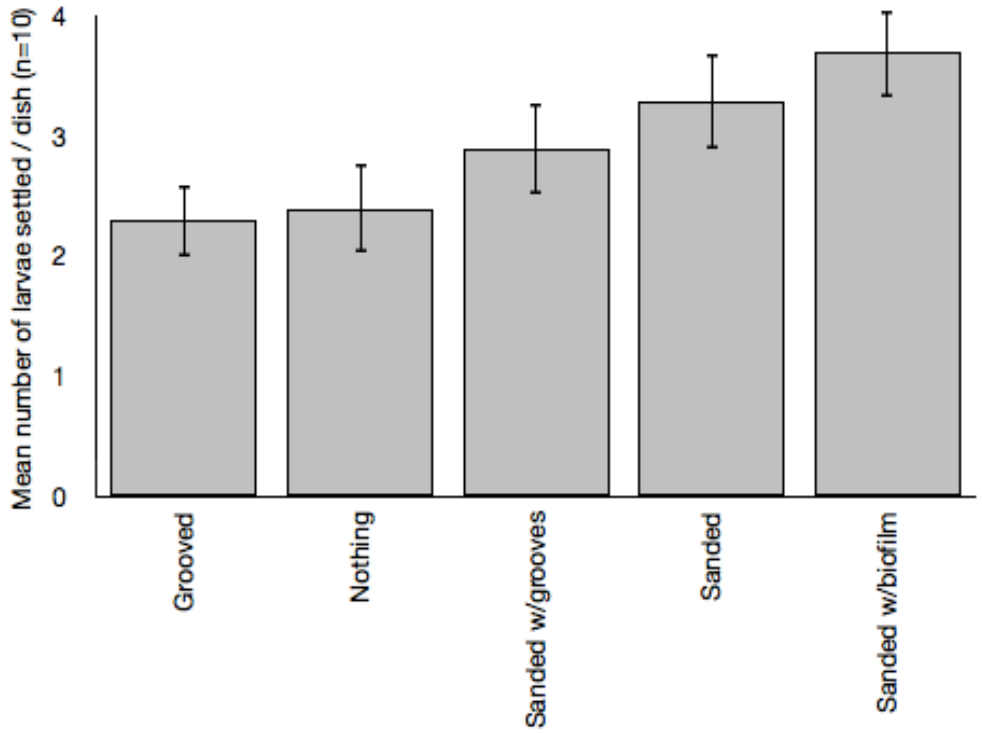

Figure 2. Effects of surface texture and biofilm on settlement success of larvae of *S. japonica* after 24 hours. Larvae were introduced to dishes with five different surface treatments, at a density of five larvae per dish (n=10 ± SE).

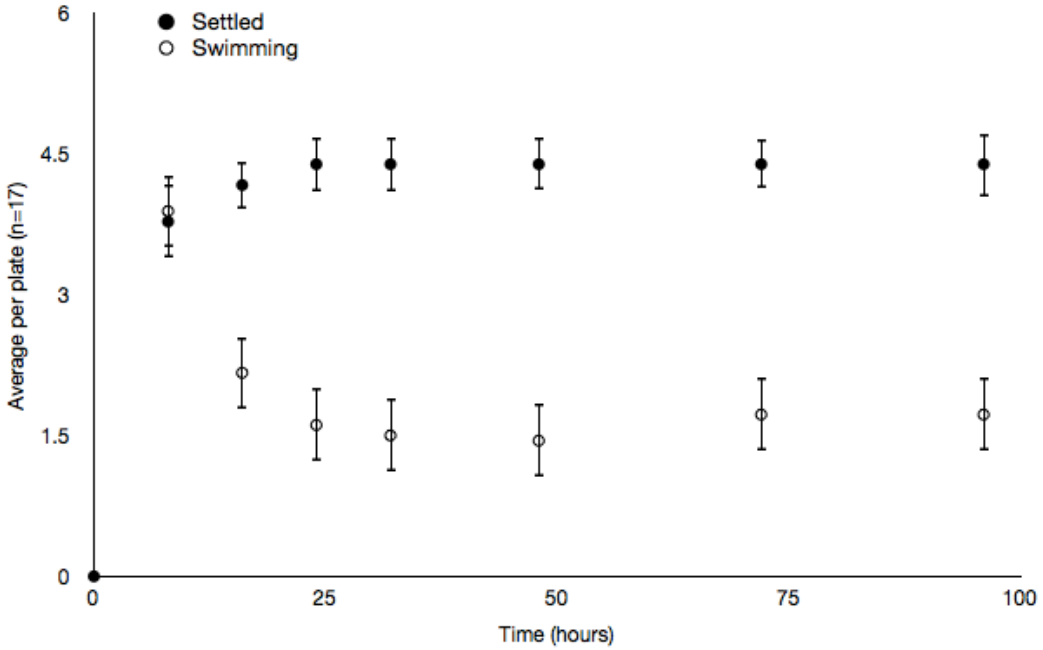

Figure 3. Settlement state of larvae of *S. japonica* over 96 hours. Larvae were held in dishes at a density of eight larvae per dish (n=17 ± SE).

Effect of cue timing on larval release and short-term settlement dynamics

We investigated the effect of timing of larval release on number of larvae released and percent settlement observed from treatments kept in darkness for 12 hours (natural light) and treatments kept in darkness for 72 hours (delayed light) (Figure 4). An average of 27 $\pm$ 8 (1 s.e.) larvae were released in the natural light treatment, and an average of 41$\pm$11 (1 s.e.) larvae were released in the delayed treatment. We observed no significant difference between the number of larvae released in the delayed light treatment than the natural light treatment [T-test, t(6)=2.44, p=0.17].

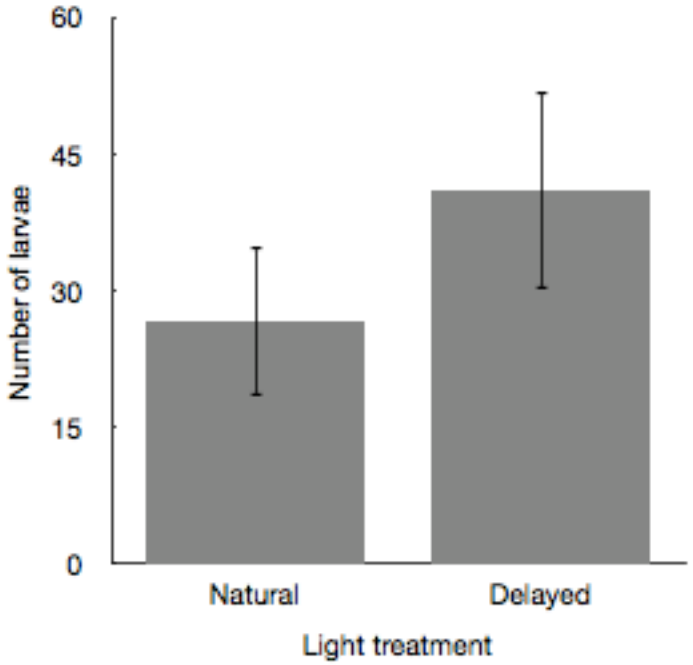

Figure 4. Effect of a 72-hour delayed larval release cue on the total number of *S. japonica* larvae released (n=4 $\pm$ SE). 'Natural' represents larvae obtained from parent colonies that collected at sundown and exposed to light the following sunrise, 12 hours later. 'Delayed' represents larvae obtained from parent colonies collected at sundown that were kept in a darkened flow-through and then exposed to light 72 hours later. Settlement was analyzed after 24 hours for each treatment.

When number of larvae released was compared between treatments across time (for a total of five hours), we observed a trend of fewer larvae released over time (Figure 5). The data was transformed to adjust for a lack of sphericity with the Greenhouse-Geisser Epsilon and Huyn-Feldt Epsilon, after which we observe a significant time effect (ANOVAR $f(4,24)=9.440$; G-Gε=0.294, $p=0.016$; H-Fε=0.384, $p=0.008$),and no significant treatment (ANOVAR $f(1,6)=1.1093$, $p=0.333$) or time*treatment interaction effect (ANOVAR $f4,24)=1.502$; G-Gε=0.294, $p=0.495$; H-Fε=0.384, $p=0.533$) at the alpha=.05 level. These results suggest that light treatments do not vary in the number of larvae released every hour.

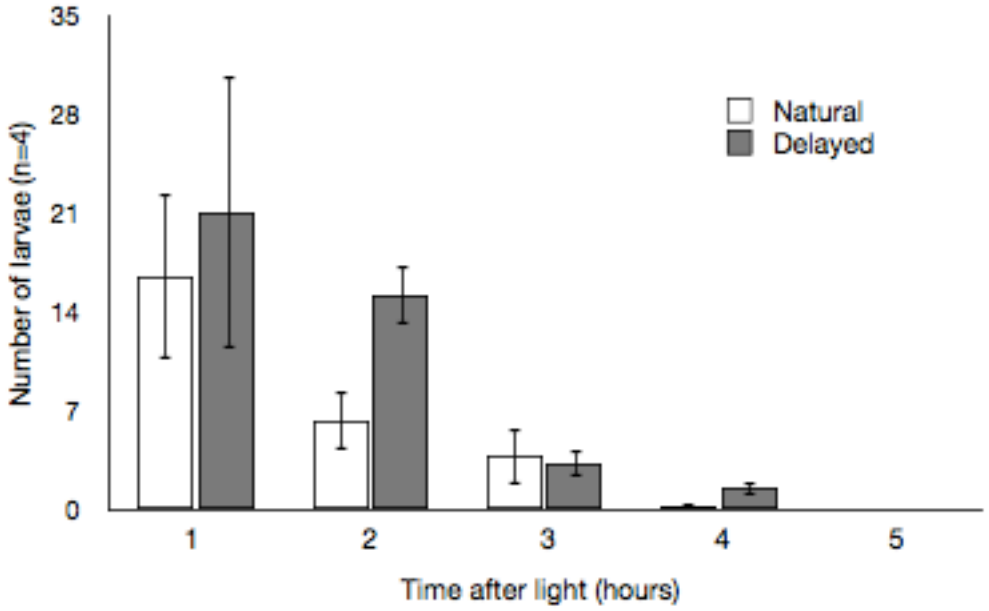

Figure 5. Effect of a 72-hour delayed larval release cue on the hourly number of *S. japonica* larvae released (n=4 ± SE. 'Natural' represents larvae obtained from parent colonies that collected at sundown and exposed to light the following sunrise, 12 hours later. 'Delayed' represents larvae obtained from parent colonies collected at sundown that were kept in a darkened flow-through and then exposed to light 72 hours later. Settlement was analyzed after 24 hours for each treatment.

On average, 19.2 ± 3.7% of larvae released from the natural light treatment settled, and 17.2 ± 3.0% (1 s.e.) of larvae released from the delayed light treatment settled (Figure 6). We observed no significant differences in percent settlement between natural and delayed light treatments [data arcsin transformed, ANOVA, $F(1,20)=0.101$, $p=0.754$]. These results indicate that the same percentage of larvae will settle that are released from the natural and delayed treatments.

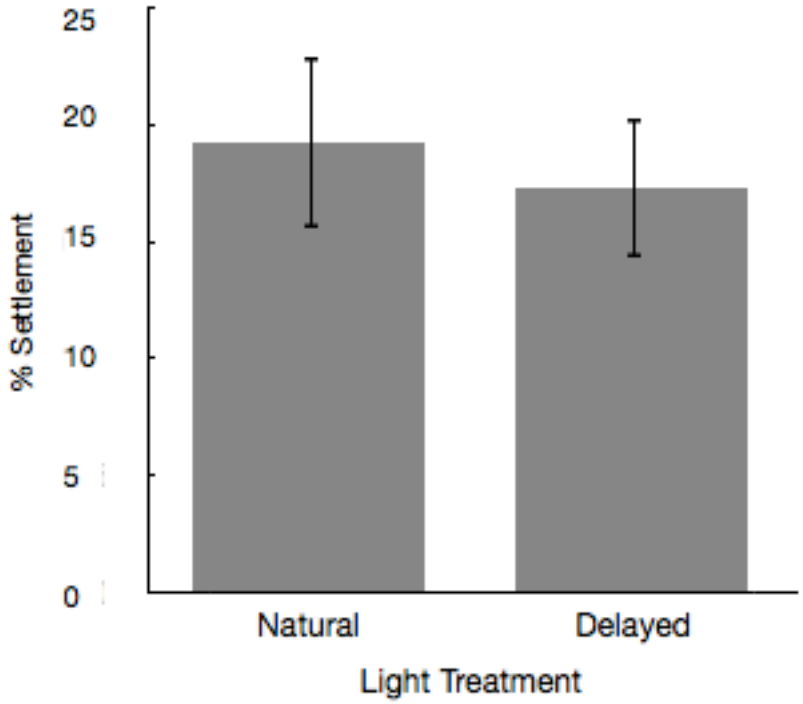

Figure 6. Effect of a 72-hour delayed larval release cue on the percent settlement of *S. japonica* (n= 16 - 20 ± SE). 'Natural' represents larvae obtained from parent colonies that collected at sundown and exposed to light the following sunrise, 12 hours later. 'Delayed' represents larvae obtained from parent colonies collected at sundown that were kept in a darkened flow-through and then exposed to light 72 hours later. Settlement was analyzed after 24 hours for each treatment.

Long-term settlement dynamics

After a long term monitoring of 19 larvae in individual dishes over a period of 13 days, 33% of the 19 larvae had settled, and we observed no additional settlement after the

first 24 hours of the study (Figure 7). After 24 hours, 42% of larvae were still swimming, and throughout the remainder of the study these larvae experienced a decline in motility over time that resulted in mortality. After 13 days, 66% of larvae had suffered mortality apparently caused by unsuccessful settlement attempts, entrapment in surface tension, or simply cessation of ciliary beat.

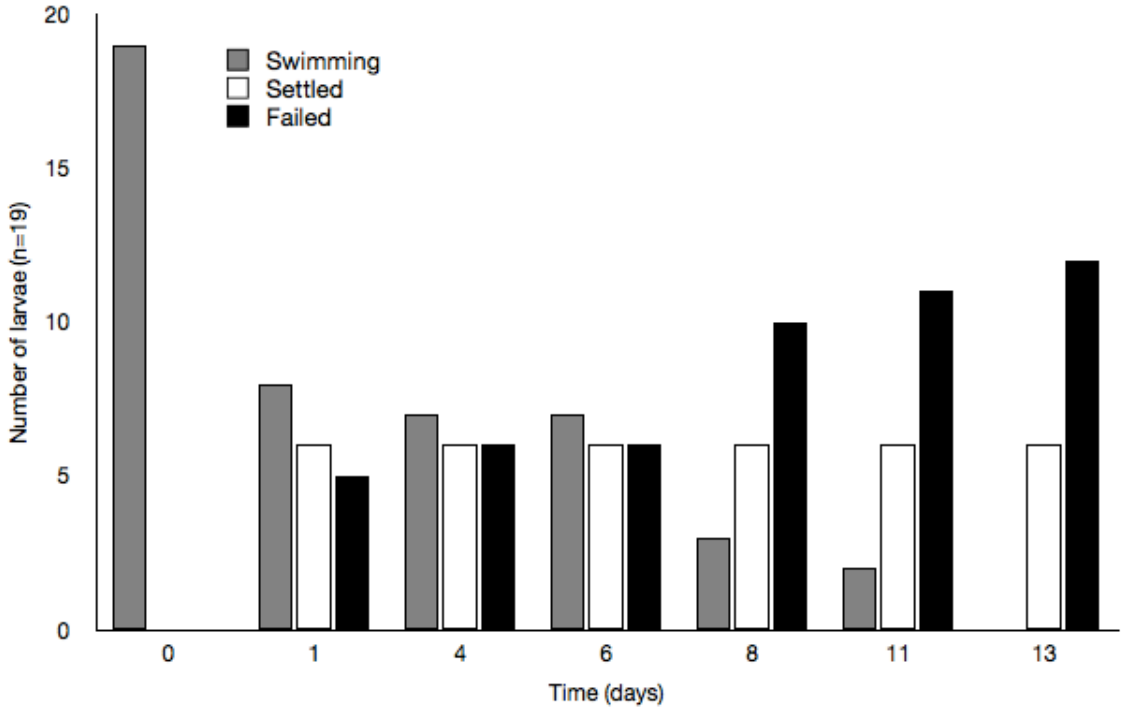

Figure 7. Daily settlement and mortality of larvae of *S. japonica* over 13 days. Larvae were held in dishes at a density of one larvae per dish (n=19). Larval behavior was analyzed with a dissecting microscope to be 'Swimming', 'Settled' or 'Failed' (see Figure 1).

Discussion

In all experiments we observed two distinct behaviors, unidirectional swimming in the water column alternating with cessation in swimming to rest on a surface of the petri dish to sense substratum for a potential settlement site. Ryland (1974) and Burgess et al. (2009) noted similar behaviors. If the substratum is deemed unsuitable for

settlement, larvae either remain on the surface to search the nearby region on a scale of square millimeters, or return to the water column in search of a more suitable settlement surface that may be on the scale of meters away (Ryland 1974; Strathmann 1985; Walters 1992). By ultimately dictating the final metamorphosis site, larval settlement behaviors have implications for the survival of the future colony, as settlement location can affect physical and ecological factors such as food availability, sedimentation, predation, and fecundity of the future colony. Larval settlement behaviors also affect how the population is structured by influencing both the distribution and abundance of individuals (Keough & Downes 1982; Raimondi & Keough 1990; McKinney & McKinney 1993).

The microtopography of the substratum can affect the settlement response of bryozoan larvae, as some larvae prefer smooth surfaces and others prefer surfaces with pits or bumps (Ryland 1974; Walters 1992). When larvae were introduced to petri dishes with varied settlement surfaces, slightly higher settlement was observed in treatments with sanded surfaces and biofilm than on treatments with grooved surfaces and unmanipulated polystyrene surfaces (Figure 2). The presence of a microbial biofilm has been shown to increase settlement of numerous sessile invertebrates including serpulid worms, oysters, barnacles and bryozoans (Meadows & Campbell 1972; Hadfield & Paul 2001; Crisp 1974). When held in clean glass dishes, the encrusting cheilostome bryozoan *Watersipora arcuata* has low settlement, as most larvae continue swimming until they die, compared to the high settlement obtained in dishes with a microbial film (Wisely 1958). We observed a trend of higher settlement within the depressions of the grooved treatment (Figure 2), a preference that is also observed in colonies of *Hippothoa spp., Alconydium hirsutum* (Ryland 1974), *Celleporaria brunnea* and *Bugula neritina* (Walters

1992). These settlement preferences can provide the ancestrula a stronger attachment to the substratum and protection from erosive forces, or perhaps a refuge from predation that may occur in the wild (Keough & Downes 1982; Walters 1992). Comparable laboratory studies of settlement in other cheilostomes result in 90% settlement with *B. neritina* (Keough 1984), and 95% settlement of *Bugula stolonifera* (Woollacott et al. 1989). However we observed notably lower percent settlement, more comparable to the 38% settlement of *Watersipora cucullata* obtained by Wisely (1958). After 96 hours approximately half of the larvae of *S. japonica* in each of the petri dishes had settled successfully (Figure 3). Larvae of *Hippothoa hyalina* and *B. neritina* have been shown to settle successfully on unfilmed surfaces (Ryland 1974), and the bryozoans *Flustrllidra hispida* (Hamer & Walker 2001) and *Bugula flabellata* (Crisp & Ryland 1960) showed a distinct preference for unfilmed surfaces over filmed surfaces. Alternatively, biofilm age may influence settlement, as Turner and Todd (1993), suggested that four weeks of submersion in sea water was necessary to accumulate the appropriate biofilm complexity required for higher percentages of settlement for bryozoans in the field. It is also possible that the structure of the petri dish did not provide larvae with a preferred substratum orientation, as a strong geonegative settlement behavior was observed in *Schizoporella unicornis*, where over 90% of larvae settled on the undersides of a submerged settlement surface as opposed to other surface orientations (McKinney & McKinney 2002). An alternate explanation for our low settlement success is most likely some difference between lab and field conditions. Perhaps larvae are missing a settlement cue or environmental variable that is present in the organism's native habitat of Japan, but lacking in Charleston, OR. Larvae may also compromise their ability to metamorphose as

they expend limited energy reserves while swimming and searching for substratum (Pechenik et al. 1998). Depletion of energy reserves in cyprid larvae of the barnacle *Balanus balanoides* is correlated with a loss in the ability to metamorphose (Lucas et al. 1979; Miron et al. 2000).

After collection we held colonies for 72 hours in complete darkness. We investigated the effects of the timing of larval release on settlement to determine if our collection methods were affecting settlement. During this extended darkness period it is possible that some larvae had become competent to release, however were delayed because they never received the cue of daylight. It is not known whether the parent colony, larva, or both organisms receive the cue and stimulate larval release. Remaining in the ovicell could potentially age larvae, altering behavior and impeding settlement (Burgess et al. 2009). We found no significant differences between the number of larvae released (Figure 4, 5) and settlement (Figure 6) in the natural light treatment and the delayed light treatment, indicating that our method of collecting larvae did not affect the number released or the settlement success. Other studies artificially age lecithotrophic bryozoan larvae by preventing them from settling by agitating the water (Woollacott et al.1989), rotating containers (Burgess et al. 2009), or exposing larvae to bright light (Burgess et al. 2009), to investigate the changes in settlement and behavior of larvae as they age. This is the first documented study to investigate the effects of a delay in larval release on settlement.

To determine the fate of larvae that do not settle in the first 24 hours, we monitored individuals over a two-week period (Figure 7). In the first 24 hours only 33% of larvae had settled and 42% of larvae remained swimming. These larvae gradually lost

mobility over the two week period, showing evidence of a decline in physiological condition (Burgess et al. 2009) and eventually suffered mortality. These behaviors observed in *S. japonica* larvae over 24 hours old seem to match behaviors observed in laboratory experiments of experimentally manipulated delayed metamorphosis of *B. stolonifera* (Woollacott et al. 1989). We observed lower settlement percentages in this experiment in particular, compared to our other settlement experiments. By isolating individual larvae in petri dishes we removed any possibility of gregarious settlement, a behavior that has been observed in *Schizoporella unicornis* (Hurlbut 1991) and other cheilostome bryozoans (McKinney & McKinney 1993). Larvae of *W. acruata* exhibit gregarious settlement (Wisely 1958), and in certain *Bugula* species, gregarious settlement appears to be dependent upon surface material, colony relatedness, and larval age (Mihm et al. 1981; Keough 1984). *B. neritina* larvae were much more likely to settle gregariously with sibling larvae than non-related conspecifics (Keough 1984).

This study describes the pre-metamorphic behavior of *S. japonica* larvae in the laboratory as larvae encounter potential substrata for settlement, with the most significant result being the observed loss of metamorphic capability in all larvae older than 24 hours. Additionally, it gives the first evidence that settlement is not affected when larval release is delayed by prolonging the time to exposure of the maternal colony to the release stimulus, light. Knowledge about the behavior of the larvae of *S. japonica* in the field will help to better estimate the dispersal of the species and may help to explain variations in recruitment of *S. japonica*, both of which increase understanding of its population structure, distribution and survival.

Bridge I

Chapter II described the settlement dynamics of *Schizoporella japonica* in the laboratory, including observations of pre-metamorphic behavior and the measurement of settlement success based on varying surface textures. Additionally, Chapter II investigated the effect of a delay of larval release on settlement success as well as the fate of larvae that do not settle within the first 24-hours of release. The experiences of non-feeding larvae have a significant effect on post-metamorphic colony success. Factors such as maternal colony condition and pre-metamorphic larval behaviors can influence larval and subsequent colony size. Chapter III explores such carry-over effects by describing the relationship between larval size of *S. japonica* and early colony growth in the field.

CHAPTER III

LARVAL SIZE EFFECTS ON COLONY GROWTH IN THE MARINE BRYOZOAN *SCHIZOPORELLA JAPONICA*

Introduction

Parents of marine invertebrates have a limited amount of energy with which to provision their offspring, and therefore must face a tradeoff between the amount of energy invested into each offspring and the number they produce (Smith and Fretwell, 1974). Greater parental investment has been shown to positively affect the survival and growth rates of offspring (Vance, 1973; Smith and Fretwell, 1974; Marshall and Keough, 2007; Jacobs and Sherrard, 2010). For marine invertebrates with lecithotrophic larvae, parents must provide the larva with the energy reserves that it will need to swim, disperse, and search for an appropriate settlement site, metamorphose from larva to adult, and begin early feeding and growth (Wendt, 2000). By experimentally manipulating maternally invested lipid reserves in lecithotrophic sea urchin larvae, Emlet and Hough-Guldberg (Emlet and Hoegh-Guldberg, 1997) found that most of energy reserves were not needed during the larval phase and were utilized post-metamorphosis. The amount of energy reserves in lecithotrophic larvae is directly correlated to the size of the egg, and as a result, larval size is a good predictor of energy content (Jacobs and Sherrard, 2010). Juvenile size is often correlated with larval size because juveniles retain a large portion of the energy reserves from the larva (Jacobs and Sherrard, 2010).

Intraspecific variation in larval size is described for many marine invertebrates (George, 1994; Jones et al., 1996; Moran and Emlet, 2001; Marshall et al., 2006;

Marshall and Keough, 2008; Burgess et al., 2009). In several species of the bryozoan genus *Bugula,* larval size has been correlated with energy content (Wendt, 2000), and in the species *Bugula neritina*, larval size was correlated with colony performance after settlement (Marshall et al., 2003; Marshall and Keough, 2004a), suggesting that larval size may be an important predictor of larval quality (Marshall and Keough, 2004a). Larger larvae appear to have specific advantages when compared to smaller larvae, such as increased performance in both the larval phase and the subsequent post-metamorphic phase (Pechenik et al., 1998). When compared to smaller larvae, larger larvae have greater energy reserves and are able to swim for longer lengths of time resulting in greater dispersal (Marshall and Keough, 2003a; Kosman and Pernet, 2009) and are able to delay settlement and retain metamorphic competence longer in the absence of appropriate settlement substratum, resulting in higher survival (Wendt, 2000; Marshall and Keough, 2003a). After metamorphosis, ancestrulae from larger larvae have greater energy reserves and a greater capacity to grow and feed than ancestrulae from smaller larvae, resulting in colonies that are more resistant to mortality and predation (Marshall et al., 2003; Marshall and Keough, 2003b).

Larger larvae do not always have an advantage, however, as larger larvae are able to swim for longer periods of time. The more time a lecithotrophic larva spends swimming, the more it can deplete valuable post-metamorphic energy reserves and as a result may suffer costs in later life stages (Lucas et al., 1979; Woollacott et al., 1989; Wendt, 1996; Pechenik et al., 1998). In the bryozoan *Bugula neritina,* prolonged larval swimming resulted in reduced growth and fecundity in the future colony (Wendt, 1998). Additionally, after metamorphosing, larger bryozoan ancestrulae are more at risk of

predation from visual predators as they are seen more easily than smaller settlers (Pechenik, 1999).

Marine invertebrate larvae vary in size within and among populations (Jones et al., 1996; Marshall et al., 2000) as a result of maternal size (Sakai and Harada, 2001; Marshall et al., 2003) and maternal nutritional, thermal or salinity stress (Bayne et al., 1978; Marshall and Keough, 2008), habitat quality (George et al., 1990), and competition and predation (Marshall and Keough, 2004b). Variation in larval size can be an adaptive response of parental colonies to varying environmental conditions such as stress or competition (Marshall and Keough, 2004a). Bryozoan colonies that have been damaged or stressed physiologically sacrifice larval quality for improvements in parental colony fitness by producing smaller larvae (Marshall and Keough, 2004a), and colonies experiencing competition presumably produce larger larvae than colonies that are not experiencing competition, to encourage dispersal of larvae (Allen et al., 2008). Raimondi and Keough (1990) propose that the production of larvae that range in size (and therefore range in dispersal potential) is a bet-hedging strategy.

Marine bryozoans serve as excellent model systems for studying the link between offspring size and post-metamorphic fitness, because lecithotrophic larvae are easily obtained in large quantities that are similar in age (Wendt, 2000), have short pelagic larval durations on the scale of hours (McCain, 1972), and metamorphose relatively quickly (Woollacott and Zimmer, 1971). For the bryozoans *Bugula neritina, Watersipora subtorquata,* and *Celleporaria* spp., offspring size has been shown to affect larval and post-metamorphic performance (Marshall et al., 2003; Marshall and Keough, 2004a; 2008; Dias and Marshall, 2010). We investigated larval size effects on early colony

growth of the bryozoan *Schizoporella japonica* Ortmann, 1890, as it is abundant in Charleston, OR and produces and releases larvae year-round (McCain 1972, Treibergs pers. obs.). The genus *Schizoporella* Hinks, 1877, is found in marine environments worldwide (Hayward and McKinney, 2002; Tompsett et al., 2009), and has been used in studies of marine invasions (Relini et al., 1998; Koçak, 2008), substratum competition (Turner and Todd, 1993), and larval settlement behavior (Hurlbut, 1991; McKinney and McKinney, 2002). Tompsett et al. (2009) note that species are often misidentified in ecological literature due to subtle differences in morphology and often scanning electron microscopy (SEM) is required for species identification. The most recently accepted conclusion indicates that *Schizoporella* specimens from the Pacific northwest region of North America are most likely *Schizoporella japonica* (Dick et al., 2005; Tompsett et al., 2009).

Carry-over effects from the larval experience affect the size, settlement location and survival of populations of many adult marine organisms (Pechenik et al., 1998), as approximately 34 phyla out of the 40 marine metazoans possess some kind of distinct, free-living larval life history phase (Strathmann, 1985; Pechenik, 1999) Larval size, in particular, influences the dispersal, recruitment, survival and resilience of adult organisms (Pechenik et al., 1998; Marshall et al., 2003; Marshall and Keough, 2003b; 2004a; Marshall et al., 2006; Burgess et al., 2009). Research into the linkage between the larval experience and post-metamorphic success is essential for a more thorough understanding of the constraints affecting growth and development in marine organisms with complex life histories.

Materials and Methods

Larval size effects

Larvae of *S. japonica* have been collected as described above (see Chapter 2). To test the effects of larval size on settlement success and colony growth, we collected 51 larvae of *S. japonica* from 30 adult bryozoan colonies with the methods described above. It is difficult to obtain accurate measurements of larvae, as swimming larvae of *S. japonica* expand and contract their body shape and frequently change orientation during swimming. To obtain the most accurate size measurements possible without damaging or over-handling larvae, we filmed each larva in a drop slide under a dissecting microscope for 30 seconds with a mounted video camera. We extracted three still images from each 30-second video where larvae were in a contracted position, circular in shape, and oriented with two red eye spots centered and facing upwards. To approximate larval size, we calculated two-dimensional larval area from each image by using ImageJ (Abramoff et al. 2004), and averaged the area from three stills. Each larva was then introduced into a 4.5 cm petri dish that was roughened with sandpaper and allowed to accumulate biofilm for 48 hours in a sea table prior to use. Petri dishes with larvae were maintained in darkness at ambient seawater temperature and monitored for settlement after 24 and 48 hours with a dissecting microscope. As a measure of ancestrular size, we photographed the ancestrulae of all successfully settled larvae 24 and 48 hours after settlement, and calculated two-dimensional area of ancestrulae with ImageJ. To ensure that ancestrulae did not grow after settlement, we compared the size of ancestrulae 24 and 48 hours after settlement and found no difference.

Colony growth

To test for the effects of larval size on colony survival and growth of *S. japonica*, we transplanted the previously measured ancestrulae into the field and measured their growth over three months, from March 18, 2011 to June 27, 2011. Each dish containing a single ancestrula was affixed with Industrial Strength Velcro® to a 40cm x 60cm backing plate and deployed facing downwards in the inner boat basin of Charleston, OR. Plates were suspended from floating docks at a depth of 50 cm below the surface of the water. Every three to six weeks for three months we transported plates in containers of seawater to the laboratory. In the laboratory, we photographed each colony under a dissecting microscope and calculated colony area with ImageJ. By comparing individual colony area over time we calculated growth rates. Backing plates with colonies were removed from the field for each analysis for no longer than a total of three hours, during which they were maintained in flow-through aquaria.

Statistical analysis

To determine the relationship between larval size and ancestrula size, we calculated the correlation between larval area and ancestrula area with a type II linear regression analysis (Geometric mean regression), which accounts for measurement error in both variables. To determine the relationship between colony size and ancestrula size we performed a series of geometric mean regression analyses between ancestrula size and colony size at 24, 42, 74 and 102 days. Because of the significant positive correlation between larval area and ancestrula area, we used ancestrula area as a proxy for larval area in these regressions.

Results

Larval size, measured as two-dimensional area, was normally distributed (Shapiro-Wilk w=0.973, p<0.720). Larval area in the sample of 51 larvae ranged from 0.0283 to 0.0470 mm^2 with a mean of 0.0366 $\pm$ 0.0005 mm^2 (1 s.e, Figure 8).

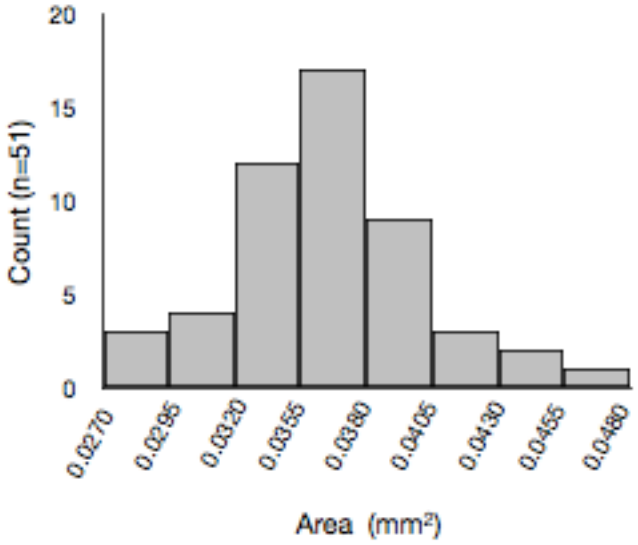

Figure 8. Size distribution of *S. japonica* larvae.

Of the 51 larvae measured, 21 settled successfully within 24 hours of being introduced to petri dishes. Larval size did not differ significantly (t(50)=2.01, p=0.758) among those larvae that settled, with a mean of 0.0368$\pm$0.008 mm^2 (1 s.e., n=21), and those that did not settle, with a mean of 0.036$\pm$0.007 mm^2 (1 s.e., n=30). Ancestrula size was not normally distributed (Shapiro-Wilk w=0.877, p<0.006), and ranged from 0.0914 to 0.1500 mm^2, with a mean of 0.1132 $\pm$ 0.003 mm^2 (Figure 9).

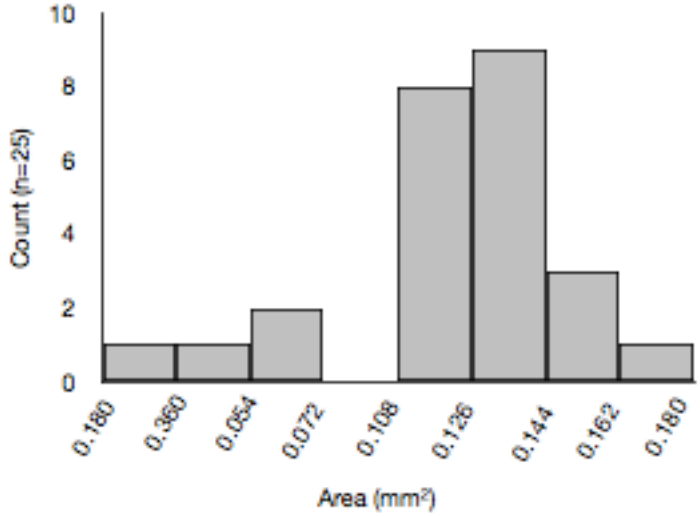

Figure 9. Size distribution of *S. japonica* ancestrulae.

Larval area was on average 0.0785 mm^2 less than ancestrula area ($t(19)=-27.4$, $p<0.0001$, Figure 10a). After settlement, colonies grew exponentially over three months, to an average of 503.1$\pm$ 30 mm^2 (1 s.e.), 102 days after settlement (Figure 10b). During laboratory analysis of plates, we observed no evidence of predation or colony mortality. Larval area was a successful predictor of ancestrula area and explained a significant proportion of variance in ancestrula area (Figure 11a, Table 1) and ancestrula size was a successful predictor of colony area at day 24 and day 42, but not on day 74 or 102 (Figure 11b-c, Table 1).

Discussion

Like many marine invertebrates, the larvae and settlers of *Schizoporella japonica* ranged greatly in size (George, 1994; Jones et al., 1996; Moran and Emlet, 2001; Marshall et al., 2006; Marshall and Keough, 2008; Burgess et al., 2009 Figure 8,9)). Results of this study however suggest that larger larvae of *S. japonica* do not

metamorphose more successfully than smaller larvae. When we compared the size of larvae that metamorphosed successfully to the size of larvae that did not metamorphose, we found no difference, indicating that in *S. japonica,* larval size is not related to settlement success in the laboratory.

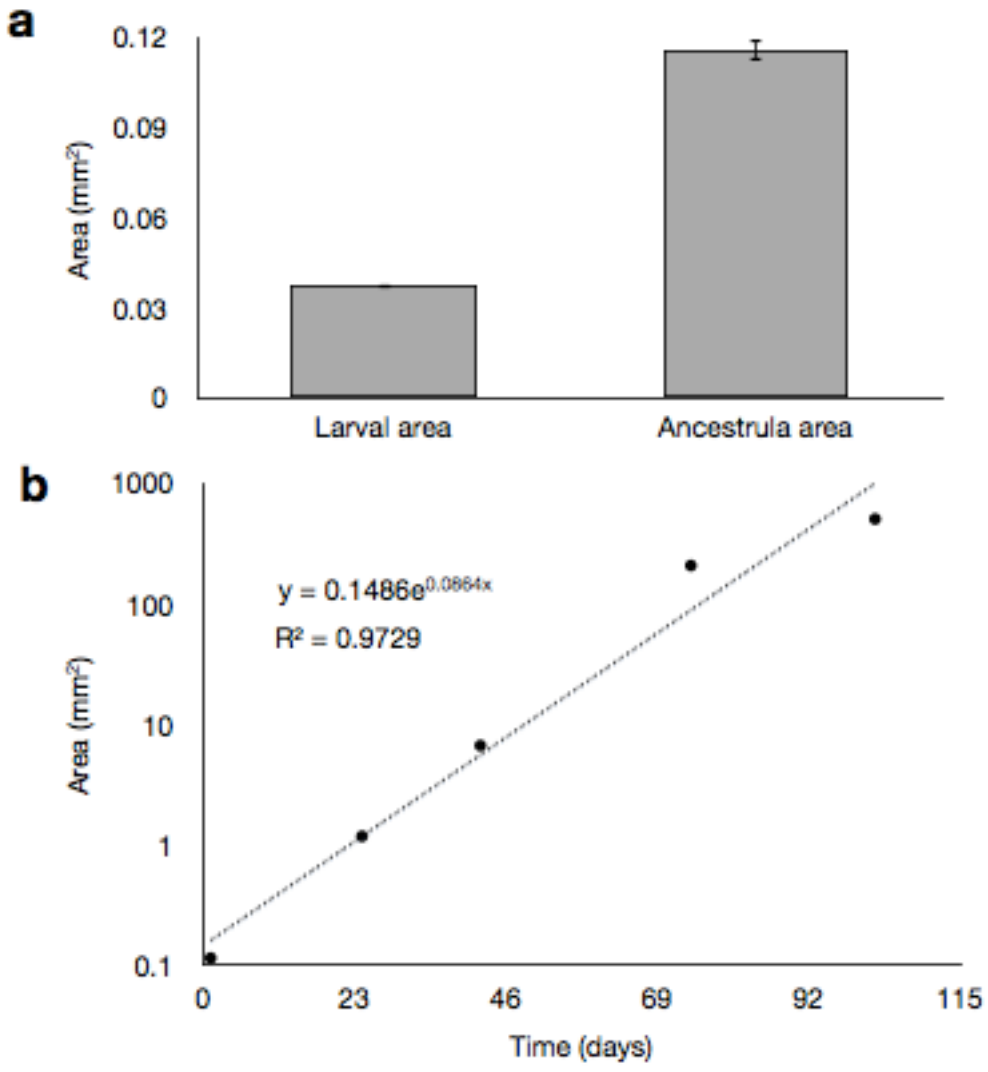

Figure 10. Size of larvae and ancestrulae of *S. japonica (a)*, and exponential colony growth over 102 days (b).

Table 1. Predictors of colony size.

	ß	t	df	R^2	P
Larval area	6.455	3.73	20	0.376	0.001
24-day colony	28.32	5.05	17	0.6140	< 0.001
42-day colony	166.7	3.68	17	0.458	0.002
74-day colony		1.62	17	0.375	0.126
102-day colony		1.82	17	0.414	0.088

We found that larval area is a good estimator of ancestrula area for *S. japonica* (r^2=0.376, Table 1, Figure 10a) just as Marshall and Keough found for *Watersipora subtorquata* (r^2=0.78) (Marshall and Keough, 2003a). Measuring ancestrula size rather than larval size is more accurate and saves time (pers. obs.), therefore this technique will maximize the efficiency of future studies of larval size of *S. japonica*. The strong positive effects of larval size on colony growth continued through 42 days (r^2=0.614, Table 1, Figure 10b,c), but not after 74 days (r^2=0.38, Table 1). Similarly, Marshall et al. (2003) found that colonies of the bryozoan *Bugula neritina* that metamorphosed from larger larvae had higher survival and faster growth rates than colonies that metamorphosed from smaller larvae, and that these effects persisted for up to six weeks in the field. Our study is yet another contribution to Dias and Marshall's "growing list of studies that show that offspring size influences performance across the metamorphic boundary in marine invertebrates with complex life-cycles" (Emlet and Hoegh-Guldberg, 1997; Moran and Emlet, 2001; Marshall et al., 2003; Dias and Marshall, 2010).

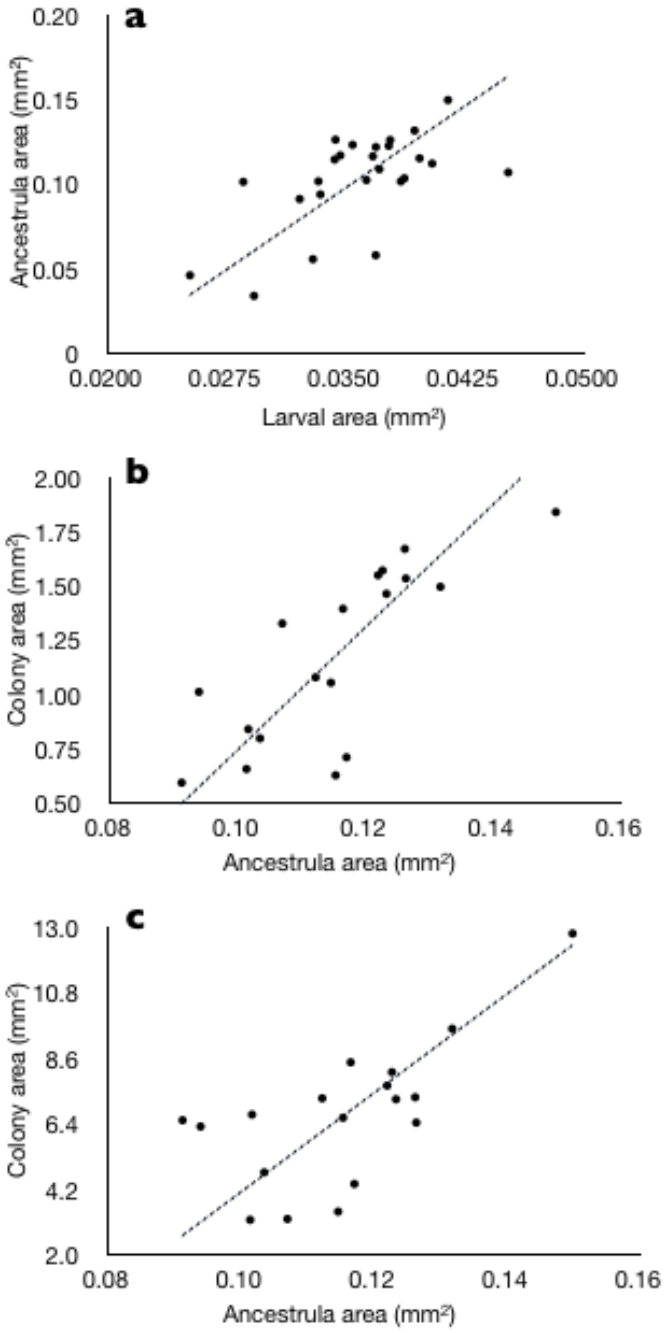

Figure 11. Relationship between larval area and ancestrula area (a) of *S. japonica,* and ancestrula area and colony area at 24 days (b) and at 42 days (c). Regression for larval area and ancestrula area (a): Ancestrula area= 6.455 Larval area + 0.0815, $p<0.001$, n=21. Regression for 24 days (b): Colony area = 28.32 Ancestrula area – 1.089, $p<0.001$, n=17 Regression for 42 days (c): Colony area = 246.23 Ancestrula area – 21.78, $p<0.002$, n=17.

In our study, there remained a positive correlation between settler size and colony size for the first 6 weeks, however the relationship was no longer statistically significant at 74 days (Figure 10). Interestingly, the strength of the relationship (as interpreted to be the slope of the regression) increased over time, similar to the results obtained by Dias and Marshall (2009) with the encrusting bryozoan *Celleporaria* sp. As suggested by Dias and Marshall (Dias and Marshall, 2010), the strengthening of the relationship between settler size and colony size over time may be a result of the allometric growth of the colony in an environment without competition for space and predation (Sebens, 1987).

We transplanted colonies into the field to simulate as realistic environmental conditions as possible (Marshall and Keough, 2004a), where the newly settled ancestrulae of *S. japonica* grew exponentially, unimpeded on the flat artificial substratum of a petri dish for a total of three months. Competition for space is a common characteristic of marine fouling communities (Richmond and Seed, 1991), and in the field, *S. japonica* must settle within an already established fouling community. Within the Charleston, OR fouling community, colonies of *S. japonica* are commonly found growing on the shells of mussels, *Mytilus* spp., which line the sides of floating docks (Treibergs pers. obs.). Such colonies are often in direct competition for space on mussel shells with other invertebrates, including colonial ascidians, other encrusting bryozoan colonies (including conspecifics), sponges, and barnacles (Treibergs pers. obs). Competition and predation pressures from these neighboring organisms may significantly decrease colony growth rates (Sebens, 1987). In the fouling community of Charleston, OR, interspecific and intraspecific competition for space likely has significant negative effects on adult

colony growth of *S. japonica* (Marshall and Keough, 2004a). Marshall and Keough (Marshall and Keough, 2004a) found that settlers of the bryozoan *Watersipora subtorquata* growing unimpeded on settlement plates had much faster growth rates than those transplanted into established fouling communities on small (9 mm diameter) plugs. By providing the ancestrulae in our study with cleared surfaces on which to grow, we may have overestimated average colony growth rates of naturally settled *S. japonica*. Larval size effects are more persistent in adult colonies that do not experience competition or predation when compared to colonies experiencing either pressure (Marshall et al., 2003).

Only in the past 15 years have scientists begun to consider the 'carryover effect' of the larval experience on adult marine invertebrates (Pechenik et al., 1998). The conclusions drawn from this study have implications for understanding how aspects of the larval life stage affect the lives of adult invertebrates, because larval size influences the dispersal, recruitment, survival and resilience to competition and predation of adult colonies (Pechenik et al., 1998; Marshall et al., 2003; Marshall and Keough, 2003a; 2004a; Marshall et al., 2006; Burgess et al., 2009). Additionally, larval size is often an important determinant of post-metamorphic success in many marine invertebrates (Moran and Emlet, 2001; Marshall et al., 2003; Marshall, 2005; Marshall et al., 2006; Allen et al., 2008; Marshall and Keough, 2008). The strong influence of larval size on early colony growth in this study serves as another example of the linkage between life stages in marine invertebrates, emphasizing the importance of studying the integration across all life stages of an organism rather than studying a single life stage in isolation.

Bridge II

Chapter III described the relationship between larval size and early colony growth of *Schizoporella japonica* in the field, focusing on carry-over effects of larval size on early colony performance. Chapter IV continues with the exploration of the field growth of *S. japonica* along with that of other fouling community invertebrates in the South Slough estuary, Charleston, OR. We monitored fouling community development on settlement plates at sites deployed along the estuarine gradient for a total of one year, paying special attention to the presence of invasive species. Additionally, we outplanted two common fouling community invertebrates at three different life stages to investigate the effect of life stage and salinity on survival.

CHAPTER IV

EVALUATING THE PRESENCE AND PHYSICAL TOLERANCES OF INVASIVE FOULING ORGANISMS IN SOUTH SLOUGH, CHARLESTON, OR

Introduction

Non-indigenous species are widespread in coastal and estuarine waters throughout the world and pose real or potential threats to native species, ecosystems, and business activities (Lotze et al., 2006; Ray, 2005). Of all aquatic environments, estuaries are the most vulnerable to species introductions because of their relatively low diversity compared to both riverine and marine ecosystems, which allows room for the establishment of introduced species (Nehring, 2006). In addition, estuaries are often areas of high international traffic because they serve as shipping ports (Nehring, 2006, Hewitt, 1993). Over centuries, as a result of this vulnerability, numerous non-indigenous species have become established members of estuarine communities (Ray, 2005). The international shipping industry began in Coos Bay, OR in the 1850's and has been responsible for introductions of organisms on hulls and in discharged ballast waters of ships (Carlton and Geller, 1993, Hewitt, 1993). In addition, oysters have been cultivated in the South Slough estuary since the 1920's, and many fouling organisms were introduced into the estuary from the shells of Japanese oysters, *Crasostrea gigas* (Rumrill, 2007).

South Slough estuary (43°20’ N, 124°19’ W) is the southernmost subunit of Coos Bay (DeRivera et al., 2009), the second largest estuary in Oregon (Roegner and Shanks, 2001). South Slough contains South Slough National Estuarine Research Reserve (Cowlishaw, 2004), and one of 28 national estuarine protected areas reserved specifically for research, water-quality monitoring, education and coastal stewardship (http://www.nerrs.noaa.gov/). The estuary itself is shallow and characterized by vast mudflats (Rumrill, 2007). South Slough is unique, because unlike many estuaries where local phytoplankton is produced in abundance, South Slough has low local phytoplankton production, and instead oceanic phytoplankton is produced offshore and is advected into the Slough via tidal currents (Roegner and Shanks, 2001).

Studies of fouling communities and non-indigenous species in Coos Bay and South Slough include a dissertation by Hewitt (1993), surveys by J.T. Carlton, colleagues and students (initiated in 1977 and conducted approximately biennially since 1995 (Carlton 2007), and most recently a 2003-2004 deployment for 100 days of ten fouling plates at each of fourteen sites in Coos Bay and South Slough (deRivera et al., 2005). In this study we measured monthly, seasonal, and yearly patterns of recruitment to fouling plates deployed along the salinity gradient of the South Slough estuary for one year. Additionally, we conducted field transplant experiments of thee life stages of two common non-indigenous species to study sites along a salinity gradient. *Schizoporella japonica* and *Botrylloides violaceus* are very abundant fouling community invertebrates found on floating docks in the inner boat basin of Charleston Harbor and have been in lower Coos Bay at least since 1986 and probably much earlier (Carlton 2007). Our goal was to determine the range and tolerances of these two fouling species, as well as to

identify life stages that are particularly resilient to environmental changes (such as salinity), and what life stages are particularly sensitive to environmental changes.

It is important to learn about the recruitment patterns and physiological tolerances of non-indigenous organisms in order to predict where they may be further spread. The results of this study will help to reflect on the current state of invasive organisms within and nearby the South Slough National Estuarine Research Reserve and will provide data that will help predict the future impact of invasive species on all estuaries.

Materials and Methods

Study sites and apparatus

We conducted settlement plate studies from January 2011 through December 2011 at six sites in South Slough estuary of Charleston, Oregon, and outplanted fouling community species to four sites (IBB, DFF, YP and VI) during September 2011 (Figure 12). We selected study sites in regions arranged along a salinity gradient in locations near hard substrata as evidenced from personal observations and previous data from studies of invasive species in Coos Bay (Hewitt 1993, deRivera et al. 2005, Carlton 2007) and a site profile of the slough (Rumrill 2006).

Sites (listed in order from oceanic to riverine) included the Charleston harbor inner boat basin (IBB), the distant fleet facility (DFF), Yonker's Point (YP), Valino Island (VI), Long Island Point (LIP) and Hidden Creek Marsh (HCM). For the duration of the study we had access to water quality data from South Slough's system wide monitoring program (SWMP) which has data sondes at the DFF, VI and LIP sites.

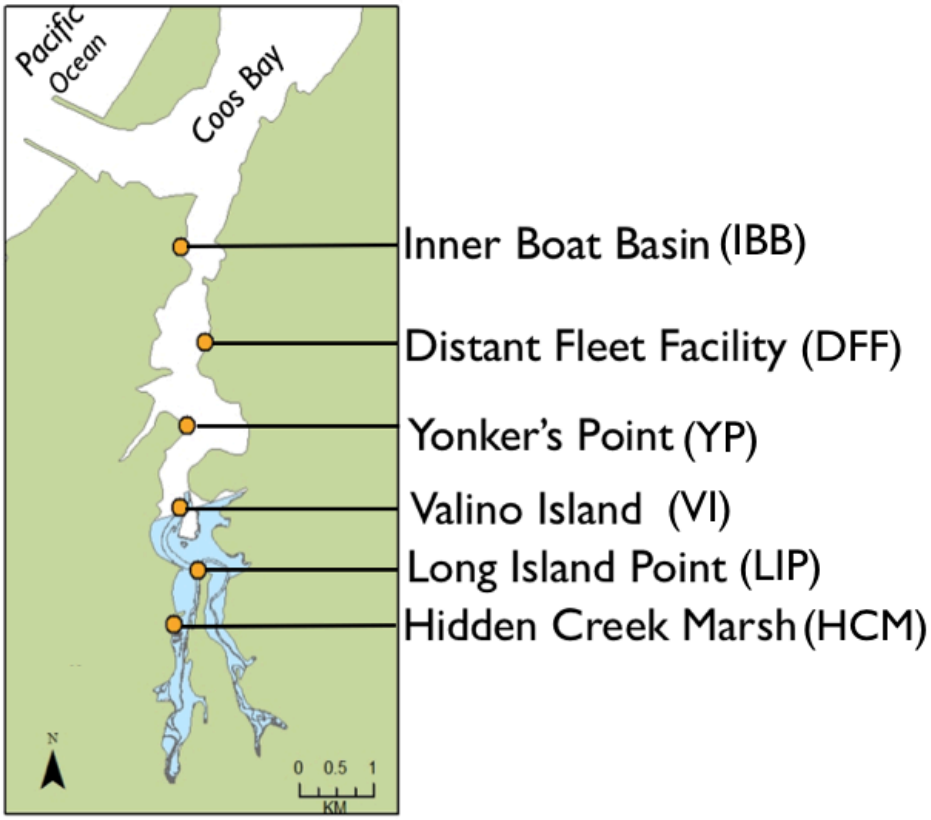

Figure 12. Study area showing the position of six study sites in South Slough, Charleston, OR. Shaded region indicates the boundary of the National Estuarine Research Reserve.

Salinity, temperature, dissolved oxygen, pH, turbidity, and chlorophyll data were recorded continuously at these stations in 15 minute intervals. We obtained wind speed, photosynthetic radiation and precipitation data for Charleston, OR from the nearby Charleston Meteorological Station, which was also collected continuously at 15 minute intervals.

At each site, we deployed a 50 lb cylindrical concrete mooring with a 50 cm length of 9.5 mm rebar oriented vertically and inserted into the center (Figure 13). Prior to being embedded in the concrete, the rebar was coated with a marine sealant to prevent rusting. For the year-long settlement plate study, two (20 x 20 cm^2) backing plates were arranged along the rebar, separated by 4 cm long PVC spacers, and two additional

backing plates were added for the species outplant study during September. Sets of five, 5 cm diameter petri dishes were used as settlement surfaces, randomly assigned to backing plates and attached with Industrial Strength Velcro®. Backing plates were oriented horizontally and petri dishes were attached to the underside of these plates and faced downward to decrease sedimentation. At the top of the rebar rested a T-shaped PVC pipe with two 8 x 8 cm slate plates affixed with stainless steel bolts to the underside of each end. This apparatus rotated with incoming and outgoing tidal currents to standardize flow across slate plates. To increase directional rotation, one end of the PVC "T" had a plexiglass fin.

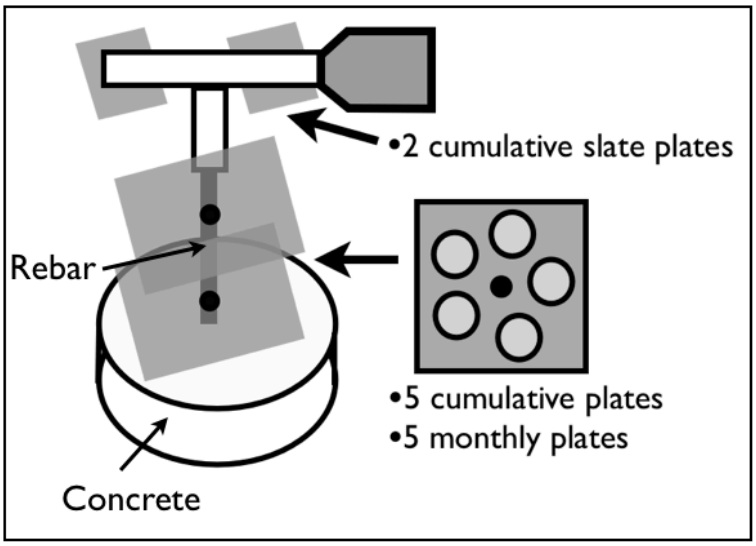

Figure 13. Design of moorings deployed at each study site in South Slough (see Figure 12). The mooring has a 50lb concrete base with backing plates stacked on a vertically oriented piece of rebar . Petri dishes were affixed to random positions on backing plates with velcro. At the top of the apparatus two downward facing slate settlement plates are bolted to a "weather vane" that rotates freely and orients according to flood and ebb currents.

Settlement plates

To ascertain which fouling community organisms settle, survive, and grow on hard substrata in South Slough, we deployed three types of settlement plates: cumulative

slate plates (s), cumulative petri dishes (c), and monthly petri dishes (m) at our six study sites for a total duration of one year from January 2011 - January 2012.

Cumulative slate plates

We monitored the slate plates in the field monthly, calculating abundance and identifying species to the nearest operational taxonomic unit. During these monitoring periods, slate plates were removed from the water and exposed to air for no longer than 15 minutes per site. At the conclusion of the study we collected slate plates from each site and transported them in seawater to the Oregon Institute of Marine Biology laboratory where we photographed plates and completed a more detailed taxonomic analysis under a dissecting microscope, along with calculations of percentage species cover on each plate.

Cumulative petri dishes

At the start of the study, five petri dishes were randomly assigned to backing plates at each site. Monthly, for the duration of the study, plates were examined in the field, and organisms were identified to the nearest operational taxonomic unit. If a petri dish for a site was lost or damaged it was replaced immediately. During the monthly field monitoring process, dishes were submerged in seawater whenever possible to prevent desiccation and were exposed to air for no longer than ten minutes at each site. For a more detailed taxonomic analysis, at the conclusion of the study plates were analyzed in the laboratory with a dissecting microscope and percentage cover of each species was calculated using ImageJ.

Monthly petri dishes

Each month we collected five additional petri dishes from each site, replaced them with clean dishes, and transported them in containers of seawater to the laboratory within three hours of collection. If a petri dish for a site was lost or damaged, it was replaced immediately. Within one hour of returning to the laboratory, organisms were identified under a dissecting microscope. Occasionally plates were kept in flow-through containers in a sea table at ambient temperature and oceanic salinity for a more detailed analysis.

Target organisms

The purpose of this study was to ascertain the state of non-indigenous organisms of fouling communities in South Slough, including the inner boat basin. As a result, we took special care to identify any nonnative invertebrates, specifically the nonnative fouling community species that have previously been reported in South Slough, or Charleston Harbor (Carleton 2007, deRivera 2005), bryozoans *Schizoporella japonica*, *Alcyonidium* sp. *Watersipora subtorquata*, and *Bugula* sp., and ascidians *Didemnum vexillum*, *Molgula* sp., *Botrylloides violaceus* and *Botryllus schlosseri*.

Statistical analysis

To better understand fouling community composition at each site, we performed a principal component analysis (PCA) based on the correlation matrix applied to phylum presence/absence and invasive species presence/absence at all sites as well as phylum percentage cover and invasive species percentage cover on cumulative slate plates.

Organisms were grouped at the phylum level for simplification, as there were too many species present to include each individual species in the analysis. For the PCA we used Statistica 6.0.

The relationships between estuarine physical variables (salinity, temperature, disolved oxygen, chlorophyll, pH, turbidiy) and study site (Site) vs. species richness (R) and presence of target invasive organisms (*Alcyonidium* sp., *Bugula* sp., *Schizoporella japonica*, *Watersipora subtorquata*, *Didemnum vexillum*, *Molgula* sp., *Botrylloides violaceus* and *Botryllus schlosseri*) was analyzed by means of generalized additive models (GAM) as implemented in the mgcv library of R (R Development Core Team 2010). Site (LIP, VI and DFF, the study sites that correspond with the location of water quality sampling stations) was introduced as a factor and environmental variables were introduced as smooth terms and estimated with thin plate regression splines. To select the optimal set of variables for inclusion in the GAM, we used the Akaike information criterion (AIC). Model validation included the verification of homogeneity, normality and independent assumptions (Zurur et al. 2009). The GAM for total species richness was built using a Gaussian distribution, and the GAMs for presence of invasive species were built using a binomial distribution with a logit-link.

Salinity tolerance transplants

To study invasibility along the estuarine axis, we transplanted larvae, recent settlers and non-reproductive subadults of two common non-indigenous encrusting organisms, *Schizoporella japonica* and *Botrylloides violaceus* to four of our study sites in South Slough in September 2011. Due to concerns regarding the spread of nonnative

species, and at the request of the reserve managers, these were transplanted only to regions where both species have been previously documented, thus excluding the two most riverine sites (HCM and LIP) (DeRivera et al. 2005, Rumrill 2006, Treibergs pers. obsv.). Colonies of *S. japonica* are reproductive year round in the Charleston harbor inner boat basin (Treibergs pers. obsv.) and colonies of *B. violaceous* are reproductive in the field from June to October (Yamaguchi, 1975, Epelbaum et al., 2009). Colonies of *S. japonica* do not become reproductive (with visible embryos brooding in ovicells) until they exceed 2 cm in diameter provided they do not become space limited (Treibergs pers. obsv.) and one month old colonies of *B. violaceus* that are not space limited also do not reproduce (Marshall et al. 2006).

Oceanographic variables including salinity, temperature, dissolved oxygen, pH, turbidity and chlorophyll concentration were measured for the month of September at two of the transplant study sites (DFF and VI) as well as at one site upriver, (LIP). Data were recorded continuously at these stations in 15-minute intervals, available for use through South Slough's system wide monitoring program (SWMP).

Larvae

Bryozoan larvae were collected from 75 adult *S. japonica* colonies that were kept in dark aquaria of ambient temperature seawater with minimal aeration. After 48 hours flow and aeration were stopped and colonies were immediately exposed to bright light from five, 100-watt spotlights suspended 30 cm above the tank. Larvae are positively phototactic upon release, and after about 30 minutes of light exposure, hundreds of coronate larvae 250 μm in diameter were collected at the tank surface. Larvae of

B. violaceus were collected from 50 adult colonies that were kept in darkness for 48 hours and then were exposed to ambient daylight during the 30-minute travel time to field sites. At each field site, ascidian colonies in a container were gently torn apart, releasing tadpole larvae approximately one mm in length. Larvae of both species were placed in petri dishes, covered with sealed 150 μm mesh, and submerged in water. Five petri dishes with ten larvae of *S. japonica* each, and three petri dishes with five larvae of *B. violaceus* were randomly assigned positions on backing plates at each site. After 24 hours we collected plates with larvae and brought them back to the laboratory to observe percentage settlement with a dissecting microscope. We chose a 24 hour larval settlement period because in the laboratory, settlement of *S. japonica* and *B. violaceus* occurs within one day, and those who do not settle in the first 24 hours never settle successfully (Treibergs, Chapter 1., Epelbaum et al. 2009). We scanned plates for settlement with a dissecting microscope and compared numbers of settlers between sites for both species with a 1-way ANOVA.

Recent settlers

We collected larvae from *S. japonica* and *B. violaceus* by the methods described above and induced them to settle on acetate sheets, from which we cut out sections and glued three settlers to each petri dish with cyanoacrylate adhesive. We transplanted five dishes with three ancestrulae (the founder zooids for bryozoan colonies) of *S. japonica* each and seven dishes with three oozoids (founder zooids of colonial ascidians) of *B. violaceus* each to each study site.

Adult colonies

At each site we transplanted seven non-reproductive adult colonies of *S. japonica* and *B. violaceus* affixed to petri dishes. We collected adult colonies of *S. japonica* growing on mussel shells less than 4.5 cm long from the Charleston harbor inner boat basin. Mussel shells were attached to petri dishes with cyanoacrylate and left to circulate in an aquarium for five days to recover from any damage inflicted by collection or attachment, as colonies tended to damage easily when handled (Treibergs pers. obsv.). We photographed each colony one day before field deployment on 9/1/11 and calculated colony area using image analysis software (ImageJ). We removed flat-growing adult colonies of *B. violaceus* that were less than four cm in diameter from inner boat basin hard substrata and secured colonies to petri dishes with a drop of cyanoacrylate glue (Epelbaum, 2009). Dishes were kept in circulating seawater aquaria with aeration for 48 hours when colonies had grown completely attached to the substratum. We photographed colonies of *B. violaceus* on 9/1/11, the day of deployment, and calculated colony area using ImageJ.

When we performed the transplant experiment of settlers in September 2011 we had difficulty in obtaining the required number of larvae of *B. violaceus* needed for all four study sites, and transplanted ascidian larvae to only two of the four sites, the Charleston Inner Boat Basin (IBB) and to Valino Island (VI). Transplanted colonies and newly settled zooids were deployed on 9/1/11 for one month, when we photographed colonies and monitored growth and survival biweekly. From those images we used ImageJ to measure colony area.

Results

Study sites

The hydrographic regime in South Slough estuary experienced seasonal patterns associated with annual rainy and dry seasons. In Charleston in 2011, the dry season ranged from May to September, and was characterized by a peak in solar radiation (Figure 14a), decreased precipitation (Figure 14b), and decreased wind speeds (Figure 14c). The rainy season ranged from October to April, and was characterized by decreased solar radiation (Figure 14a), increased precipitation (Figure 14b) and increased wind speeds.

We observed seasonal variations in hydrographic conditions at three study sites, Long Island Point (LIP), Valino Island (VI) and the Distant Fleet Facility (DFF). At all three sites, salinity was lowest in April, and peaked during the summer dry season (Figure 15a). The two most oceanic sites, DFF and VI showed similar trends in salinity, however, the most riverine site, LIP, had noticeably lower salinities throughout the year. The temperature at all three sites remained relatively constant in January 2011, after which it steadily rose to a peak from June to September, decreased in October and remained constant from November through January (Figure 15b).

During the summer, LIP was warmer than VI, which was warmer than DFF, and during the winter, LIP seemed slightly colder than VI and DFF (Figure 15b). Dissolved oxygen was higher in winter and lower in the summer (Figure 15c). We observed no consistent trend in pH across all three sites, however LIP consistently had lower pH than

VI and DFF (Figure 15d). Chlorophyll and Turbidity fluctuated at all three sites, but both parameters exhibited a strong peak in July at all three sites (Figure 15e, 15f).

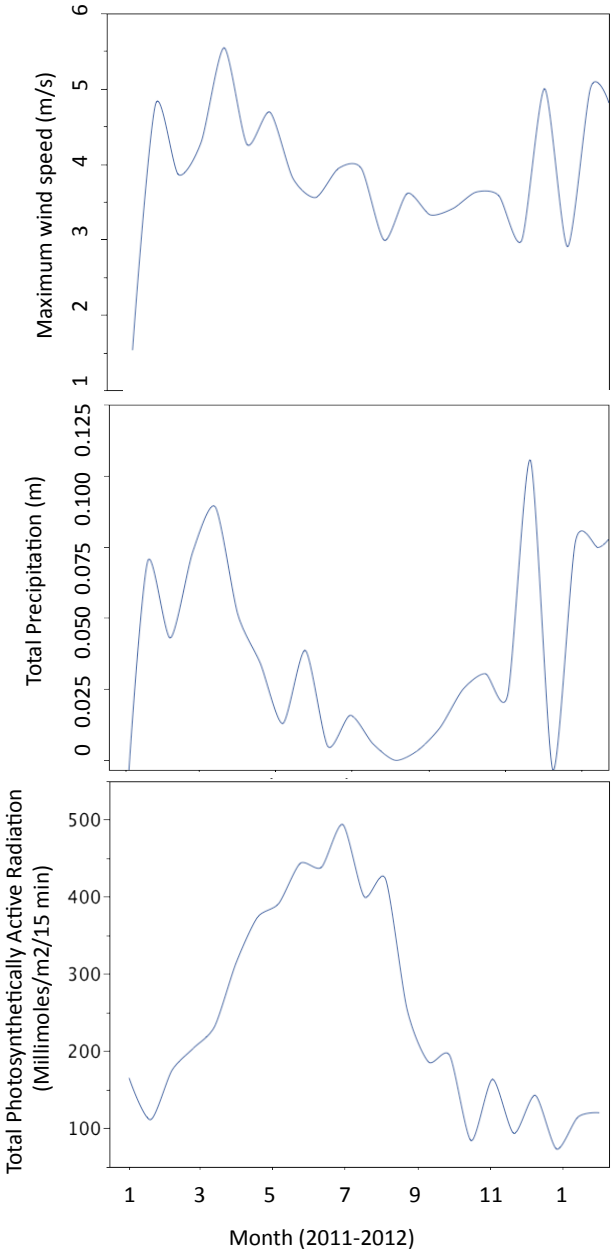

Figure 14. Meteorological data from Charleston, OR collected every 15 minutes from 1/1/2011 to 1/31/2012 from the Charleston Meteorological Station, a NOAA National Estuarine Research Reserve System-wide Monitoring Program monitoring site. Parameters include total photosynthetically active radiation (a), total precipitation (b), and maximum wind speed (c).

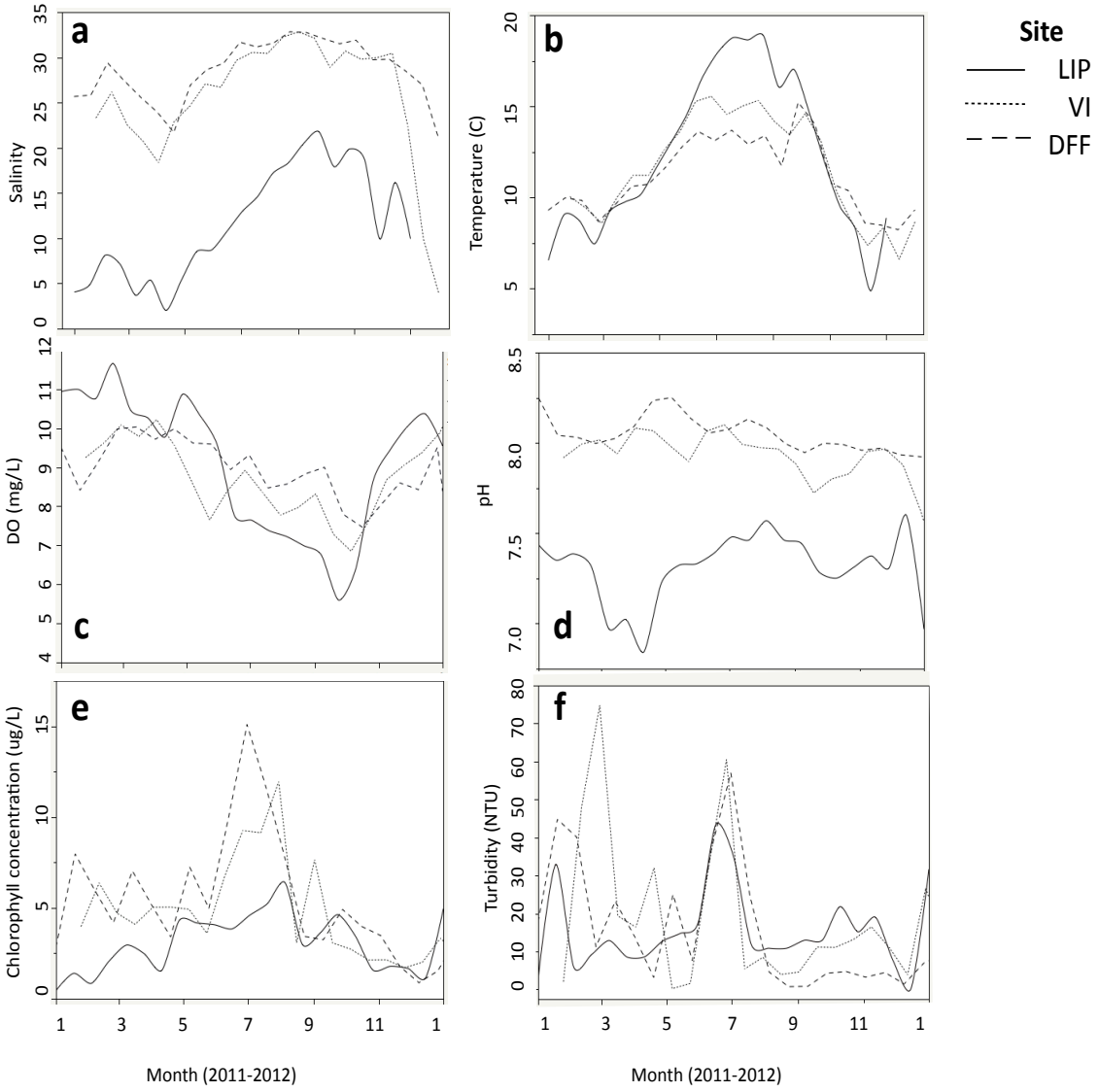

Figure 15. Oceanographic descriptions of three study sites located in the South Slough estuary in Charleston, OR, with data measured every 15 minutes from 1/1/2011 to 1/31/2012 (see Figure 1). Data obtained from NOAA National Estuarine Research Reserve System-wide Monitoring Program Central Data Management Office.

Settlement plates

Study sites were each monitored within the first week of each month during the year 2011. In three separate instances, a mooring was not monitored because the buoy

became tangled and was not visible at the surface of the water. When this occurred (at HCM in April, YP in August, and HCM in September) no settlement data were collected for that month at the site without a visible buoy. A complete summary of species settled on each type of settlement plate by site is included in the appendix (Table 9). On all settlement plate types (cumulative slate plates, cumulative petri dishes and monthly petri dishes) we only observed four of the eight target invasive organisms listed (see methods), including *Alcyonidium* spp., *Botrylloides violaceus*, *Bugula* spp., and *Schizoporella japonica.*

We observed a seasonal trend in species richness on all plates at each site in 2011, where richness increased at almost every site with a peak around July, and then decreased between July and October (IBB, DFF, YP, VI, and HCM) (Figure 16). Four sites experienced a second peak in species richness between October and December (IBB, DFF, YP, and VI). We also observed a peak in species richness around July on the monthly plates (five petri dishes that were collected and replaced each month) at five out of six sites (IBB, DFF, YP, VI and LIP), however in the months following July, those sites each experienced a decline in species richness from July to October, and all sites had only three or fewer species settle from October through December (Figure 16). In July, the most riverine site, HCM, experienced no settlement, and all dishes were darkened with fine particles of anoxic sediment which was observed clinging to the corners of the plate and to barnacle shell margins.

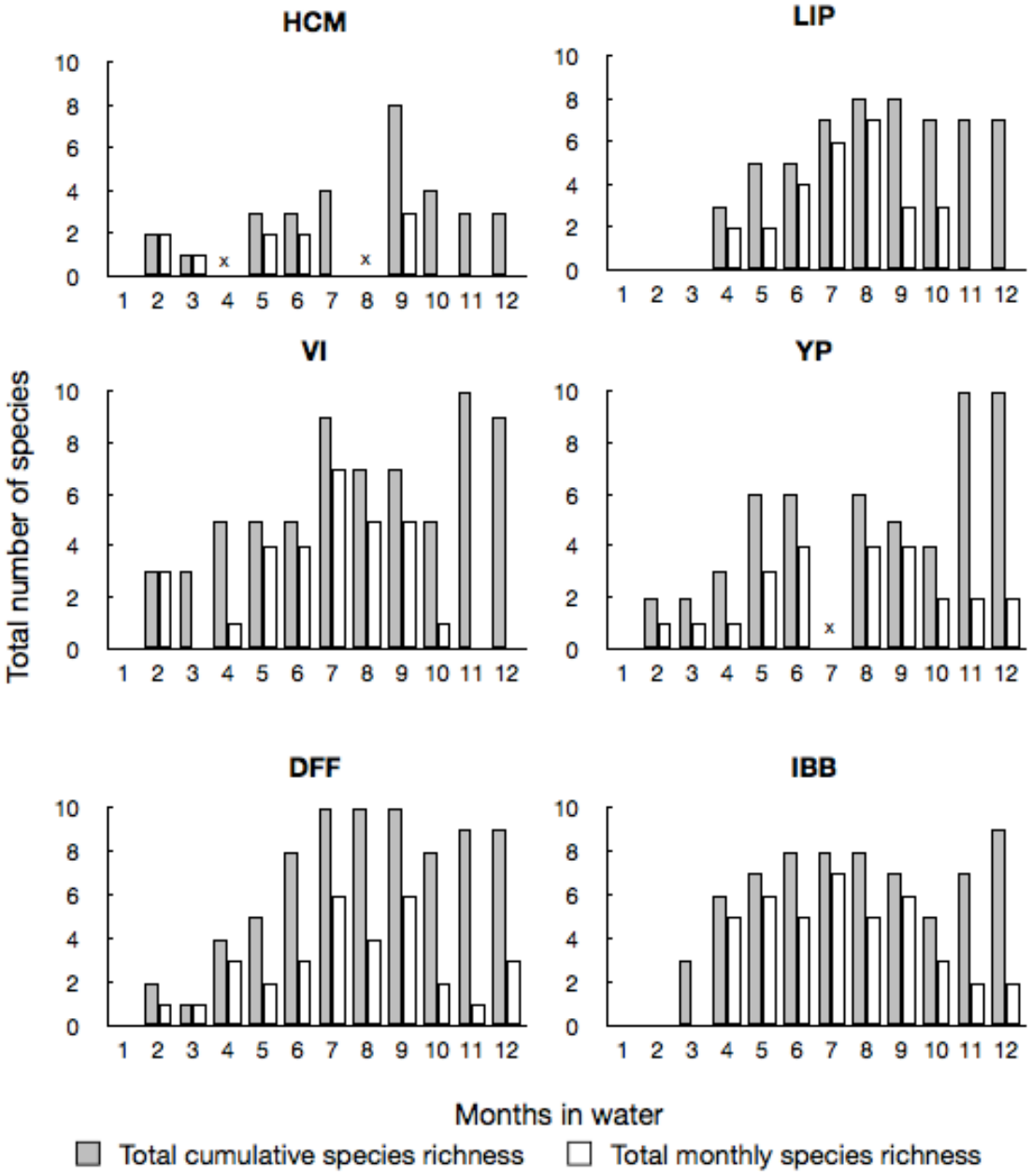

Figure 16. Total cumulative species richness on all plates (cumulative slate plates n=2, cumulative dishes n=5, monthly dishes n=5) and total monthly species richness from monthly plates alone (n=5). X's indicate months when no data were available for a site**.**

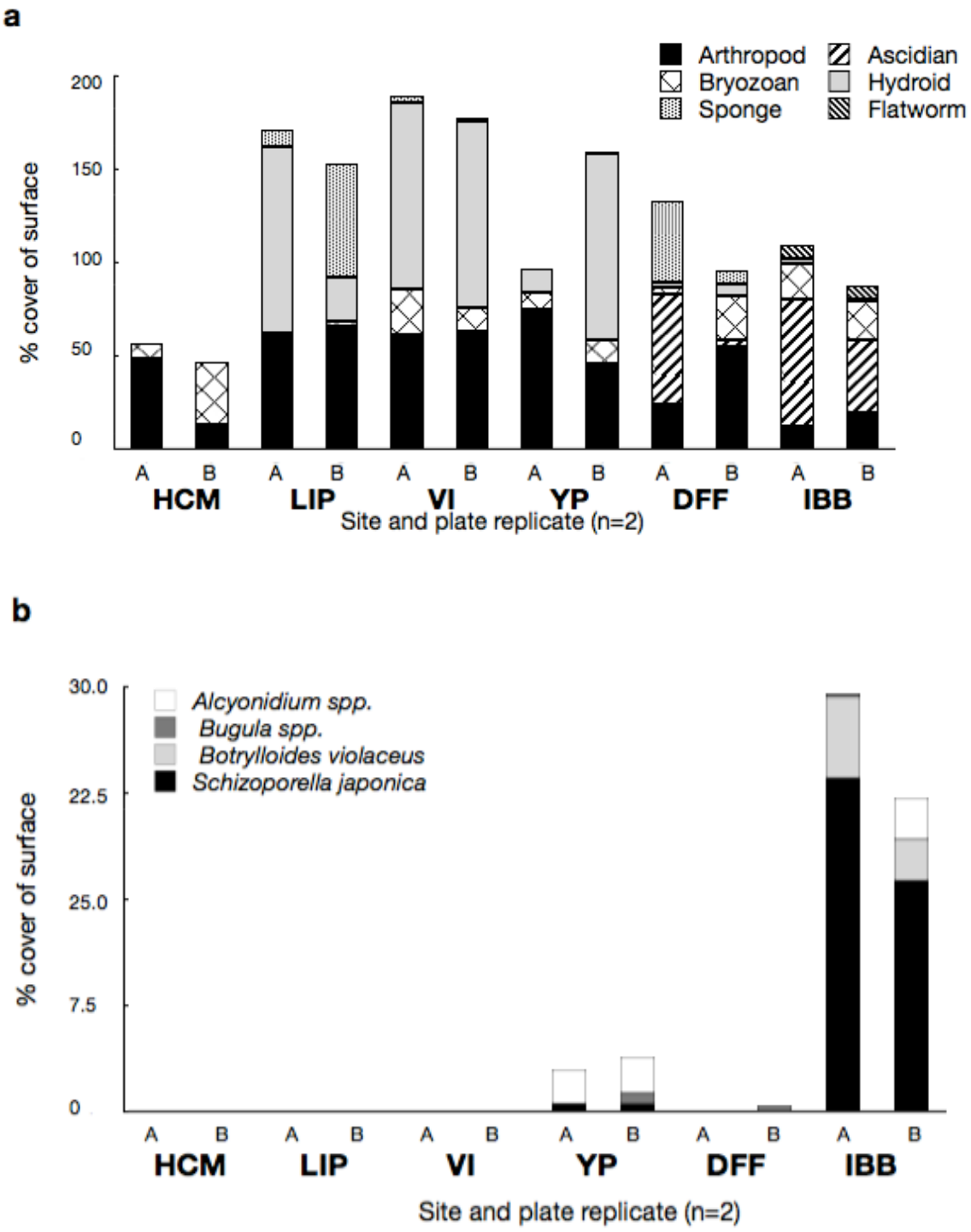

Figure 17. Percentage cover of cumulative slate plates (image) after one year in South Slough. Percent cover is displayed by phylum (a) and by target invasive species (b), and is described for each of two plate replicates (A,B) at six sites displayed from the most rivering (HCM to the most oceanic (IBB).

We calculated percentage cover of each species on cumulative slate plates after one year (Figure 30, 31). When species are grouped by phylum, site assemblages appear to be similar between site replicates (Figure 17a). Arthropods (barnacles) and bryozoans were present at all six sites, however ascidians were only present at DFF and IBB. Sponges were present at LIP, VI, YP, and DFF, and cnidarians (hydroids) were heavily present at LIP, VI and YP. Percentage cover is greater than 100% at all sites except for HCM because hydroids were observed growing directly on top of barnacles, and both factored into the calculation of total percentage cover. When we calculated percentage cover of nonnative species on cumulative slate plates after one year, YP and IBB appeared to have the highest cover of invasive species, with *Schizoporella japonica* most abundant at IBB.

Principal components analysis

Three principal components were extracted from the PCA of phylum presence or absence on all settlement plates, explaining 63.4% of the variability (Figure 18). The first component explained 32.26% of the variability, and phyla with the greatest contributions were Bryozoa (0.512), Arthropoda (0.505), Cnidaria (0.436), and Porifera (0.376), whose presence was positively correlated among them (Figure 18a, Table 2). The second component explained 17.13% of the variability, and described the inverse relationship between the presence of Annelida (0.650), Mollusca (0.466), and Urochordata (0.332) vs. Porifera (-0.364) and Platyhelminthes (-0.315) (Figure 8b, Table 2).

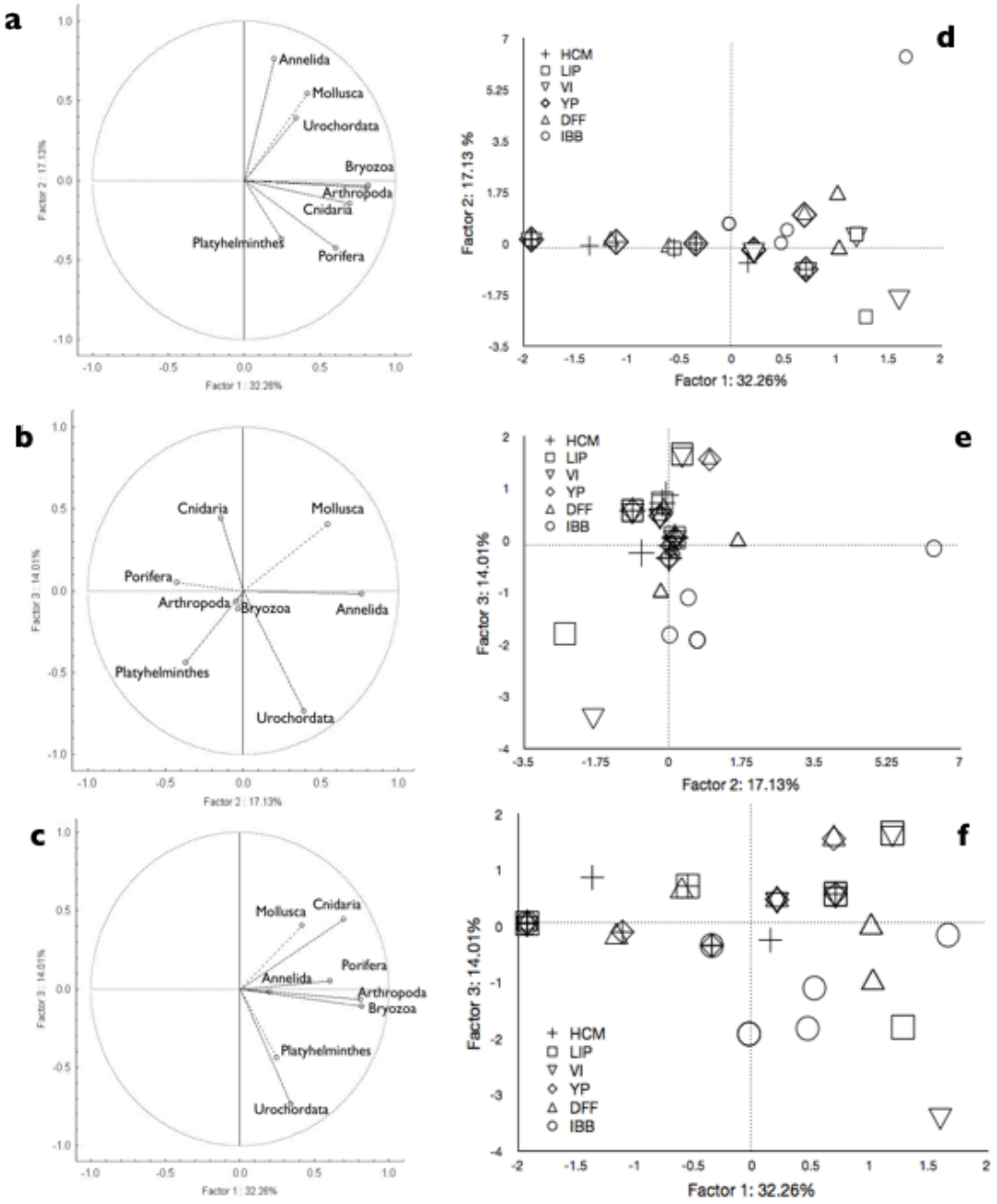

Figure 18. Principal components analysis (PCA) of phylum presence/absence. Factor scores of the observations are plotted for the first three components. Projection of the variables (phyla) on the factor plane include principle component 1 vs. 2 (a), 2 vs. 3 (b), 1 vs. 3 (c), and projections of the cases (study site) on the factor plane include principle component 1 vs. 2 (c), 2 vs. 3 (d), and 1 vs. 3 (e).

Table 2. Eigenvector matrix of phylum presence/absence for the first three components of the Principal Components Analysis (Figure 3).

Variable	Factor 1	Factor 2	Factor 3
Bryozoa	0.512	-0.0269	-0.106
Arthropoda	0.505	-0.0396	-0.0634
Cnidaria	0.436	-0.123	0.422
Porifera	0.376	-0.364	0.0490
Mollusca	0.259	0.466	0.382
Urochordata	0.214	0.334	-0.697
Platyhelminthes	0.153	-0.315	-0.415
Annelida	0.124	0.650	-0.0175

The third component explained 14.01% of the variability, and was characterized by the inverse relationship between presence of Urochordata (-0.697) and Platyhelminthes (-0.415) vs. Cnidaria (0.422) and Mollusca (0.382) (Figure 18c, Table 2). Case projections for all three components reflect a differentiation of IBB from the remaining sites (Figure 18d-f).

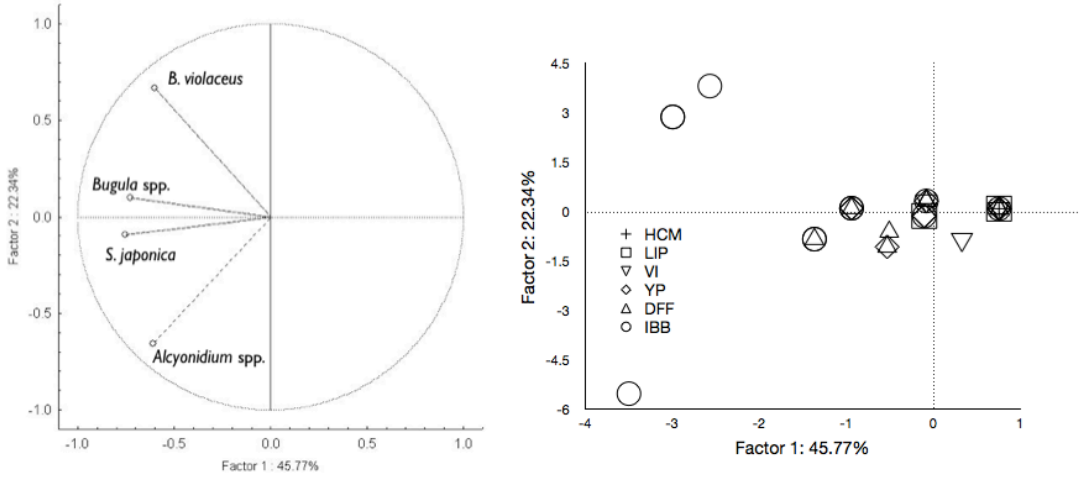

Figure 19. Principal components analysis (PCA) of invasive species presence/absence. Factor scores of the observations are plotted for the first two components. Projection of the variables (phyla) on the factor plane include principle component 1 vs. 2 (a), and projections of the cases (study site) on the factor plane include principle component 1 vs. 2 (b).

Two principal components were extracted from the PCA of invasive species presence or absence on all settlement plates, with 68.11% of the variability explained (Figure 19). The first component explained 45.77% of the variability, and it was mostly driven by the presence of *S. japonica* (-0.558) and *Bugula* spp. (-0.537), however all invasive species dominated the negative loadings on PC1 and were positively correlated (Figure 19a, Table 3). The second component explained 22.34% of the variability, with the greatest contributions from the presence of *B. violaceus* (0.705) and *Alcyonidium* spp. (-0.694), which were strongly negatively correlated (Figure 19a, Table 3). Case projections for both components reflect a differentiation of IBB from the remaining sites (Figure 19b).

Table 3. Eigenvector matrix of invasive species presence/absence of species for the first two components of the Principal Components Analysis.

Variable	Factor 1	Factor 2
Schizoporella japonica	-0.558	-0.100
Bugula spp.	-0.537	0.105
Alcyonidium spp.	-0.452	-0.694
Botrylloides violaceus	-0.443	0.705

Two principal components were extracted from the PCA of percentage cover of phyla on cumulative slate plates, explaining 73.34% of the variability (Figure 20). The first component explained 46.91% of the variability, with the strongest contributions coming from percentage cover of Arthropoda (-0.537) which is negatively correlated with Platyhelminthes (0.498) and Urochordata (0.487) (Figure 20a, Table 4). The second component explained 26.43% of the variability, and the greatest contributions included percentage cover of Porifera (0.694) and Cnidaria (0.694), which negatively correlated with percentage cover of Bryozoa (-0.593) (Figure 20a, Table 4). Case projections

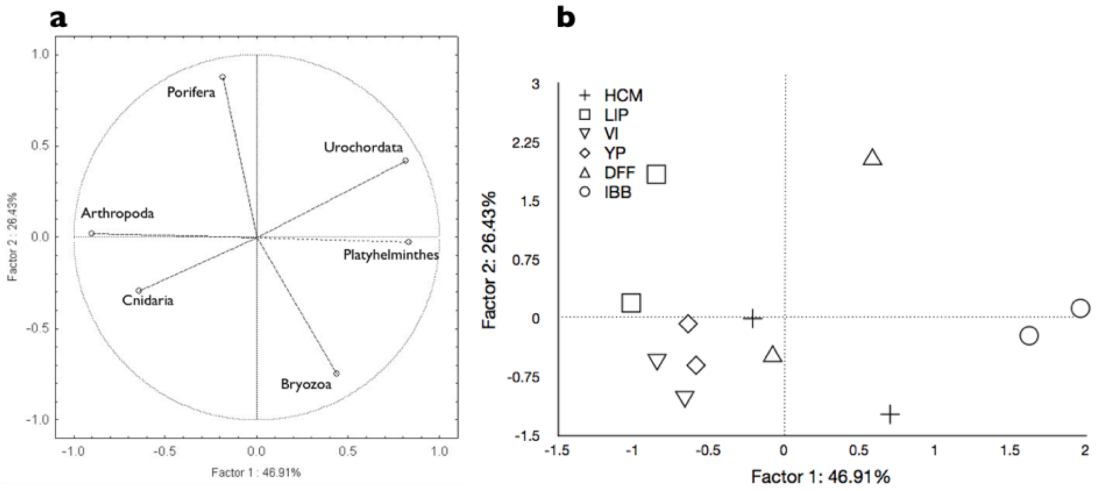

Figure 20. Principal components analysis (PCA) of phylum percent cover on slate plates (n=2). Factor scores of the observations are plotted for the first three components. Projection of the variables (phyla) on the factor plane include principle component 1 vs. 2 (a), 2 vs. 3 (b), 1 vs. 3 (c), and projections of the cases (study site) on the factor plane include principle component 1 vs. 2 (c), 2 vs. 3 (d), and 1 vs. 3 (e).

Table 4. Eigenvector matrix of percent cover of each phylum for the first two components of the PCA.

Variable	Factor 1	Factor 2
Arthropoda	-0.537	0.0157
Platyhelminthes	0.498	-0.0208
Urochordata	0.487	0.332
Cnidaria	-0.382	0.694
Bryozoa	0.262	-0.593
Porifera	-0.110	0.694

for each component reflected differentiation of IBB, DFF and LIP from each other and from the remaining sites (Figure 20b).

Two principal components were extracted from the PCA of percentage cover of target invasive species on cumulative slate plates, explaining a total of 84.01% of the variability (Figure 21). The first component explained 51.36% of the variability, with the greatest contributions coming from percentage cover of *S. japonica* (0.473) and *B. violaceus* (0.455) (Figure 21a, Table 5). The second component explained 32.35% of the variability, with the greatest contributions coming from the percentage cover of *Bugula*

spp (0.530) and *Alcyonidium* spp. (0.413). Case projections for each component reflected the differentiation of IBB from the remaining sites, as well as DFF from the remaining sites.

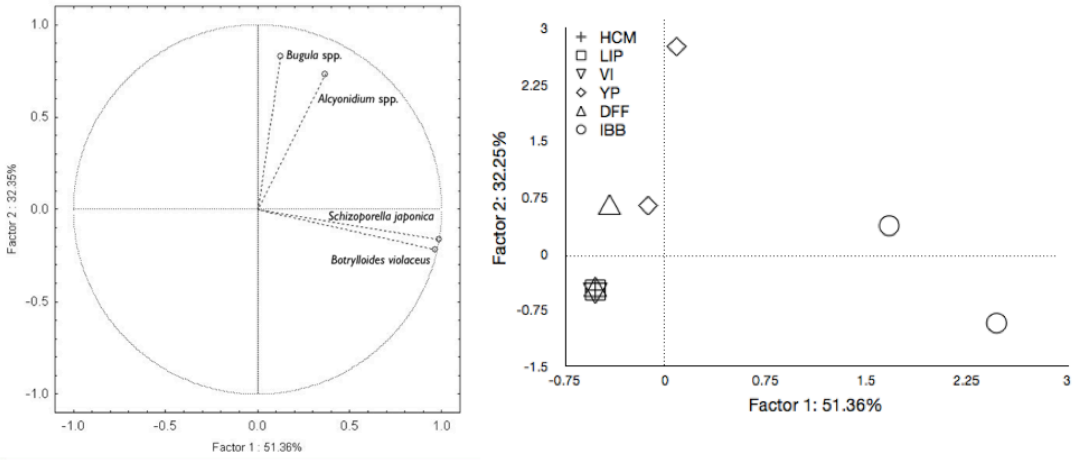

Figure 21. Principal components analysis (PCA) of invasive species percent cover on slate plates (n=2). Factor scores of the observations are plotted for the first three components. Projection of the variables (phyla) on the factor plane include principle component 1 vs. 2 (a), 2 vs. 3 (b), 1 vs. 3 (c), and projections of the cases (study site) on the factor plane include principle component 1 vs. 2 (c), 2 vs. 3 (d), and 1 vs. 3 (e).

Table 5. Eigenvector matrix of percent cover of each invasive species for the first two components of the Principal Components Analysis.

Variable	Factor 1	Factor 2
Schizoporella japonica	0.473	0.0207
Botrylloides violaceus	0.455	0.0367
Alcyonidium spp.	0.0649	0.413
Bugula spp.	0.00784	0.530

Predictive models

Regarding the effect of the measured estuarine physical variables at three study sites (LIP, VI and DFF) on species richness, our model revealed salinity to be the only significant variable, which explained 60.1% of the total variability (Figure 22, Table 6). The model predicted two optima for species richness, at salinities 15 and 30. Salinity was also the only significant variable affecting the presence of *Bugula* spp (Figure 23, Table

7). Salinity explained 37.3% of the deviance on *Bugula* spp presence. *Bugula* spp. presence was limited to salinities over 20 (Figure 23), after that threshold probability of presence increased linearly with salinity (e.d.f.=1 Table 7; Figure 23).

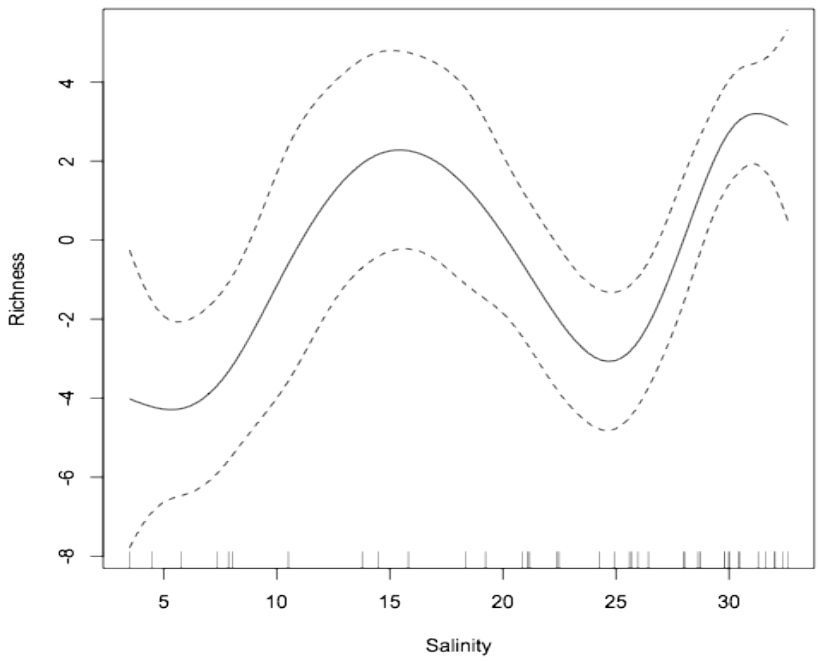

Figure 22. Pattern of species richness at DFF, VI and LIP. Results of General Additive Model (GAM) showing the effect of the independent variable salinity, on the dependent variable, species richness, at settlement plates in the South Slough estuary. Dotted lines indicate the 95% confidence interval, and tick marks along the x-axis indicate effect values where observations occurred.

In the case of *S. japonica*, turbidity was the only significant variable affecting its presence and explained 25.4% of the total deviance (Figure 24, Table 8), Turbidity showed a linear negative relationship with *S. japonica* presence (e.d.f.=1, Figure 24, Table 8) reaching zero probability of presence for turbidity values higher than 30 NTU. No significant relationships were observed between the measured variables and presence of *Alcyonidium* spp.

Table 6. Structure of the general additive model (GAM) to describe species richness. S.E. is standard error and e.d.f is estimated degrees of freedom.

Parametric Coefficients				
Parameter	**Estimate**	**S.E.**	**Z**	**P**
Intercept	5.256	0.405	12.97	2.83 e-14
Smooth terms (Non Parametric Coefficients)				
Parameter	**e.d.f.**	**F**	**P**	
Salinity	6.055	6.21	1.12E-04	
R^2 adjusted	0.526	**% Deviance explained**	60.1%	

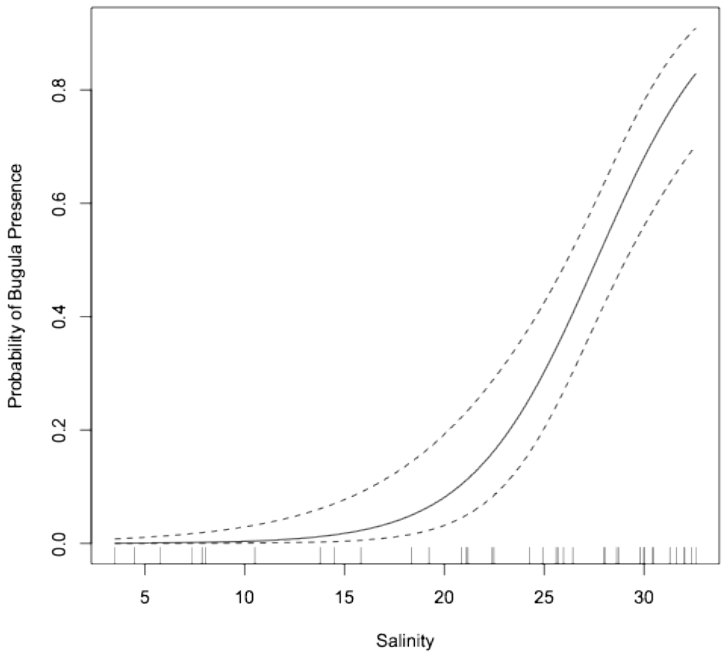

Figure 23. Probability of the presence of *Bugula* spp. in relation to salinity in the South Slough estuary. Results of General Additive Model (GAM) showing the effect of the independent variable, salinity, on the dependent variable, presence of *Bugula* spp., at settlement plates in the South Slough estuary. Dotted lines indicate the 95% confidence interval, and tick marks along the x-axis indicate effect values where observations occurred.

Table 7. Structure of the general additive model (GAM) to describe the probability of *Bugula* spp. presence on fouling plates in South Slough . S.E. is standard error and e.d.f is estimated degrees of freedom. To get the estimated values and S.E. on the scale of actual probability, the inverse of the logit function has been applied.

Parametric Coefficients				
Parameter	**Estimate**	**S.E.**	**Z**	**P**
Intercept	0.162	0.744	-2.208	0.0273
Smooth terms (Non Parametric Coefficients)				
Parameter	**e.d.f.**	**X^2**	**P**	
Salinity	1	7.473	0.00626	
R^2 adjusted	0.39	**% Deviance explained**	37.3%	

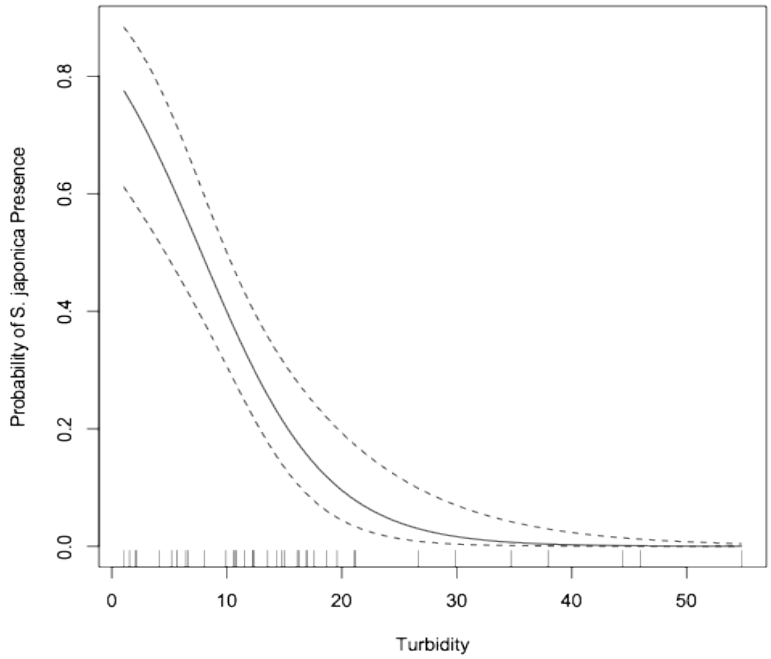

Figure 24. Probability of the presence of *Schizoporella japonica* in relation to turbidity in the South Slough estuary. Results of General Additive Model (GAM) showing the effect of the independent variable, turbidity (NTU), on the dependent variable, presence of *S. japonica*, at settlement plates in the South Slough estuary. Dotted lines indicate the 95% confidence interval, and tick marks along the x-axis indicate effect values where observations occurred.

Table 8. Structure of the general additive model (GAM) to describe the probability of *Schizoporella japonica* presence on fouling plates in South Slough . S.E. is standard error and e.d.f is estimated degrees of freedom. To get the estimated values and S.E. on the scale of actual probability, the inverse of the logit function has been applied.

Parametric Coefficients				
Parameter	**Estimate**	**S.E.**	**Z**	**P**
Intercept	0.173	0.600	-2.61	0.00905
Smooth terms (Non Parametric Coefficients)				
Parameter	**e.d.f.**	**X^2**	**P**	
Turbidity	1	6.368	0.0116	
R^2 adjusted	0.252	**% Deviance explained**	25.4%	

Transplant experiment

During the September 2011 transplant experiment, average salinity was highest at DFF and lowest at LIP, and variance in salinity was much greater at LIP than at DFF or VI (Figure 25 a, Table 9). Water temperature increased in the second half of the month, and was higher at LIP than at VI and DFF (Figure 25b). Dissolved oxygen was lowest at LIP and highest at DFF (Figure 25c), and pH was lowest at LIP and highest at DFF (Figure 25d).

Chlorophyll concentration was constant across all three sites except for a pronounced peak at VI in the first week of September (Figure 25e). Turbidity appeared to be higher on average and fluctuate more at LIP and VI when compared to DFF, which had very low turbidity throughout the month (Figure 25f, Table 9). Variance in turbidity was much lower at DFF than at VI and LIP (Table 9).

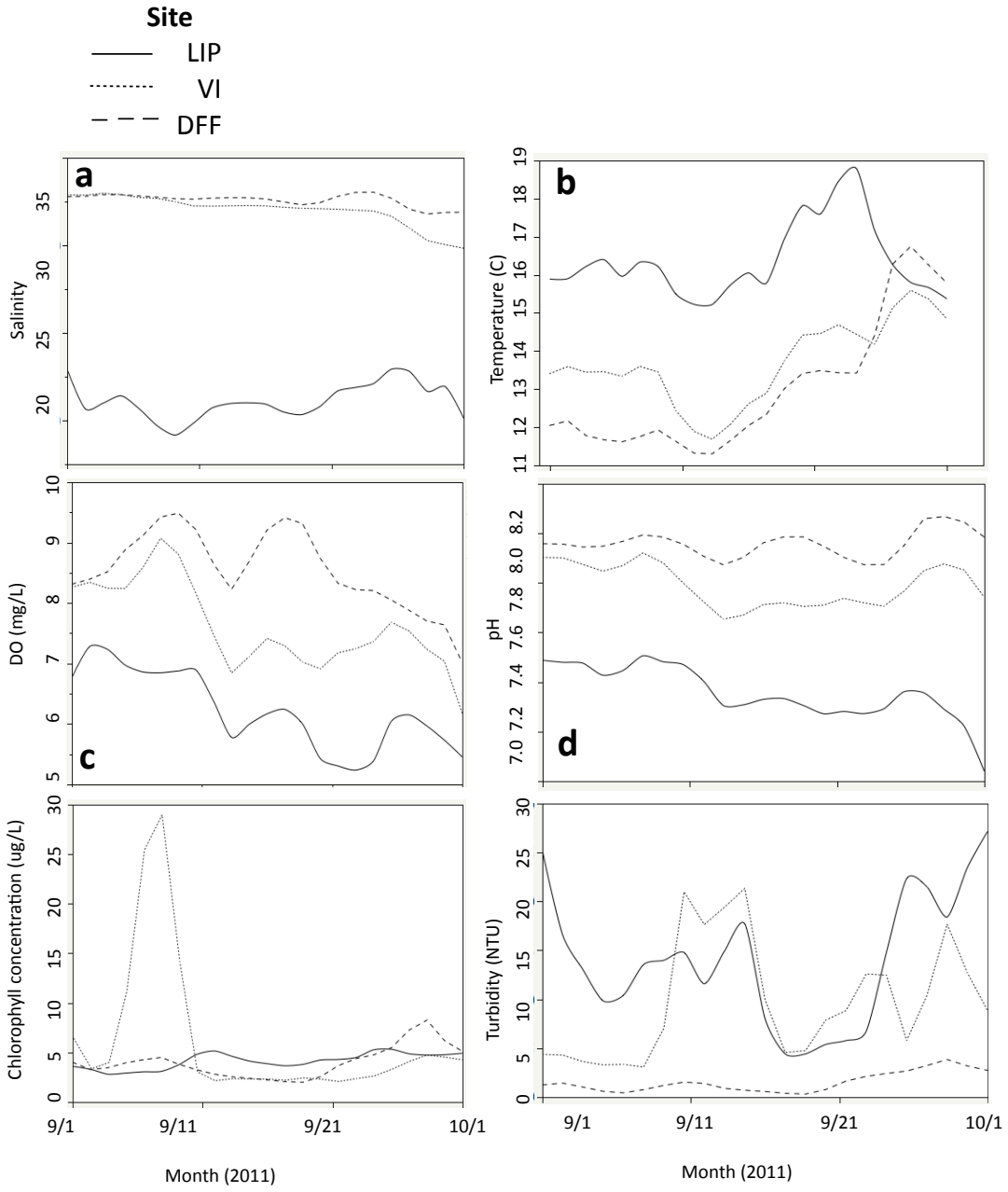

Figure 25. Oceanographic descriptions of three sites (LIP, VI, DFF) from 9/1/11 to 10/1/11, collected every 15 minutes from 9/1/11 to 10/1/11.

In the larval transplant study, on average, 4.3 $\pm$ 0.8 of 5 *B. violaceus* larvae settled (n=3) within 24 hours. The larvae of *B. violaceus* had significantly higher percentage settlement than *S. japonica*, ($t(22)=-7.44$, $P<0.001$), however there were no significant differences

between settlement sites in average number of *B. violaceus* larvae settled per plate (Figure 26a). The larvae of *S. japonica* did settle successfully at all four sites (Figure 26b), and there was a significant effect of study site on the average number of larvae settled per plate ($F(3,11)=26.93$, $P<0.0002$). Post-hoc comparisons (Tukey-Kramer HSD) indicated that significantly fewer *S. japonica* larvae settled at DFF when compared to the other three sites, VI, YP, and IBB (Figure 26b).

Table 9. September salinity and turbidity in South Slough at three sites (LIP, VI, DFF).

	DFF	**VI**	**LIP**
Salinity			
Mean	32.6	32.0	21.1
Variance	0.197	0.913	62.1
Turbidity (NTU)			
Mean	1.54	9.92	13.5
Variance	4.1116	2013.9	2859.3

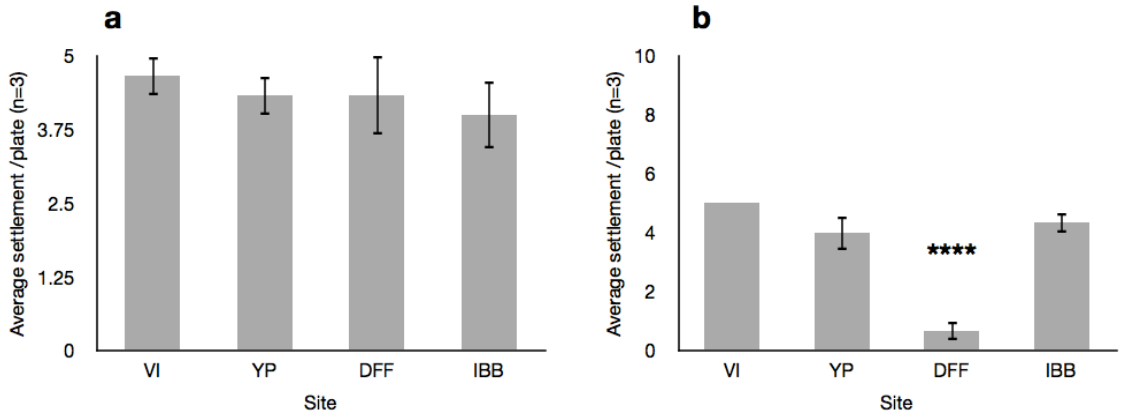

Figure 26. 24-hour field settlement of the larvae of *B. violaceus* (a) and *S. japonica* (b) at four study sites in South Slough. Treatments of *B. violaceus* had 5 larvae per dish (n=3±SE), and treatments of *S. japonica* had 10 larvae per dish *(*n=3±SE). Asterisks over bar indicate a group that is significantly different (1-way ANOVA, Tukey-Kramer HSD).

When young settlers of *S. japonica* and *B. violaceus* were transplanted to study sites in South Slough, settlers of both species survived at all sites after 33 days, although survival decreased over time (Figure 27). The *B. violaceus* transplants appeared to survive similarly at VI and IBB (Figure 27a), and the *S. japonica* transplants had on average, higher survival at IBB and YP, and lower survival at VI and DFF (Figure 27b).

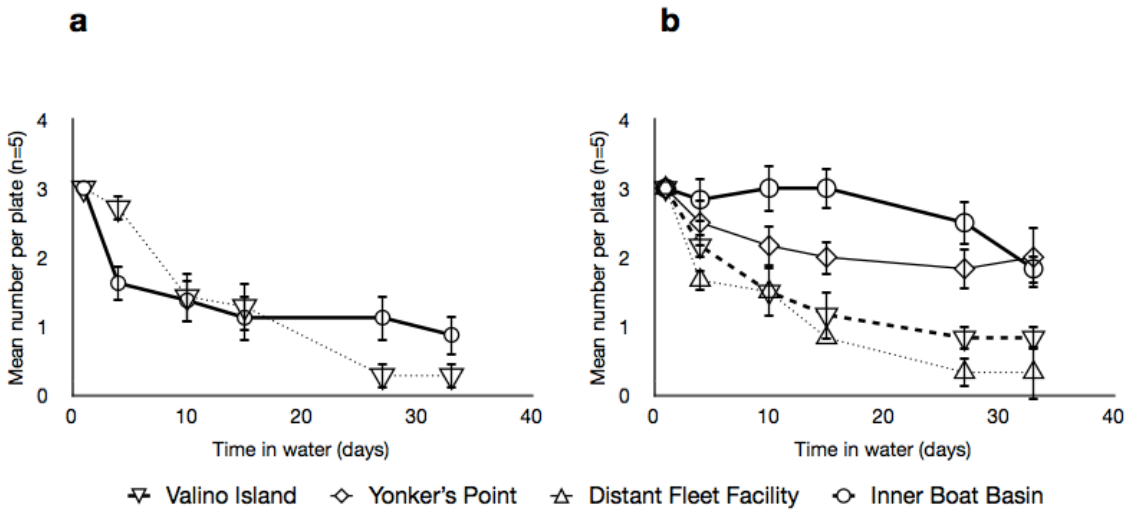

Figure 27. Young settler survival after 33 days, of a) *B. violaceus (*, and b) *S. japonica* at four sites in South Slough. Both treatments had three settlers per plate (n=5 ± SE).

Adult colonies of *B. violaceus* and *S. japonica* survived for 33 days at all four study sites in South Slough (Figure 28). Some transplants of *B. violaceus* experienced mortality, with a steady decrease in total colony number at each site over the duration of the month (Figure 28a). Some transplants of *S. japonica* also experienced mortality, however we only observed a decrease in colony number in the first half of the month, and not for the second (Figure 28b).

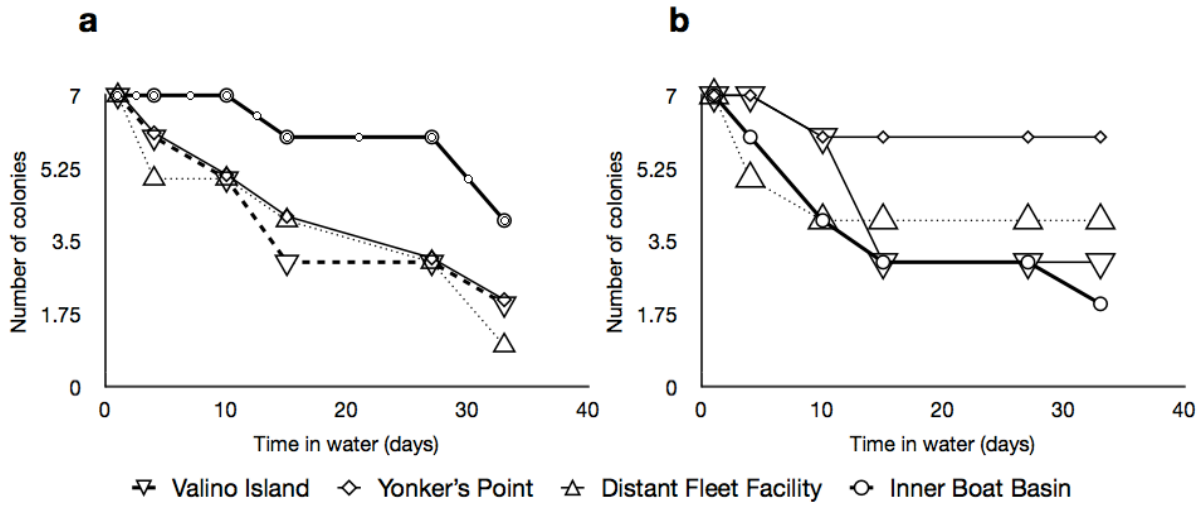

Figure 28. Adult survival of a) *B. violaceus* (n=7), and b) *S. japonica* (n=7) at four sites in South Slough over 33 days from 9/1/11 to 10/3/11.

We measured the change in area for the same adult colony transplants, to find that most colonies of *B. violaceus* experienced a decrease in area with each measurement at VI, YP and DFF, however at IBB (on average) colonies increased in area after day 15 (Figure 29a). Colonies of *S. japonica* experienced a decrease in area over the first 15 days at all four sites, but after 15 days, at VI, YP and DFF, colonies increased in area, but decreased in area at IBB (Figure 29b).

Discussion

The hydrographic conditions in South Slough throughout the year 2011 suggest the presence of a winter/spring wet season from January to May and October (2011) to January (2012), characterized by increased precipitation (Figure 14b) and decreased salinity, temperature and chlorophyll concentration (Figure 15a, b, e). Decreased precipitation and wind speeds (Figure 14b, c) and increased salinity,

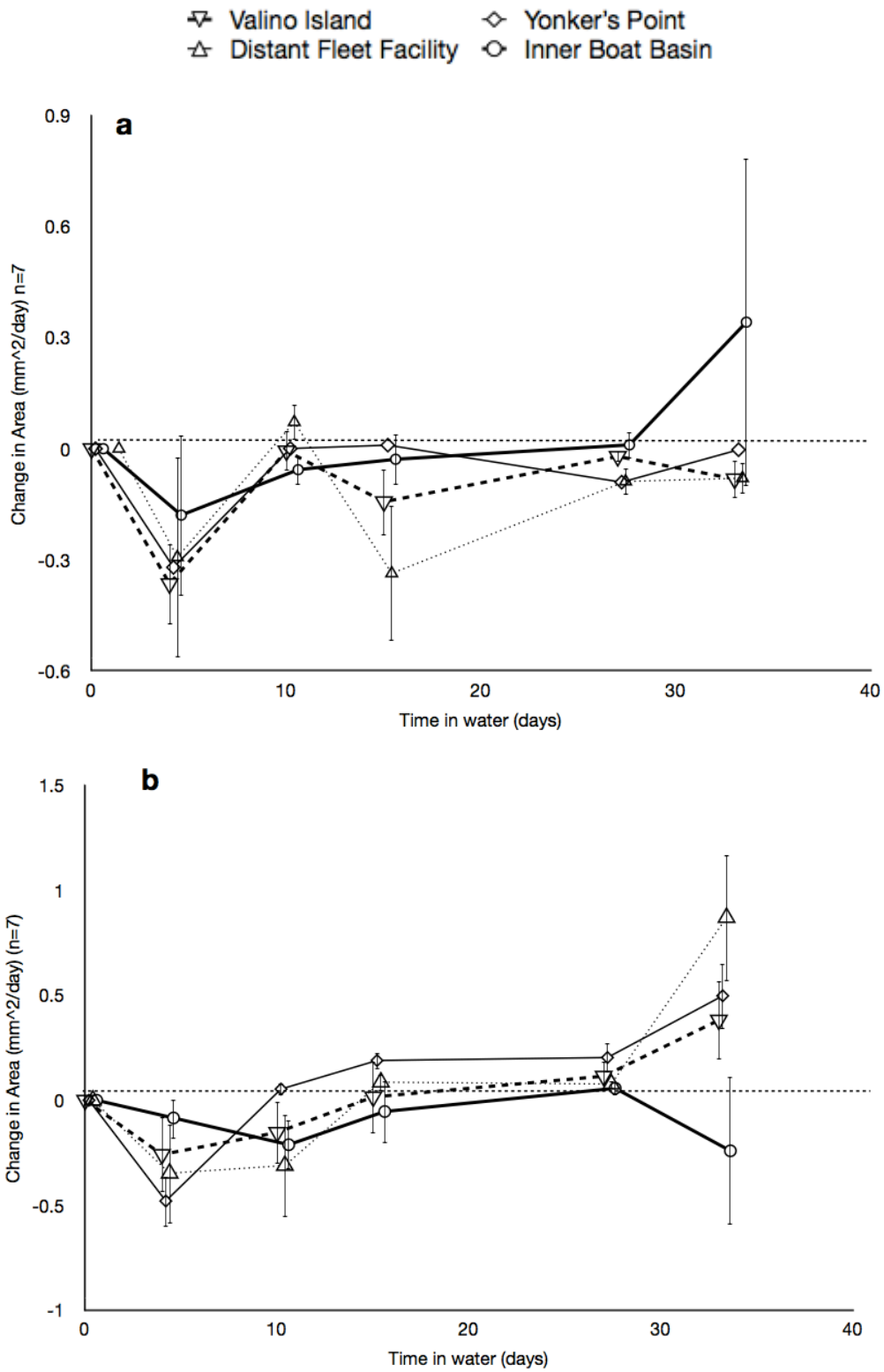

Figure 29. Change in colony area (in mm^2/day) of a) *B. violaceus* (n=7± SE), and b) *S. japonica* (n=7± SE) at four sites in South Slough over 33 days from 9/1/11 to 10/3/11. Data points have been staggered where error bars overlap.

temperatures, and chlorophyll concentrations (Figure 15a, b, e) characterized the summer dry season from June to September. These data are in agreement with hydrographic descriptions of South Slough by Roegner and Shanks (2001) and Cowlishaw (2004), as well as with the coastal upwelling regime of the Oregon coast, as described by Small and Menzies (1981) and Huyer (1983). Unlike many estuaries that have high amounts of local phytoplankton production which is fueled by terrestrial nutrient input (Roegner and Shanks, 2001), South Slough has low local phytoplankton productivity (Roegner and Shanks, 2001) due to the frequent tidal flushing of the Coos Bay water system (Cziesla, 1999), and poor hydrographic connectivity between the Coos Bay estuary and the South Slough (Roegner and Shanks, 2001). As a result, suspension feeding organisms in South Slough consume phytoplankton produced in the ocean (Roegner and Shanks, 2001) that is advected into South Slough during flood tides (Cziesla, 1999.), and consume benthic diatoms that have been resuspended into estuarine waters (Roegner and Shanks, 2001) during ebb tides (Cziesla, 1999) (Figure 15e). A sharp peak in turbidity was observed around July (Figure 15f). Turbidity can be influenced by dredging activity, wind, sediment size, geography and tidal currents (Emmett et al., 2000).

In addition to seasonal variation in hydrographic data across all study sites, we observed hydrographic trends that reflect the horizontal gradient transitioning from oceanic to riverine habitat. Of the three study sites where we had access to water quality measurements, LIP appeared to have a much stronger riverine influence throughout the year than VI and DFF. This riverine influence is characterized by lower salinity, lower pH, and lower chlorophyll concentrations (Figure 15a, d, e). During the summer dry season when the riverine influence is milder due to reduced precipitation (Figure 14), this

site experienced increases in salinity and water temperature (Figure 15a,b). Additionally, a decrease in dissolved oxygen was observed during the summer months at LIP (Figure 15c), which may be a result of increased water temperatures and possible water column stratification (Dias and Rosenberg, 1995). Together, these conditions make LIP a less hospitable environment than VI or DFF for the recruitment and survival marine species (Cognetti and Maltagliati, 2000).

Total species richness at all study sites increased during the summer, with a peak around July for all sites, with a second peak at four sites (VI, YP, DFF and IBB) around November (Figure 16). In the late summer we observed barnacle-scouring events where large sets of barnacles were removed from cumulative slate plates at three sites, DFF, VI and YP. These events were likely caused by large tangled masses of drift algae that had become entwined around the mooring apparatus. Two species of *Balanus* (*B. glandula* and *B. crenatus*) are known to form dense 'sets' in the spring that become so tightly packed together that they can easily break loose from the substratum (Morris et al., 1980). Continual exchanges of tidal currents may have caused these algal masses to dislodge the densely packed barnacle communities and associated fauna from the plates, causing a temporary slight reduction in total species richness. No masses of algae were observed on the IBB mooring, suggesting that the decrease in species richness during the months of September and October had a different cause, and likely was due to the smothering of several bryozoan species by the fast-growing ascidian *Distaplia occidentalis* (Table 10). This species is fast growing and short lived, often arriving and disappearing from a site within the period of several months (Morris et al., 1980; Strathmann, 1987). *Distaplia occidentalis* was also present at VI and DFF around

September, and may have been partially responsible for the decline in species richness during that time as well (Table 10). The greatest number of species at a site at one time was ten, which was observed at VI in November, YP in November and December, and at DFF in July through September. It is possible that events such as barnacle scouring (caused by drift algae) and smothering (caused by *Distaplia occidentalis)* opened up valuable space on settlement plates, allowing for more species to settle and increase the overall community richness (Menge and Sutherland, 1987; Richmond and Seed, 1991).

Mean monthly species richness peaked at all sites around July, and then sharply declined in October through December (Figure 16). The only organisms we observed settling on monthly plates during November and December were *D. occidentalis, Schizoporella japonica*, and *Microporella californica* at IBB, DFF and YP (Table 10). The timing of larval settlement is closely linked to seasonal reproductive patterns and pelagic larval durations, and in many species can be influenced by physical conditions of the water such as temperature, light and salinity (Richmond and Woodin, 1996; Strom and Thompson, 2000; Feng et al., 2010). In WA populations, the ascidian *D. occidentalis*, is reproductive from April to August (Morris et al., 1980), however we found settlers of *D. occidentalis* on monthly settlement plates through December 2011 (Table 10). Reproductive season varies by species and physical conditions for barnacles in the genus *Balanus* (Strathmann, 1987), but both *B. glandula* and *B. crenatus* are known to produce larvae that settle year round (Morris et al., 1980), and we observed barnacle settlement at all sites on monthly plates from April to November (Table 10). The timing of our first observations of settlement of *Bugula* spp occurred first in March at IBB, next in April at DFF, and then in May at YP. As small settlers, it was difficult to

identify colonies to a species level, however we suspect that we observed two species, *B. pacifica,* which may be reproductive year round (Young and Chia, 1981) and *B. californica*, which settles only during fall and spring seasons in California (Morris et al., 1980). Our observations suggest that in South Slough, *Bugula* spp. are reproductive in the spring. *Schizoporella japonica* is known to be reproductive year-round (Powell, 1970), however we observed a peak settlement of the bryozoan *S. japonica* in the fall and winter, on monthly plates at YP from October through December, at DFF in September and December, and at IBB from April through October (Table 10).

Fouling communities are often structured by competition for free space (Richmond and Seed 1991, Osman 1977, Jackson 1977a, Buss 1986), where competitive dominant organisms include sponges, bryozoans, ascidians and mussels. Many colonial organisms are successful competitors for space because they often grow quickly and are capable of surviving a significant degree of damage (Richmond and Seed, 1991). When cumulative slate plates were recovered after one year in the field, we calculated percentage cover of each species, and compared percentage cover of each phylum (Figure 18a) and non-indigenous species across sites (Figure 18b). Certain phyla had representatives at all sites, including the Bryozoa and Arthropoda (which consisted mostly of barnacles, *Balanus* spp.). Barnacle tests increased the available surface area of all plates, providing additional hard substrata for the settlement of organisms such as bryozoans, hydroids, ascidians and sponges. Other phyla only had representatives at certain sites, most notably the Urochordata (ascidians) at DFF and IBB, with over 50% cover of *D. occidentalis* at DFF (on replicate A only) and IBB. Additionally, Cnidaria (hydroids) were only present at three intermediate sites, LIP, VI, and YP (Figure 18a).

Non-indigenous species were observed at YP, DFF and IBB in low densities, with most species covering less than 1% of plates, with the exception of *S. japonica* (Figure 18b) in IBB, which covered approximately 20% of the slate plate surface, acting as a strong competitor for space and resisting overgrowth and colonization by other invertebrates.

PCA was used as a descriptive analysis of species composition at sites. In the PCA of phylum presence/absence (Figure 18, Table 2), PC1 explains the greatest variability in data, with strongest contributions (Table 2) coming from phyla that were present at almost every site, namely Bryozoa, Arthropoda, and Cnidaria (Figure 18a). PC2 and PC3 demonstrate much of the residual variability in the data, highlighting the contributions of the remaining phyla. PC2 highlights the importance of the presence of Annelida and Mollusca (PC2, Figure 18b), and PC3 highlights the importance of Urochordata (Figure 18c) in explaining the remaining data variability. Annelida (*Spirorbis* sp.) and Mollusca (*Mytilus* spp.) are phyla that only occurred at the most oceanic sites (IBB and DFF) at certain months throughout the year. In the PCA of non-indigenous species presence/absence (Figure 19, Table 3), PC1 explains the general presence of non-indigenous species at the most oceanic study sites, YP, DFF and IBB (Figure 19a, Table 3). PC2 accounts for much of the remaining variability in the data by demonstrating the negative relationship between presence of *Alcyonidium* spp. and *B. violaceus* (Figure 19a, Table 3). When we take into account the relative abundance of phyla on slate plates (Figure 20, Table 4) PC1 explains the most of the data variability through the negative correlation between the percentage cover of Annelida, Platyhelminthes and Urochordata, which are found in higher percentages at more oceanic sites, and the percentage cover of Arthropoda, which are found in higher percentages at

mesohaline sites (PC1, Figure 20a, Table 4). Much of the residual variability is best explained by the negative relationship between percentage cover of Bryozoa and Porifera (PC2, Figure 20a, Table 4). For the PCA of relative abundance of non-indigenous species, the majority of variability is explained by the relatively high abundance of *S. japonica* and *B. violaceus* at the more oceanic sites (PC1), and much of the remaining variability is explained by the low relative abundance of *Bugula* spp. and *Alcyonidium* spp. at the more oceanic sites (PC2, Figure 21a, Table 5). For all PCA's, case projections of study site on the factor plane appeared to highlight the uniqueness of certain sites. This trend was notably apparent in the projection of phylum percent cover, where a gradient was observed from IBB, which hosted more oceanic species, to LIP, which hosted more mesohaline species (Figure 20b, Table 4). In regards to non-indigenous species presence or absence, IBB was strongly characterized by the increased presence of non-indigenous species (Figure 19b, Table 3), and percentage cover of non-indigenous species (Figure 21b, Table 5).

Using general additive models (GAMs) to describe seasonal patterns between species richness and water quality, we found salinity to be the only significant predictor of total cumulative species richness throughout the year (Figure 22). Interestingly, two salinity optima were observed for species richness, around salinities of 15 and above 30. We expected to see a positive relationship between species richness and salinity, with a peak at or near oceanic salinities (Wells, 1961; Hewitt, 1993; Cognetti and Maltagliati, 2000; Josefson, AB and Hansen, 2004; Rumrill and Lands, 2007; Wołowicz et al., 2007) however the salinity optimum at 15 was unexpected. We estimate this salinity zone to lie somewhere between LIP and VI sites during the winter and spring months, and around

LIP during the summer months (Figure 15a). Increased species richness on settlement plates in this region may have been caused by adult source populations of fouling community organisms growing on oyster reefs or seagrass (Rumrill and Lands, 2007; Rumrill and Sowers, 2008). Additionally, the GAM described an increasing positive relationship between salinity and *Bugula* spp. presence above salinities of 20 (Figure 23, Table 7), suggesting perhaps, a physiological barrier to *Bugula* at salinities lower than 20 (Ross and McCain, 1976). Turbidity, rather than salinity best described the presence of *S. japonica* (Figure 24, Table 8), although Ross and McCain (1976) described the salinity range for *Schizoporella japonica* (which was misidentified in the study as *Schizoporella unicornis* (Tompsett et al., 2009)) to be from salinity 18 to salinity 30.

Data from the September transplant experiment show that adults, new settlers and larvae of both *B. violaceus* and *S. japonica* are capable of surviving for one month at VI, YP, DFF and IBB, though with varying success. We expected to find the highest survival of both species in IBB, where the organisms were originally collected, however this trend was only observed in the survival of *S. japonica* ancestrulae (Figure 27b) and adult colonies of *B. violaceus* (Figure 28a). Survival of *B. violaceus* in South Slough (from IBB to VI) is not limited by larval settlement success (Figure 26a), but may be limited by the physiological tolerances of young settlers and adults (Figure 27a, 28a). Of the four study sites, transplants of *S. japonica* were most successful at YP and least successful at DFF (Figure 27b, 28b), suggesting that YP may be a site of interest for directing future non-indigenous species monitoring. A potential reason for low transplant success at DFF is the proximity of the Distant Fleet facility boatyard, which may be leeching industrial contaminants such as heavy metals or antifouling paint toxins into the surrounding water.

This study was completed during the summer dry season (Figure 14,15), when estuarine salinity was higher than average due to reduced precipitation. If we were to repeat this study during the rainy season, when salinity is lower than average due to increased precipitation, we would predict that both species would have significantly lower survival due.

Together, the results of this study provide compelling evidence that salinity plays a strong role in structuring invertebrate fouling communities in South Slough, as well as the distribution of invasive species, however it is a more complicated relationship than previously expected. This study is the first to identify two salinity optima for species richness in South Slough at 15 and 30, rather than the simple positive relationship we expected with increased species richness along an increasing gradient. Additionally, this is the first documented study identifying turbidity as a significant factor in the distribution of the non-native bryozoan *S. japonica*. Conclusions drawn from this study about the seasonal recruitment patterns of invertebrates to artificial substrata, the physiological tolerances of fouling community organisms in the field, and the relative abundance and presence of non-indigenous species will help to inform managers of the current state of fouling communities in South Slough. It is important to understand the recruitment, physiological tolerances and survival patterns of invasive species in order to predict where they may further be spread, and to predict the effects they may have on native communities.

CHAPTER V

CONCLUDING SUMMARY

My objectives for this thesis were to describe aspects of the biology of the bryozoan *Schizoporella japonica*, with a particular focus on the early life history stages of pre-settlement, metamorphosis, and early colony growth. In addition, I determined settlement patterns, distribution, and physical tolerances of *S. japonica* and other fouling community invertebrates in South Slough.

For Chapter II, I focused larval settlement in the laboratory. First, I measured the effect of substratum texture and biofilm presence on laboratory settlement success of *S. japonica* and found higher settlement on sanded plates with biofilm, along with an observed preference for larvae to settle within grooves or dish corners, perhaps exhibiting a preference for microtopography features that protect or shelter the vulnerable young ancestrula. All laboratory settlement occurred within the first 24 hours of introducing larvae to the settlement surface, with a success rate between 33% and 50%. I then investigated the effect of timing of larval release on the number of larvae released and settlement success by delayed the cue of larval release (light) by 48 hours. I found no difference in the number of larvae released, or settlement success between treatments, suggesting that larval quality is not affected by aging within the ovicell. While other studies have investigated the effects of aging lecithotrophic bryozoan larvae by physically preventing them from settling (Woollacott et al 1989), this is the first documented study of a delay in the actual release cue. Lastly, in Chapter II, I investigated the fate of the remaining 50% of larvae that did not settle within the first 24 hours of

being introduced to appropriate settlement substrata, by monitoring them over a two-week period. Presumably these larvae are no longer able to metamorphosis after 24 hours, perhaps due to the lack of a preferred settlement substratum or chemical cue. In many bryozoan larvae, a delay in metamorphosis can have specific ecological advantages (Young & Chia 1981; Burgess et al. 2009), by allowing the larva to find a more suitable settlement location, however in this case, after two weeks, all larvae had died. Knowledge gained from this study will be useful in estimating the dispersal potential of this species, and may help to explain field recruitment patterns of *S. japonica*.

In Chapter III, I measured variation in larval size of *S. japonica*, as well as in the relationship between larval size, settlement success, ancestrula size, and colony growth. In this study, I measured larvae, and compared larval area to settlement success and corresponding ancestrula area, finding no relationship between larval size and settlement success, and a significant positive relationship between larval size and ancestrula size. I outplanted each ancestrula into the field and measured colonies over 102 days. The positive relationship between larval/ancestrula size and colony size persisted through 42 days, but was no longer significant at 74 days. Throughout the entire study period, colony growth was exponential, as colonies grew unimpeded on individual petri dishes. The strong effects of larval size on early colony growth may play a significant role in determining the success of *S. japonica* settlement into crowded fouling communities. Colonies that grow larger sooner (i.e. colonies that come from larger larvae) are at an advantage in this space-limited environment, as they are able to grow faster and possibly outcompete other organisms for space on hard substrata.

In Chapter IV, I further investigated the role of *S. japonica* and other non-indigenous invertebrates in fouling communities in South Slough. At six study sites arranged along the estuarine gradient, I used cumulative and monthly settlement plates to monitor fouling community structure as well as monthly settlement of species. In addition, in the month of September, I outplanted the larvae and young settlers of two common fouling community species from the Charleston boat basin (*S. japonica* and the ascidian *B. violaceus*) to compare tolerances of different life stages of each organism at four sites along the estuarine gradient. Finally, I was able to using the water quality measurements available at three of my study sites, to describe the species richness and presence of invasive species in South Slough. Together, the conclusions drawn from this study indicate that fouling communities in South Slough are structured by salinity, however the relationship between species richness and salinity is not linear. Two salinity optima exist for species richness, at salinities of 15 (which was unexpected) and 30 (which was expected). I suspect that the increase in species richness observed at salinity 15 in South Slough may be related to larvae settling from nearby fouling organisms associated with oyster shells that are acting as a source population. Conclusions drawn from this study will help to inform managers of the current state of South Slough fouling communities, as well as the location, recruitment, tolerances and survival of non-indigenous fouling community species.

APPENDIX

SOUTH SLOUGH FOULING COMMUNITY SPECIES

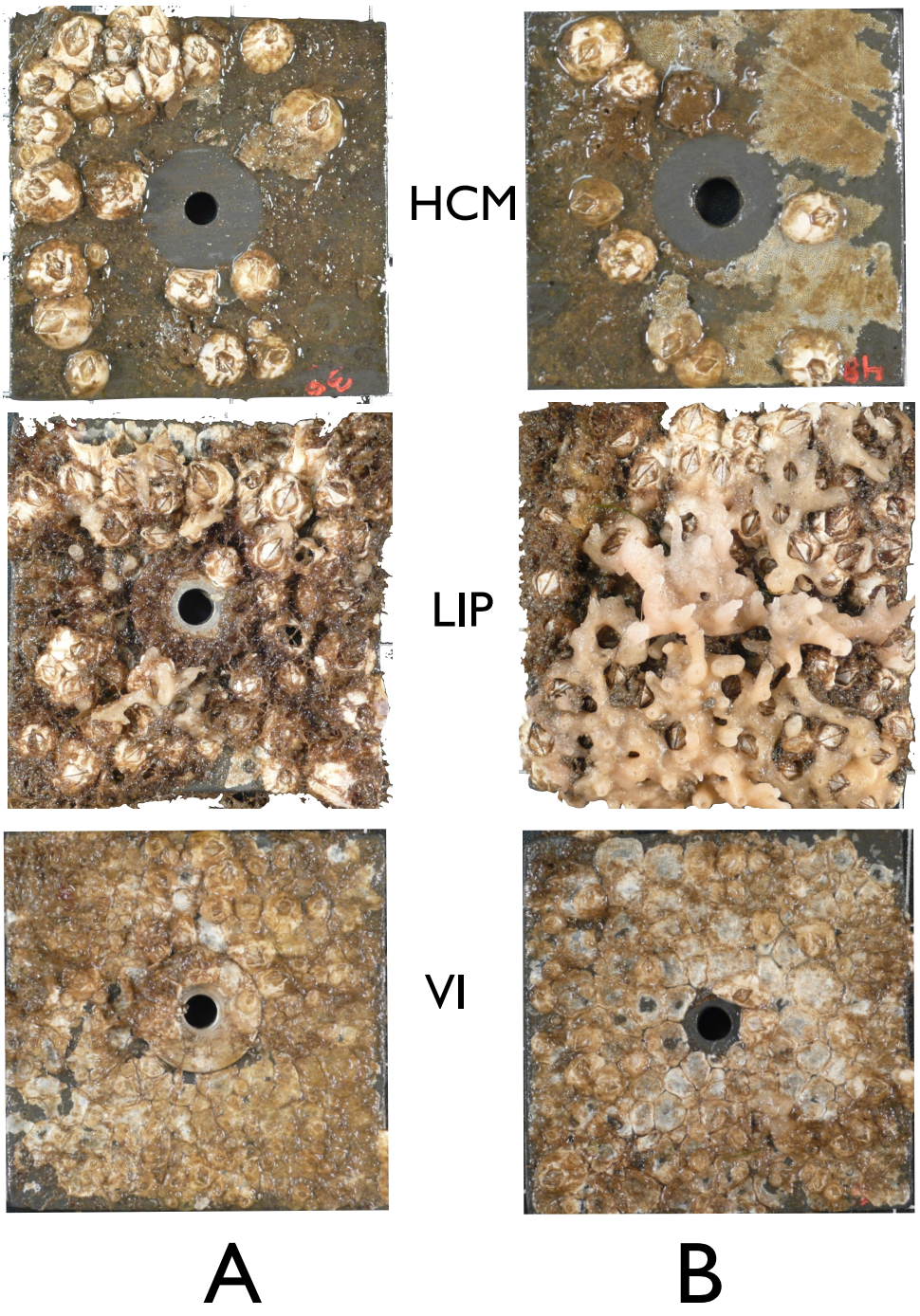

Figure 30. Cumulative slate plate cover after one year for three most riverine sites, HCM, LIP and VI. Plate length and width are 8 cm, and each site had two replicates (A, B).

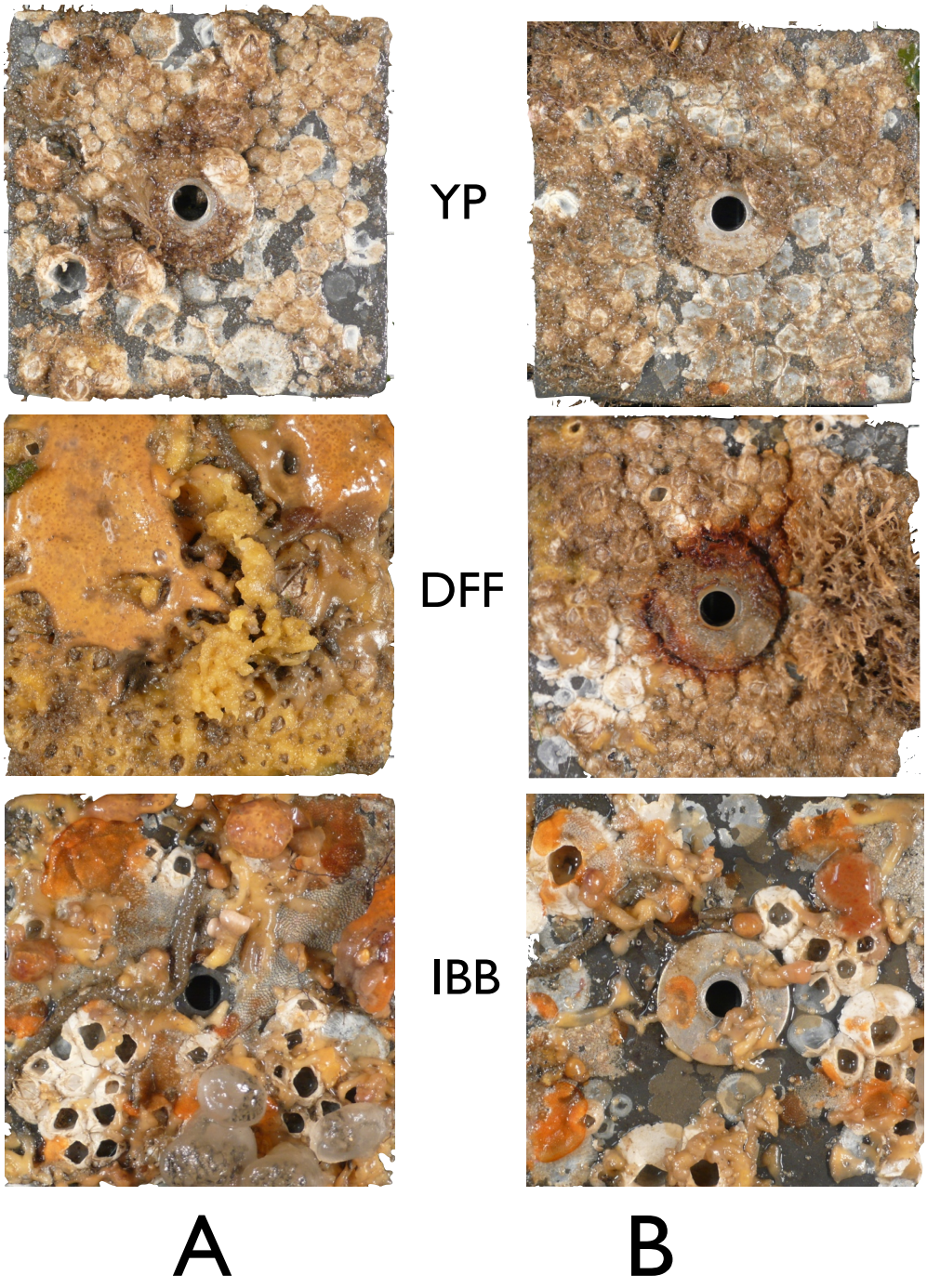

Figure 31. Cumulative slate plate cover after one year for three most oceanic sites, VI, DFF and IBB. Plate length and width are 8 cm, and each site had two replicates (A, B).

Table 10. Species presence on settlement plates in South Slough at six sites (HCM, LIP, VI, YP, DFF and IBB) monitored monthly over the course of one year (2011). Settlement plate type is specified by letter: s-cumulative slate plate (n=2), c- cumulative petri dish (n=5), m-monthly petri dish (n=5). Asterisks indicate periods where sites were unable to be sampled.

Month	1	2	3	4	5	6	7	8	9	10	11	12
1. Hidden Creek Marsh												
ARTHROPODA												
Amphipod sand tube		m	cm	*	m	cm	c	*	m			
Balanus spp.				*	cs	s	s	*	sc	sc	sc	sc
BRYOZOA												
Celloporella hyalina				*				*	m			
Conopeum reticulum				*		m	s	*	sc	sc	s	s
Bowerbankia sp.				*			s	*	c	c	c	c
Tricellaria sp.				*				*	s			
CNIDARIA												
Unidentified colonial hydroid		m		*	m			*	m			
PORIFERA												
Halichondria bowerbanki				*				*	sc	sc		
2. Long Island Point												
ARTHROPODA												
Amphipod sand tube				c		m	m		m			
Balanus spp.				cm	sc	sc	scm	scm	sc	c	c	c
BRYOZOA												
Bowerbankia sp.								scm	sc	sc		
Celloporella hyalina								m	m			
Conopeum reticulum					cm	cm	c		c		s	s
Microporella californica								m		m		
Microporella californica								m		m		
Schizoporella japonica								**c**	**c**	**c**	**c**	**c**
CNIDARIA												
Calcyella syringia											s	s
Metridium senile					m							
Obelia longissima					sc	sm	scm				s	s
Unidentified colonial hydroid				m	c	cm	cm	m	scm	scm	c	c
MOLLUSCA												

Month	1	2	3	4	5	6	7	8	9	10	11	12
Mytilus sp.											s	s
PLATYHELMINTHES												
egg mass							m					
PORIFERA												
Halichondria bowerbanki							m	scm	sc	sc		
3. Valino Island												
ARTHROPODA												
Amphipod sand tube		m					m					
Balanus spp.			s	sc	sc m	sc	sc m	sc m	sc m	sc	sc	sc
BRYOZOA												
***Alcyonidium* sp.**				**c**	**c**							
Bowerbankia sp.				c	m		c	sc	sc	c	c	
***Bugula* spp.**								**c**	**c**	**c**	**c**	**c**
Celloporella hyalina		sm	sc	s	m	cm		m	m		s	s
Conopeum reticulum						m	cm	m	m		s	s
Microporella californica							c			cm	c	c
CNIDARIA												
Calcyella syringia											s	s
Obelia longissima											s	s
Unidentified colonial hydroid		m	c	m	m	cm	scm	m	m	s		
UROCHORDATA												
Distaplia occidentalis							m					
PORIFERA												
Halichondria bowerbanki						m	cm	scm	scm		s	s
MOLLUSCA												
Mytilus sp.											s	s
PLATYHELMINTHES												
egg mass							m					
4. Yonker's Point												
ARTHROPODA												
Amphipod sand tube		cm	m				*					
Balanus spp.		s	s	scm	sc	sc	*	scm	scm	s	s	s

Month	1	2	3	4	5	6	7	8	9	10	11	12
BRYOZOA												
***Alcyonidium* spp.**							*				**s**	**s**
Bowerbankia sp.					m		*	scm	scm		s	s
***Bugula* spp.**						**s**	*	**s**	**s**			
Celloporella hyalina				scm	cm	c	*	cm	cm		s	s
Conopeum reticulum							*				s	sc
Microporella californica							*			cm	cm	scm
Schizoporella japonica						**s**	*	**s**		scm	scm	scm
CNIDARIA												
Calcyella syringia							*				s	s
Metridium senile					s	s	*					
Obelia longissima							*				s	s
Unidentified colonial hydroid				m	scm	scm	*	cm	scm	s		
PORIFERA												
Halichondria bowerbanki							*				s	s
MOLLUSCA												
Dendronotus frondosus eggs					m		*					
5. Distant Fleet Facility												
ARTHROPODA												
Amphipod sand tube							m					
Balanus spp.				scm	sc	sm	scm	scm	sm	sc	sc	sc
BRYOZOA												
***Alcyonidium* sp.**						**c**					**c**	**c**
Bowerbankia sp.							c	sc	sc	s	s	s
***Bugula* spp.**				**m**		**c**	**c**	**sc**	**c**		**s**	
Celloporella hyalina		scm	scm		scm	scm	cm	cm	m			
Conopeum reticulum						c						
Microporella californica					s		sc	c	cm	cm	cm	cm
Schizoporella japonica								**sc**	**cm**	**c**	**c**	**m**
Tricellaria sp.				s	s	s		s	s	s	s	s
CNIDARIA												
Obelia longissima											s	

Month	1	2	3	4	5	6	7	8	9	10	11	12
Unidentified colonial hydroid		s		sm	scm	scm	scm	cm	cm	m		s
UROCHORDATA												
Botrylloides schlosseri							**s**					
Distaplia occidentalis							m	sm	sm	s		cm
PORIFERA												
Halichondria bowerbanki								s		s	s	s
MOLLUSCA												
Dendronotus frondosus eggs						c						
Mytilus sp.							m		s			
6. Inner Boat Basin												
ARTHROPODA												
Amphipod sand tube				m	m							
Balanus spp.			s	scm	scm	sc	scm	scm	scm	scm	s	s
BRYOZOA												
***Alcyonidium* sp.**								**sc**			**s**	**s**
Bowerbankia sp.							m	m				
***Bugula* spp.**			**sc**	**m**	**m**	**m**		**m**	scm		**s**	**s**
Celloporella hyalina			s	sc	sc	sc			m			
Microporella californica							sm		m			
Schizoporella japonica				**sm**	**sm**	**sm**	**sm**	**sm**	**sm**	scm	scm	scm
CNIDARIA												
Obelia longissima												s
Unidentified colonial hydroid							m					
UROCHORDATA												
Botrylloides schlosseri										**c**	**sc**	**c**
Botryllus violaceus					**m**	**m**						**s**
Corella inflata						s	s	sc	c	sc	sc	sc
Distaplia occidentalis				sm	m	scm	scm	scm	scm	scm	scm	scm
***Styela* sp.**								**s**				
ANNELIDA												
Spirorbis sp.							m					
MOLLUSCA												

Month	1	2	3	4	5	6	7	8	9	10	11	12
Mytilus sp.						m						

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