Settlement patterns of the invasive tunicate, *Botrylloides violaceus,* as affected by substrate type

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Writer's Comment: *I was first introduced to tunicates during the UC Davis Bodega Marine Laboratory program. This program allowed me to observe marine invertebrates up close in their natural habitats. One of the animals we were introduced to was the tunicate. I instantly became fascinated by the life cycles, body structures, and evolutionary histories of these animals. I particularly became interested in the invasive nature of many tunicates found in Bodega Bay, such as Botrylloides violaceus. I began to do my own research on tunicates in hopes of better understanding what makes them so invasive. When we were assigned to write a technical article in UWP104E, I immediately knew my tunicate research would be the subject. Although I already knew quite a bit about tunicates, this assignment was a great opportunity to perfect my scientific writing skills and learn even more. I hope my paper can help show others that marine invertebrates, including odd-looking ones such as tunicates, are fascinating and ecologically important creatures.*

Instructor's Comment: *The students in my winter quarter UWP 104E class kept me on my toes. This group of advanced students were all working on technical projects in labs and internships that they brought into our classroom and into their writing assignments. As an instructor, I was challenged by them and their level and breadth of scientific insight, and many of our class discussions were fascinating. Megan was a quieter student in those in-class activities, but from the first week of class, she distinguished herself through her writing. She is among the best critical thinkers I have encountered at UCD, and writes in a clear and engaging style about her research. As my own limited scientific background is on California marine ecology, I was particularly interested in her technical paper about invasive species in the Bodega area. Megan writes eloquently even in her more technical reports, and I believe she has a bright future as a scientist who can communicate her work with style.*

—Katie Rodger, University Writing Program

Abstract

Invasive species make up a large portion of fouling communities. One such species is the invasive colonial tunicate *Botrylloides violaceus*. Because of negative effects associated with fouling communities, previous research has focused on factors that influence settlement and survival. This study attempts to understand how biofilm, the presence of adult tunicates, and occupied space affect settlement patterns of *B. violaceus* larvae. *B. violaceus* colonies were collected from Spud Point Marina in Bodega Bay and light shocked to release larvae. These larvae were then exposed to tiles containing either a layer of biofilm, biofilm and tunicates, biofilm and foam, or a blank control. After twenty hours, larval settlement was assessed and data collected. The tiles containing the biofilm had the highest survival rates and larval settlement, followed by the control. This helps explain why *B. violaceus* is such a successful invasive species, as individuals are able to settle even without necessary cues from biofilm. The foam and tunicate tiles had lower survival rates and larval settlement. However, there was no statistically significant difference between the two. This suggests occupied space is an important factor influencing settlement. Studying settlement patterns of fouling species is important as it can provide information about community structure, distribution patterns, invasive potential and spread, and even possible management strategies.

Introduction

Fouling organisms grow on artificial substrates and are often found on docks, marinas, and boats. Many of these fouling organisms are invasive, brought in by boats, debris, and cargo (Gollasch, 2002; Karlson & Osman, 2012; Lacoursiere-Roussel, Forrest, Guichard, Poila, & McKindsey, 2012). Once these organisms arrive in new areas, they are in the absence of natural diseases and predators and thus often spread quickly. Invasive species that make up fouling communities often have negative effects, disrupting local operations as well as ecosystems (Murray, Therriault, & Martone, 2012; Schultz, Bendick, Holm, & Hertel, 2011; Sievers, Fitridge, Dempster, & Keough, 2013; Wong & Vercaemer, 2012).

One such damaging invasive species is the colonial ascidian *Botrylloides violaceus*. After a short free-swimming larval period, *B. violaceus* tadpoles settle on a substrate and begin development (Swalla, 2004). Because of ascidians' role as invasive fouling organisms, studies have tried to determine factors that influence larval settlement and survival. The presence of biofilm (Todd & Keough, 1994; Wieczorek & Todd, 1997) and adult conspecifics (Bullard, Whitlatch, & Osman, 2004; Osman & Whitlatch, 1995; Rius, Branch, Griffiths, & Turon, 2010) appears to be contributing factors.

Biofilm, an aggregation of microorganisms on a surface, is thought to be important to larval settlement because it chemically signals that a surface is suitable for settlement (Todd & Keough, 1994; Wieczorek & Todd, 1997). These cues are thought to indicate available resources or the presence of other organisms (Roberts, 2001). Adult conspecifics are also thought to influence settlement. However, whether adults positively or negatively influence ascidian settlement has been a matter of debate. For example, some studies indicate that adult conspecifics do not have any effect on larval settlement (Bullard et al., 2004). Others have found that adult conspecifics limit the success of settlement, possibly by competing with larvae for space or releasing anti-fouling chemicals (Osman & Whitlatch, 1995). Other studies have found that the influence of adults differs depending on the species involved (Rius et al., 2010). These conflicting results may be due to the fact that many of these studies do not differentiate between the influence of adults due to chemical cues and the role of ascidians as an occupied space independent of these cues.

This study seeks to understand how adult conspecifics, occupied space, and the presence or absence of biofilm affect *B. violaceus* larval settlement and survival. Because biofilm contains necessary chemical cues for survival (Roberts, 2001), it is predicted that biofilm will increase *B. violaceus* larval settlement and survival. In addition, it is hypothesized that adult conspecifics will lower survival and settlement rates due to their role as an occupied space, as well as the presence of chemical deterrents released by these adults. Occupied space alone will also negatively affect settlement and survival because larvae will likely have a more difficult time attaching to an already occupied substrate. However, because occupied spaces do not necessarily contain chemical deterrents, it is predicted that occupied space will not influence *B. violaceus* larval settlement and survival as much as the presence of adult conspecifics.

Methods

Tile preparation

10 by 10 cm tiles made of Plexiglass served as a substrate for this experiment. Tiles were fastened to a PVC pipe with a zip tie (**Figure 1**) and tied to the Spud Point Marina dock in Bodega Bay. Tiles hung 50cm below the water's surface at the same water height as *B. violaceus* species. This allowed for accumulation of a biofilm.

After seven days, tiles were cut from the PVC pipe and randomly assigned a treatment. There were three treatments and one control. The control tile had no biofilm and had not been in seawater. The first treatment tile had only a layer of biofilm. The second treatment tile had biofilm and an adult *B. violaceus* colony. The third treatment tile had biofilm and a piece of foam. The foam was used to control for substrate, differentiating between settlement because of occupied space and settlement because of chemical cues from adult *B. violaceus* individuals. The piece of foam was cut to resemble the shape of the adult tunicate. A metal tool was used to create a pattern similar to the rows of siphons on a tunicate. Tunicates and foam were fastened to tiles with a rubber band. A rubber band was also placed on the control and biofilm tiles to account for any effect the rubber band may have had on larval settlement. There were three replicates of the control and each treatment, making for a total of twelve tiles.

B. violaceus collection and storage

Fifty-five *B. violaceus* colonies were collected from Spud Point Marina in Bodega Bay, CA. Tunicates were scraped off of the dock and temporarily stored in water-filled buckets for transport. Research has shown that tunicates can be induced to spawn by shocking colonies with light after a period of darkness (West & Lambert, 1975). To start this process, tunicates were collected from the marina and placed in coolers supplied with aerated, filtered seawater. The tunicates were then left in complete darkness inside the coolers.

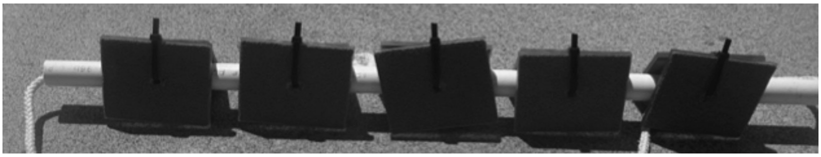

Figure 1: PVC pipe with tiles

Spawning and settlement of B. violaceus

After twenty hours of darkness, tunicates were removed from the coolers and placed in glass bowls containing filtered seawater. Microscope lights aimed at these bowls induced the release of larvae. Larvae were pipetted one at a time into containers containing one of the four tiles previously discussed — the control, biofilm, biofilm and tunicates, or biofilm and foam (**Figure 2**). Eleven larvae were pipetted into each container. Containers sat on water trays overnight. Eighteen hours later, the number of larvae on the tile, on the container, dead, and still swimming was recorded.

Results

All proportions in this experiment were first transformed using Arcsin square root.

The mean proportion of larvae that settled on the tiles differed among treatments (ANOVA, $F_{3,8}=50.0$, $p<0.0001$, **Figure 3**). The biofilm tile had the greatest mean proportion of larvae tile settlement (Tukey-Kramer, $p<0.01$, **Figure 3**), followed by the control (Tukey-Kramer, $p<0.01$, **Figure 3**). There was no statistically significant difference between settlement on the tunicate and foam tiles (Tukey-Kramer, $p>0.05$, **Figure 3**).

The survival rates for larvae also differed among treatments (ANOVA, $F_{3,8}=31.8$, $p<0.0001$, **Figure 4**). Survival rates included larvae that had settled on tiles, the container, as well as those that were still swimming. Survival rates were greatest for the biofilm treatment. There was no statically significant difference in survival rates among the control, foam, and tunicates treatments (ANOVA, $F_{2,6}=3.17$, $p=0.115$, **Figure 4**).

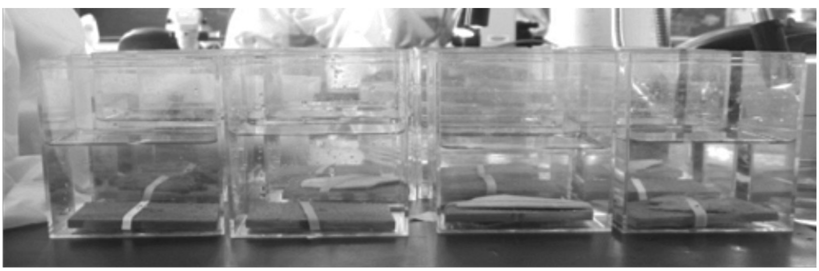

Figure 2: Tiles in containers filled with filtered seawater

Discussion

Biofilm Tile

This study supported previous research and the initial hypothesis, emphasizing the importance of biofilm for successful settlement (Todd & Keough, 1994; Wieczorek & Todd, 1997). Biofilm significantly enhanced the survival and settlement of *B. violaceus* larvae, suggesting that new communities will most likely be found in unoccupied areas that have accumulated a significant biofilm. The biofilm accumulated in this experiment was from the same area as the adult colonies. Further research may examine the effects of biofilm from different areas on larval settlement, potentially helping to assess invasive potential.

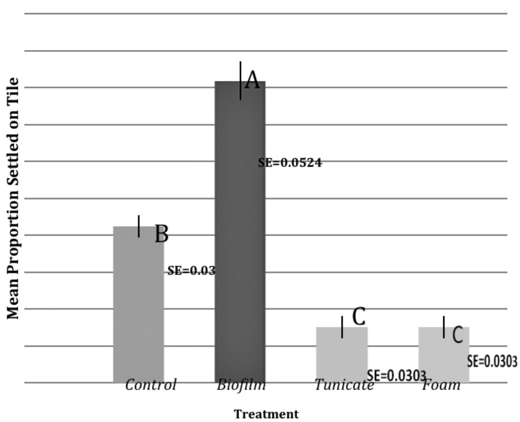

Figure 3: Mean Proportion of larvae that settled on each tile (n=11 larvae per replicate container). Mean proportion rates differed among treatments. Levels not connected by the same letter are significantly different.

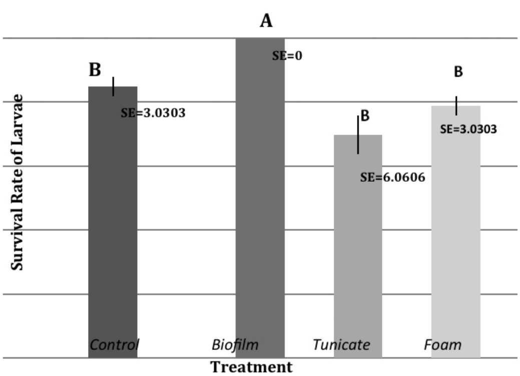

Figure 4: Survival rates were greatest for the biofilm treatment. Levels not connected by the same letters are significantly different.

Control tile

The observation that the control tile had the second greatest settlement may help to explain why *B. violaceus* is such a successful invasive species. Even without a biofilm indicative of resources, larvae can still attach and begin development. Many fouling treatments focus on eliminating the bacterial biofilm from substrates in hopes that this will deter larval settlement (Carver, Mallet, & Vercaemer, 2006). However, the high levels of settlement on the control tile suggest that just eliminating the biofilm will not be enough. Other mechanical and chemical methods must be considered.

Understanding settlement patterns is important in determining management strategies, as these patterns tell researchers where larvae will most likely settle and survive. The areas with the highest settlement and survival rates will become the highest priority when developing management strategies. High priority areas for *B. violaceus* would be relatively unoccupied stretches of harbor that have already accumulated significant regionally specific biofilm. However, attention would still need to be given to clean boats and new aquaculture cages that have just entered the area, as even substrates without biofilm can be colonized. Areas that are already established with dense tunicate colonies would be of lower priority, as settlement and survival are lower. Settlement patterns are often species specific (Rius et al., 2010). This suggests that additional studies need to be carried out to develop a more comprehensive management strategy for combating fouling communities as a whole.

Foam and tunicate tiles

The initial hypothesis that occupied space and adult conspecifics would negatively affect *B. violaceus* settlement and survival was supported, as larval settlement rates were lowest for both the tunicate and foam tiles (**Figure 3**). However, there was no statistically significant difference in settlement between the two. This indicates that larval settlement was not affected by chemical cues from adult tunicates. Instead, occupied space is the more important factor influencing *B. violaceus* settlement. Larvae may be more likely to settle away from established tunicates and start new communities. This is supported by observations within the study site as *B. violaceus* colonies tended to be spread across the general area of a dock or marina rather than clumped in one spot. Other factors such as resources and competition may influence this distribution, yet settlement patterns are also an important factor.

Although larvae did not settle directly on or near occupied space, larvae did eventually settle approximately 10 cm away from the foam and tunicate tiles. This suggests that in the beginning of the larval life cycle, space to grow is extremely important. Once zooids grow into larger colonies, competition may become increasingly important. As fouling communities become more established and take up all available space, newly introduced *B. violaceus* larvae may have lower success.

It is important to keep in mind that in this case the occupied areas were soft, slippery surfaces that resembled a tunicate. Soft spaces may specifically deter settlement as larvae attach to substrates by elongated ampullaes (Carver et al., 2006). Ampullae may have trouble attaching and sticking to areas that are soft and slippery such as a tunicate or a wet piece of foam. On hard surfaces, larvae may have an easier time attaching and thus survival rates may increase. This observation was supported in the field as tunicates were increasingly observed growing on top of hard surfaces, such as limpets and mussels, but were rarely observed growing directly on top of one another. Further research may look at these differences in settlement between hard and soft substrates.

In this study, biofilm and soft occupied substrates influenced *B. violaceus* settlement. This has implications for invasive species management, community structure, distribution patterns, and intraspecies interactions. Yet understanding settlement patterns is not only important for *B. violaceus*, but for all marine organisms. Larvae comprise future generations, thereby giving clues to where organisms may disperse, how far they may go, and how settlement may affect a community. This is especially important in the case of invasive species, as demonstrated by *B. violaceus*.

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