The Effects of Predation by the Pacific North West Nudibranch *Janolus fuscus* (O'Donoghue) on the Bryozoan larvae of *Bugula pugeti* (Robertson)

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Abstract

The sensory organs of larvae of the bryozoan *Bugula c.f. californica* are very important during the short transition from the swimming phase to the brief settlement phase. These sensory organs detect environmental cues that initiate and regulate settlement within a favorable habitat. However, these organs may also allow the larvae to perceive unfavorable habitat and avoid predation. This study looked at the settlement response of the competent larvae of *Bugula c.f. californica* when they came in contact with traces of a predatory nudibranch, *Janolus fuscus*, with a known positive settlement cue (biofilm), and with traces of a non-predatory nudibranch, *Archidoris montereyensis*. A known number of larvae were kept in small petri dishes filled with filtered seawater at about 12 degrees Celsius with no flow inside the dishes. Prior to adding the newly released larvae, the petri dishes were divided into four groups that were smeared with the three following treatments: mucus from the predatory nudibranch, mucus from the non-predatory nudibranch, and a thin layer of biofilm. Clean (un-filmed) dishes with FSW served as the control. A second study looked at preferential settlement with the known positive- biofilm, the biofilm substratum with predatory water, and ½ biofilm substratum next to ½ predatory mucus substratum. We found that the larvae in the biofilm with FSW settled early in their planktonic period. The larvae in the dishes with predator mucus, dishes with biofilm and predator water, in dishes with a choice of biofilm or predator mucus delay their settlement- perhaps in search of optimal habitats. Furthermore, when the biofilm and predator mucus exist in the same dish, the larvae preferentially settle in the side with biofilm, which suggests that they try to avoid potential predation.

1. Introduction

There are a variety of environmental cues that promote or discourage settlement for the larvae of many sessile marine invertebrates in fouling communities. Settling in a favorable habitat or avoiding unfavorable habitat by perceiving and responding to the environmental cues is important to the survival, growth, and metamorphosis of these larvae (Ueda & Degnan, 2014). In order to detect chemical cues in their environment the larvae are equipped with sensory organs (Hadfield *et al.*, 2000). The lecithotrophic larvae of the arborescent bryozoans in the genus, *Bugula*, have two larval sensory organs: the aboral apical disc and the anterior pyriform organ that is thought to assist in substrate selection during settlement (Strathmann, 1992; Young *et al.*, 2002). Natural cues such as biofilms and diatoms that cover the substratum are often considered promoting factors of larval settlement in several invertebrate phyla (Dahms *et al.*, 2004; Wang et. al, 2014). Biofilms that consist of a number of microorganisms and bacteria can induce the larvae of *Bugula* to settle (Young *et al.*, 2002; Dahms *et al*., 2004; Qian *et al.*, 2007).

Larvae may delay their settlement in order to select a favorable habitat (Woollacott *et al.*, 1989; Romero *et al.*, 2013; Wendt, 1996; Wendt, 1998). There is evidence that barnacle larvae avoid settling in areas previously occupied by motile benthic predators (Johnson & Strathmann, 1992), and *Bugula pacific* will avoid settling around traces of their ascidian spatial competitor, *Diplosoma macdonaldi* (Young & Chia, 1981). Chemical cues emitted by the organisms that occupy these communities may have a great influence on larval settlement.

In this study, we examined the settlement of larvae of *Bugula c.f. californica* in response to predator cues and other cues. The bryozoan *B. c.f. californica* was chosen because their larval pre-settlement and settlement phases are short (Young *et al.*, 2002), the gravid zooecia are easily

induced to release multiple larvae around the same time (Wendt, 2000), and both the adults and larvae are easily collected. *Bugula c.f. californica* is an arborescent bryozoan, characterized by light orange, erect spiraling branches with multiserial zooecia (Kozloff, 1987). The adult colonies release free swimming, lecithotrophic larvae that spend up to a few hours in the water column before settling down permanently on suitable stable substrata (Woollacott *et al.*, 1989; Lamb and Hanby, 2006). The nudibranch, *Janolus fuscus*, (Fig. 1) was selected as the predator of *B. c.f. californica* since it is a primary predator of arborescent bryozoans in the fouling habitats of the Pacific Northwest (Strathmann, 1992; Wolf, 2010; Wolf & Young, 2012). The nudibranch, *Archidoris montereyensis*, (Fig. 2) was selected as the non-predator since it predates on sponges within the intertidal and subtidal habitats (Strathmann, 1992).

In the past, many studies mainly focused on the effect of predation on the adult bryozoan colonies (Harvell, 1981; Yoshioka, 1982; Harvell, 1984), but fewer studies investigated the interactions between the larvae of prey species and predators. One such study looked into the larvae mechanisms for avoiding the long term risks of predation (Johnson & Strathmann, 1989). For this study, we propose that in an ideal habitat the larvae of *Bugula c.f. californica* may select a spot and settle down a few hours into their planktonic phase, but in an unfavorable environment with traces of the predator, *Janolus fuscus*, the larvae may delay their settlement.

2. Method and materials

2.1 Sampling procedure

Reproductive colonies of *Bugula c.f. californica*, showing a slight orange coloration from the orange colored embryos that are brooded in the zooecia, were collected from the dock at

Friday Harbor Laboratories, Friday Harbor, WA, between August 12th and August 14th of 2014. The colonies were kept in flow-through plastic containers within a dark tank with running seawater at about 12 degrees Celsius overnight. The next day, the colonies were transferred into clean jars half filled with filtered sea water, and placed in an outdoor tank with circulating open system seawater, and exposed to the peak of natural sun light for a period of several hours, in order to induce larval release. On one day it was raining, but that did not seem to impede larval release. We checked for larvae after the first hour of exposure and every 15 minutes after that until a 40+ larvae were apparent. The gravid adult colonies released larvae approximately between 1 and 2 hours after exposure to the sunlight. Each day we collected the first batch of larvae after 30 minutes from the first observation of the apparent larvae to use in the experiment in order to ensure the analysis of the same cohort. To harvest the larvae we used crystalizing dishes filled with FSW to contain them until they were transferred via small e-ware glass pipettes into 3.5 cm diameter plastic petri dishes that were half filled with FSW and the respective treatments.

2.2 Settlement treatments

Two studies were conducted. In the first study there were a total of three different treatments and one control: traces of predatory mucus, traces of non-predatory mucus, and biofilm, with the control being FSW only. The second study had three treatments: biofilm with predator water (water exposed to the predator), half predator mucus traces on the left side of the petri dish and half biofilm on the right side. Each plastic petri dish was considered a replicate and there were three replicates per treatment, and the experiment was run once per day for two days. Three swimming larvae were gently pipetted individually into each of the dishes. Each petri dish floated in a labeled crystalizing dish filled with FSW that sat in a semi-filled flow

through wet table that stayed about 12°C in temperature throughout the experiment. The petri dishes were randomly placed within the grid on the wet table and the entire wet table was covered in a black tarp to reduce interference of light in settlement with the phototactic bryozoan larvae (Wendt & Woollacott, 1995).

2.3 Observing settlement

Each petri dish was examined under a dissection microscope once every hour after initial transfer of larvae into the treatments. The cumulative count of settled larvae was taken each time for each replicate of all the treatments. For the half and half treatment the side on which the larvae settled was recorded. The treatment dishes and their larvae were examined for about eight hours however, to simplify statistical analysis we only included data up until the sixth hour, since the settlement activity ceased after the sixth hour. The larvae of *B. c.f. californica* exhibited normal pre-settlement and settlement behavior (Fig. 3) and we counted a larva as settled once they released their internal metasomal sac (Fig. 4) to attach to the substratum (Woollacott *et al.*, 1989; Wendt & Woollacott, 1995; Young *et al.*, 2002).

2.4 Statistical analyses

Data collected from this experiment were displayed by graphs and analyzed with statistical tests for normality and homogeneity. All the graphs were made using Microsoft Excel 2010, and all the statistical analysis were analyzed using SPSS v.20.0. A one way ANOVA (analysis of variance), Kruskal-Wallis test (according to the normality of the data) as well as the One Sample T-test were used to detect whether statistically significant differences of the larval settlements exist among the different treatments. To check for homogeneity of the variance of the data, we performed a Leven´s test, and to check for normality we utilized a Shapiro-Wilk test

or Kolmogorov-Smirnov test. Furthermore, a post-hoc-test (Tukey´s HSD; honest significant difference), was conducted after ANOVA to determine which treatment groups differ.

3. Results

By the 6^{th} hour, all of the larvae in the treatment with biofilm had settled; in contrast, only 94% and 89% of the larvae settled in the FSW and non-predator mucus respectively. The percentage of the larval settlement in the treatment predator mucus showed the lowest value of 72% settlement compared with the other three treatments (Fig. 3). Since there are the significant differences among those treatments of predator mucus, non-predator mucus, biofilm, and FSW (ANOVA, $F= 8.96$, $p<0.05$), the data of the cumulative larval settlement in these treatments were compared with each other. The majority of the larvae settled on biofilm in the first and second hours (Fig. 3). The larvae in the other treatments did not settle as quickly. More larvae settled in the dishes with the FSW control within the 2^{nd} and 5^{th} hour, more larvae settled in the treatment with non-predatory mucus during the 2^{nd} and 6^{th} hours, and more larvae appear to have settled in the treatment with predatory mucus during the 3^{rd} and 6^{th} hours (Fig. 3).

At the 6^{th} hour, 100% of the larvae in the biofilm with FSW treatment settled. Only 50% of the larvae in the treatment of half biofilm with half predator mucus settled. For the treatment of biofilm with predator water 83% settled after 6 hrs (Fig. 4). These settlement percentages are significantly different (Kruskal-Wallis, $p<0.05$). The majority of the larvae in the biofilm with FSW treatment settled in the first two hours, but the larvae in the treatment of half biofilm with half predator mucus, and biofilm with predator water kept on settling constantly over the first 4 hours (Fig. 4).

There are no significant differences of larval settlement in the treatment of half biofilm with half predator mucus in the six replicates (One sample T-test, $t= -1.41$, $df=5$, $p>0.05$), and therefore, an average value of the larval settlement on the right side can be obtained and shows that 33% of the larvae are settling on the right side in the biofilm, and only 17% of the larvae settle on the left side in predator mucus. However, the remaining 50% of the larvae continue to swim past the sixth hour of observation (Fig. 5).

4. Discussion

In this study, we found that the larvae in the biofilm settled in their early planktonic stage, but the larvae delayed their settlement when exposed to traces of predator mucus, biofilm with predator water, or a choice of biofilm and predator mucus in the same dish. Although a larger sample size is needed to verify these observed trends, it appears that the larvae preferentially select the optimal habitats for their short term settlement and metamorphosis, and long term growth and reproduction processes (Young & Chia, 1981; Johnson & Strathmann, 1989). Larvae may prolong their planktonic phase until they encounter a more favorable site. This extension of their planktonic period may be the larvae's defense mechanism to avoid the potential predation of a predator until the bryozoan colony has had a chance to grow in size enough to survive "prudent predator" grazing (Harvell, 1981), or even to compete for fouling habitat space with competitors like ascidians (Young & Chia, 1981). Many larvae exhibit a trade-off of showing a tendency to avoid predation based on signs from environmental cues by prolonging their pre-settlement phase, a tactic that can lower the juvenile and consequently the colony fitness (Wendt, 1996; Pechenik, 1998; Wendt, 1998), in order to invest time in reaching a size and age of maturity before encountering predation, a phenomenon described as "the ghost of

predation future" by Johnson and Strathmann (1989). However, not all colonial larvae are created equal.

For lecithotrophic larvae the amount of maternal embryonic provisioning is strongly correlated with larval size (Pechenik, 1998; Allen *et al.*, 2008). Bryozoan colonies may release fewer larger lecithotrophic larvae with more energy in their yolk provided by the maternal zooids during embryogenesis (Strathmann, 1992), and therefore, these larvae may spend more time searching for their favorable habitats. As lecithotrophic larvae, the *Bugula c.f. californica* offspring are endowed with more energy reserves than the typical planktotrophic larva, but larval size varies within the same age cohorts, which could be the result of a colonial strategy to produce offspring with different dispersal capabilities (Burgess *et al.* 2009). Within the *Bugula* species larger larvae tend to swim for longer periods than the smaller ones and consequently advect and colonize habitats farther away, while the smaller larvae are more likely to settle near the maternal colony (Burgess *et al*. 2009; Kosman and Pernet 2009) and may not be able to avoid potential predation. The small size of the larvae and lower energy content may force them to accept the sub-optimal habitats before depleting their energy for metamorphosis and growth.

This study examined the preferential selection of settlement habitat for the bryozoan *Bugula c.f. californica* larvae in that they prefer to settle in biofilm and that they appear to be able to detect and distinguish between predator mucus and non-predator mucus. Unfortunately, due to the limited experiment time and small sample size, sufficient data was not collected from this study to prove the hypothesis referred to above. In future studies it would be beneficial to improve upon these limitations and to take into account the fast swimming speeds of these bryozoan larvae when considering the volume and surface areas of the experimental tanks. Taking a look at the parental colonies' reactions to the presence or traces of a predator versus a

non-predator would be interesting as well, since other bryozoans and sponges have been found to exhibit defenses against predation (Harvell, 1981; Yoshioka, 1982; Harvell, 1984; Knowlton & Highsmith, 2005) .

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Figures

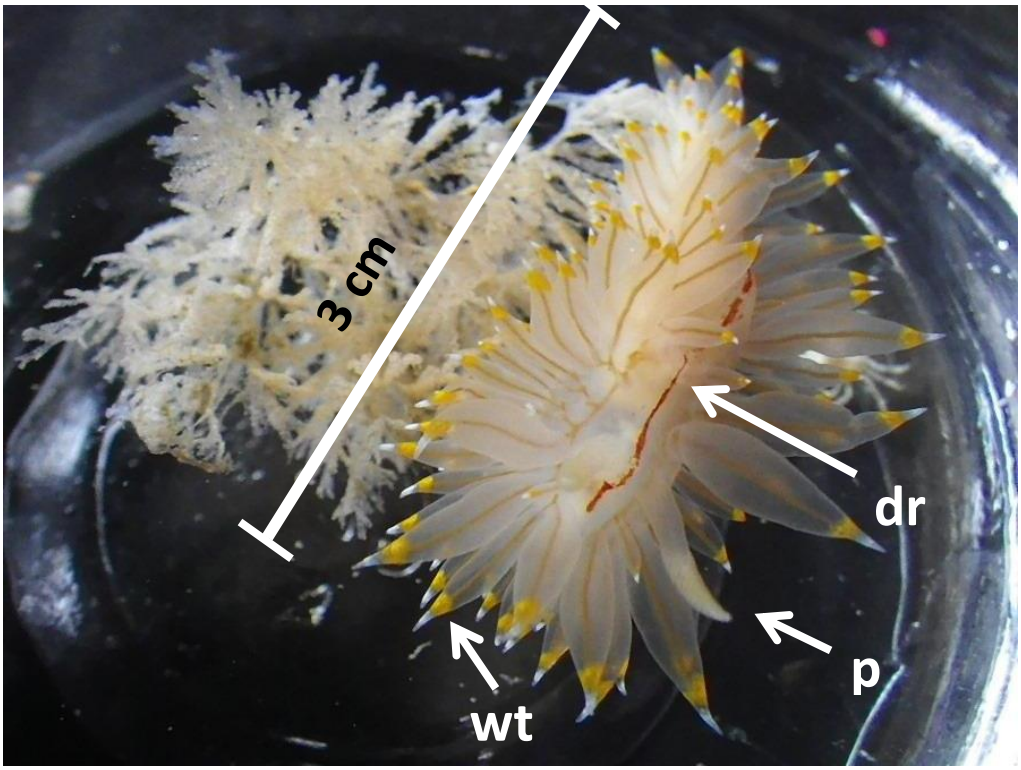
Figure 1. Nudibranchs. **A.)** The predatory nudibranch, *Janolus fuscus*, found in Pacific Northwest fouling communities with (wtc) white tipped cerata, (drl) dorsal red line, and (pr) perfoliate rhinophores imbedded in the cerata. **B.)** The predatory nudibranch, A*rchidoris montereyensis*, found in the Pacific Northwest intertidal and subtidal communities with (pr) perfoliate rhinophores, (gs) exposed dusky colored gills, and (bt) black spots on and between dorsal tubercles as described in the taxonomic key by Kozloff (1987).

Figure 2. Larvae of *Bugula c.f. californica*. **A.)** Larva with vibratile plume (vp) that is a component of the pyriform organ that assists in settlement. **B.)** Larva showing the apical disc (ap) that serves as a larval sensory organ, and the vibratile plume (vp) within the superior glandular field (sgf) of the pyriform organ as described by Strathmann (1987) and Young *et al.* (2002). The larva uses vibratile plume to 'crawl' along a surface as it searches for an optimal settlement site. **C.)** A settled larva of *Bugula c.f. californica* that has everted the internal sac (is).

Figure 3. The cumulative average of larval settlement in the different treatments of predator mucus, non-predator mucus, biofilm, and FSW over time is shown. There is a tendency for the predator mucus treatments to have lower cumulative settlement than the biofilm treatments.

Figure 4. This graph shows the cumulative average of the larval settlement in the three different treatments of half biofilm with half predator mucus, biofilm with predator water, and biofilm with FSW over time. The cumulative average of larval settlement in the biofilm with FSW is much higher than in the half and half treatment.

Figure 5. This graph shows the percentage of the larval settlement in the treatment of half biofilm with half predator mucus at the 6^{th} hour and looking at which side of the dish that the larvae settle on. The left sides of the dishes had predator mucus trails and the right side had biofilm. Besides the 50% that did not settle, a majority of the remaining larvae settled on the right side of the dishes. In hind sight more repetitions would be useful to verify this tendency to avoid the traces of predatory mucus. (It should be noted that the volume and diameter- about 3.5 cm- of the petri dishes were not of a large scale.)



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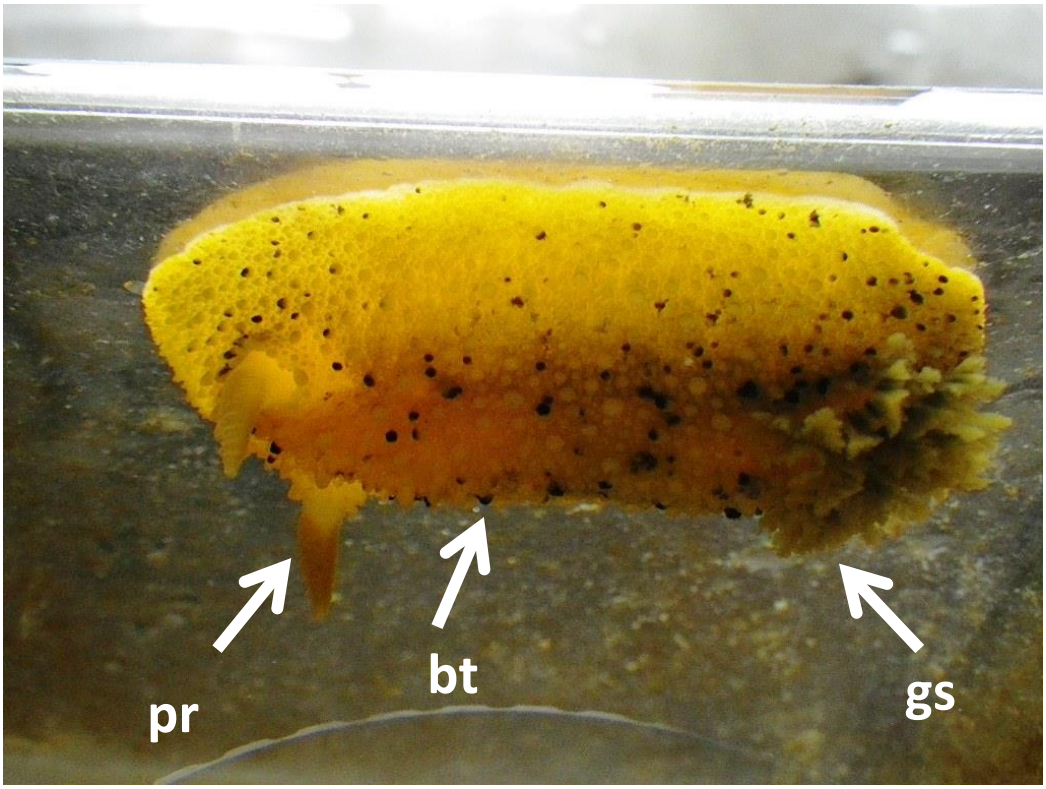

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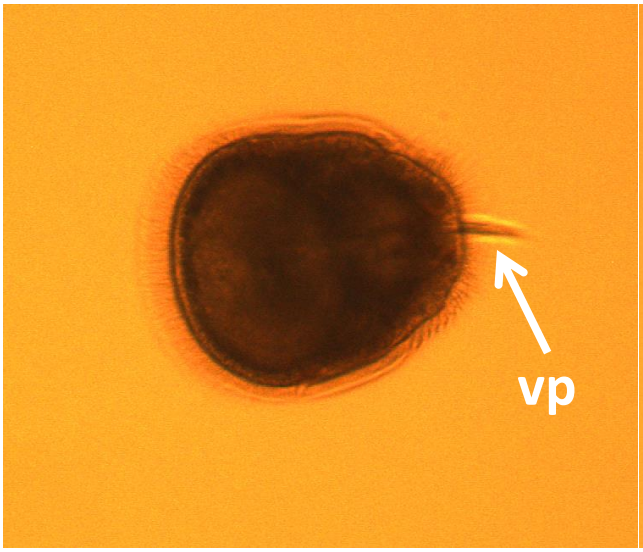

Figure 2 A

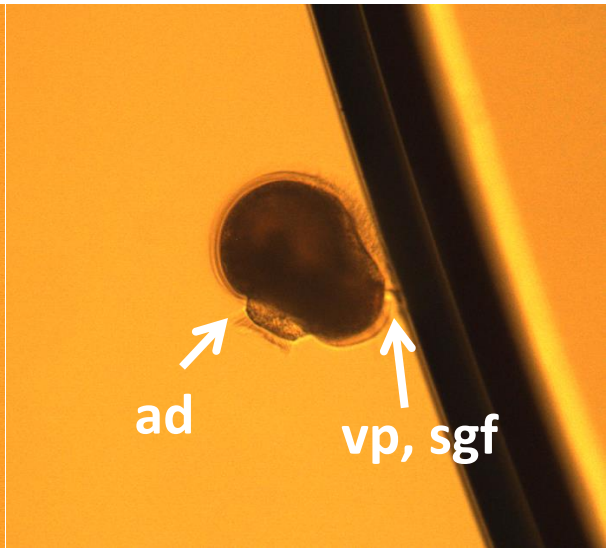

Figure 2B

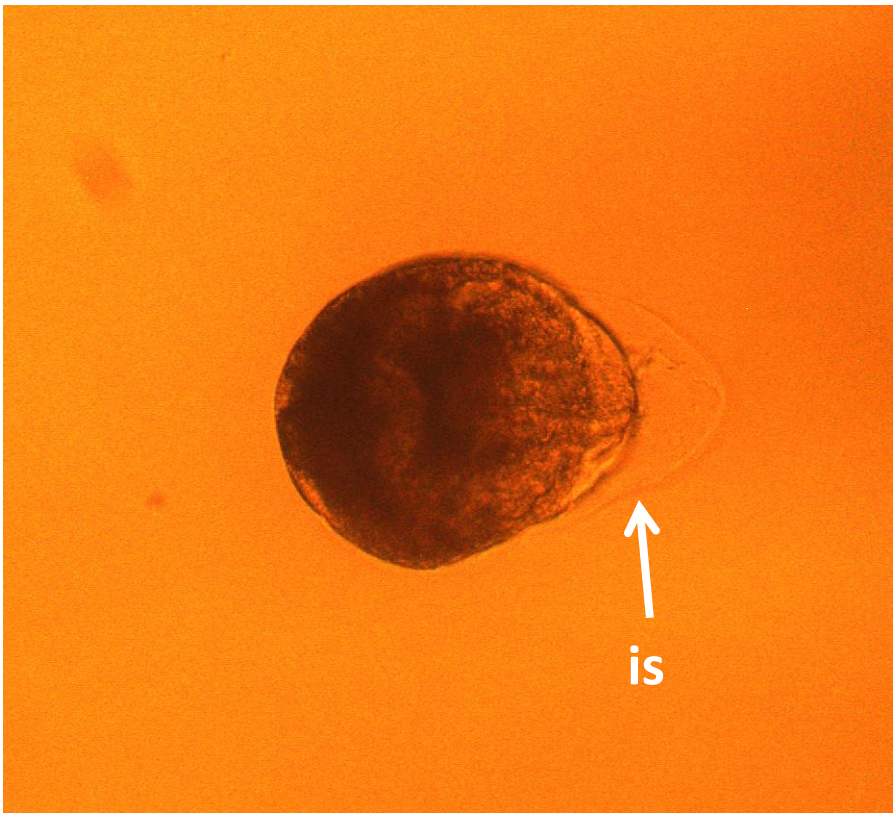

Figure 2 C

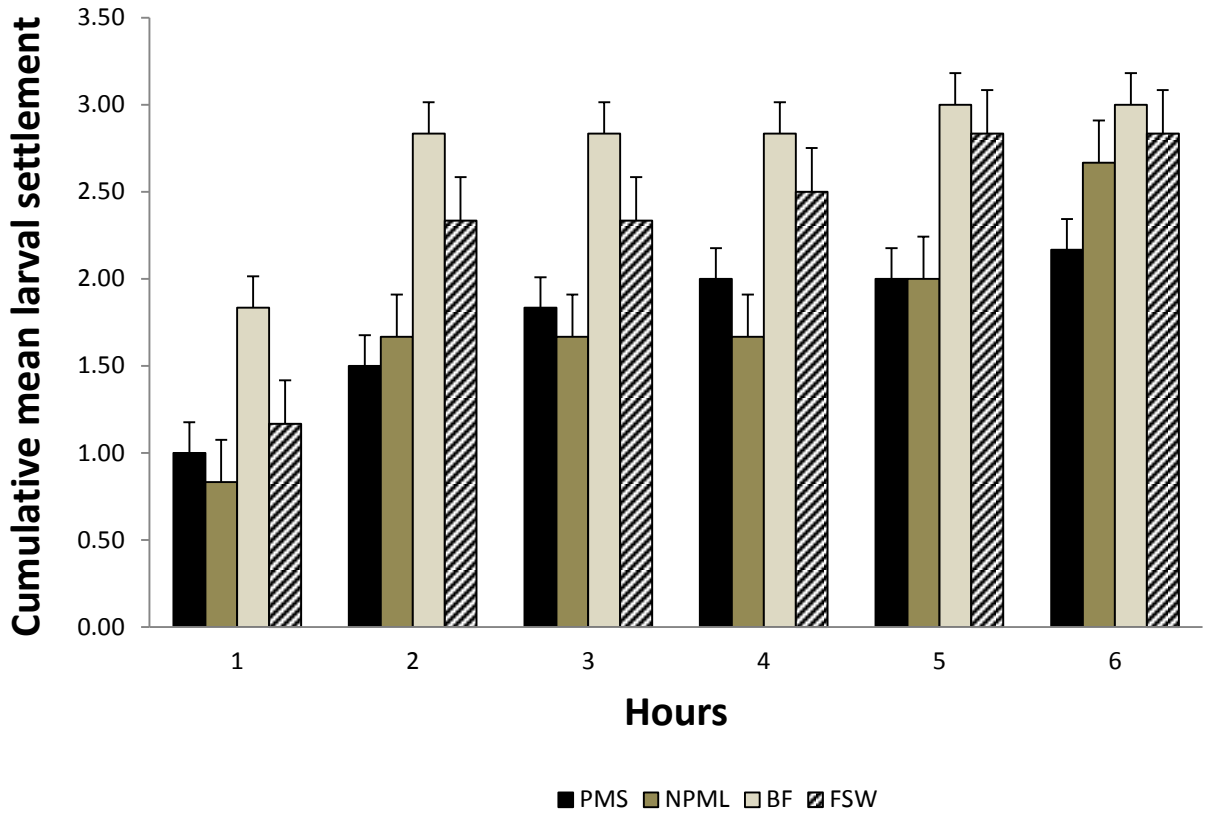
Cumulative mean larval settlement
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Figure 3

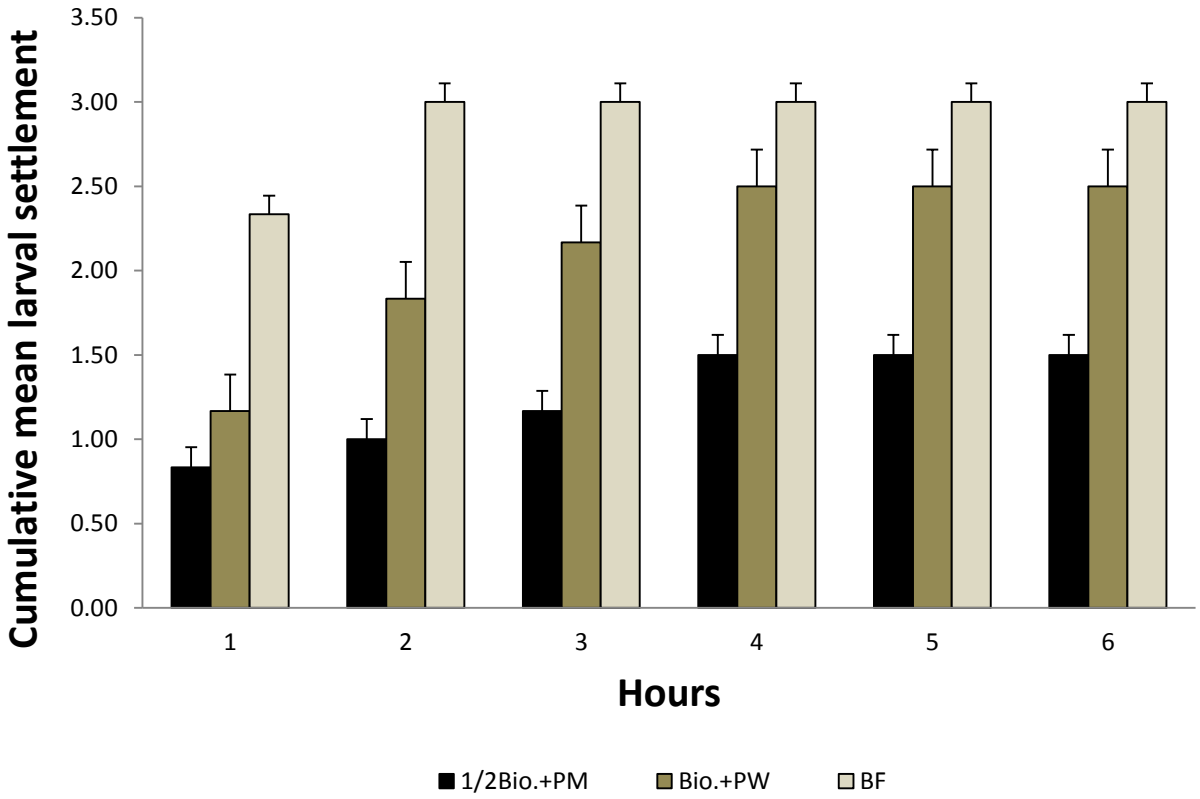
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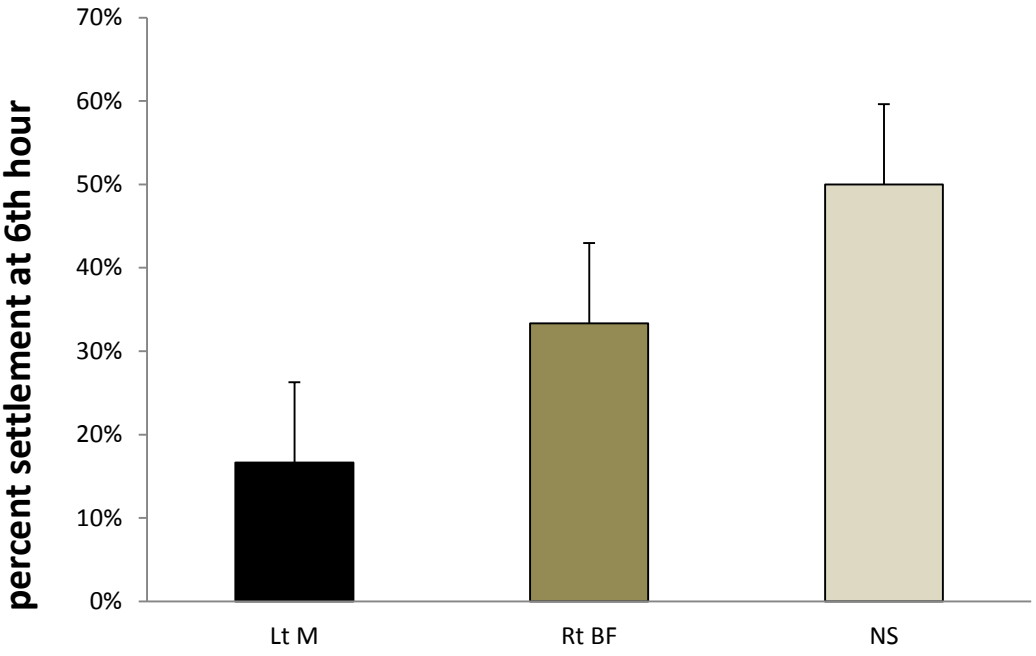
Figure 4


percent settlement at 6th hour
70%
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Lt M
Rt BF
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Figure 5