Crowd control: does cyprid larval density affect settlement?

By:

Helen Kesting

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Abstract

The site selection and attachment mechanisms of barnacles are studied extensively because of the negative impact of barnacles as biofouling organisms that attach to ships hulls and increase frictional drag. Site selection in barnacles occurs during a specialized larval stage known as the cypris stage. This paper investigates the effect of cyprid population density on settlement by comparing differences in settlement percentages between treatments with 5 cyprids/ 10 mL water, 10 cyprids/ 10 mL water, and 20 cyprids/ 10 mL water. Cyprids were exposed to each treatment for one week. A single factor ANOVA was used to test for significance at alpha = 0.05. There was no significant difference in the average settlement percentage between treatments. Future research into antifouling technologies should investigate the mechanisms behind settlement cues that have a significant effect on settlement percentage such as light, salinity, flow rate, surface texture, conspecific proteins, and biofilms.

Introduction

Biofouling is the accumulation of barnacles, algae, and other marine organisms on man-made structures, exacerbating corrosion or decreasing efficiency of moving parts by increasing frictional drag. Schultz et al. (2011) estimated that biofouling costs the US Navy approximately $180 million to $260 million annually due mostly to an increase in fuel consumption. This estimate also includes the cost of painting and cleaning ships' hulls. Since barnacles are some of the most common biofouling organisms, the site selection and attachment mechanisms of barnacles have been studied by many scientists seeking to discover new antifouling techniques.

Barnacles (infraclass Cirripedia) have a three-stage life cycle which includes two different larval stages. The first larval stage is the nauplius stage during which larvae feed on plankton and undergo six molts before metamorphosing into the cypris stage. Cypris larvae are a non-feeding form specialized for attachment site selection. As adults, barnacles are permanently attached to the substratum and cannot move to another habitat. Therefore, larval habitat selection is an essential function that is key to the success of adult barnacles. Environmental cues that affect settlement include flow rate, surface roughness, salinity, and light, among others (Qian et al., 2000; Hills & Thomason, 1996; Nasrolahi, 2007). Chemical factors such as biofilms and conspecific proteins are important settlement cues as well (Lindgren et al., 2009; Elbourne & Clare, 2010).

During the cypris stage, the larvae use energy stored as lipids to swim through the water column. Once they find a substrate that is potentially fit for settlement, they use their two antennules to stick to the substrate and explore its surface (personal observation). Clare et al. (1994) found that the antennular secretion used to adhere to surfaces also functions as a pheromone to induce settlement of conspecific cyprids. Assuming most species of barnacle have a similar pheromone in their antennular secretion, an increase in cyprid density would be expected to increase settlement rate. However, too much crowding will eventually inhibit the growth of an adult barnacle. Hooper and Eichhorn (2016) found that the average adult radius of *Semibalanus balanoides* was 0.35 +/- 0.02 cm and the distance away from a given barnacle for which the presence of another barnacle was most likely was 0.36 +/- 0.02 cm. These results suggest that barnacle density is related to average adult radius and that cypris larvae likely prefer to settle in locations where they will have adequate space to grow.

This paper examines the percent settlement of cyprid larvae at densities of 0.5 cyprids/ mL water (low), 1 cyprid/ mL water (intermediate), and 2 cyprids/ mL water (high) to answer the question, “does cyprid larval density affect settlement?”. My hypothesis is that the treatment with a density of 1 cyprid/ mL water will have the highest settlement percentage because the high-density treatment would send larvae signals of overcrowding that may inhibit future growth and the low-density would reduce the concentration of conspecific proteins that induce settlement. If cyprid larvae density affects percent settlement, then further research should aim to understand the mechanism responsible for this effect. Once understood, this mechanism could potentially be manipulated in order to deter barnacle settlement on ships hulls and other structures that are damaged by biofouling.

Methods

Study Organisms

Most of the cyprid larvae collected were identified as *Balanus glandula* according to a key provided by Arnsberg (2001). Characteristics used to identify *B. glandula* included intermediate size, upturned anterior end, and the presence of pigment patches. When selecting larvae for treatments, I chose cyprids based on size rather than morphological characteristics, limiting my study organisms to larvae of approximately 700 μm +/- 100 μm in length (Fig. 1).

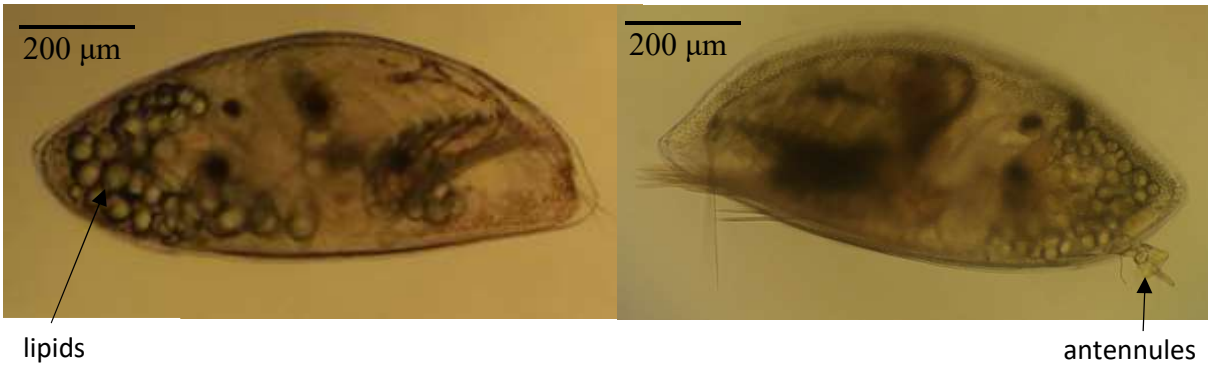

Fig. 1. Images of cyprid larvae taken with a cellphone camera through the eyepiece of a compound microscope.

Experimental Procedures

Cypris larvae were collected in May via plankton tows from the UW Friday Harbor Labs floating docks using a mesh net. The contents from the plankton tows was stored in a container and placed in a sea table that circulates water from the Puget Sound, which is approximately 10 degrees Celsius. Larvae were stored in the sea table for up to 3 hours before placement in one of three density treatments

Three plastic embryo-safe well plates with six round, 2.2 cm diameter wells each were prepared by rinsing them with reverse osmosis water and allowing them to dry, then pouring 10 mL filtered sea water into three of the six wells on each plate. Cypris larvae were hand-selected using a dissecting scope and a pipette, then transferred to the wells. See Appendix A for the method used to sort out cypris larvae from the contents of the plankton tow. Each plate contained a well with 5 cyprids, a well with 10 cyprids, and a well with 20 cyprids. The well plates were left in the sea table for 6 to 7 days, then the number of cypris larvae that had settled and metamorphosed into the adult form were counted. The adult form was recognized by its round shape and its feeding cirri. The number of adult barnacles was averaged among all three

replicates and converted into a percentage. The percentages of cypris larvae metamorphosed into the adult form in each density treatment were compared using a single factor ANOVA test in Microsoft Excel (1904) with alpha = 0.05.

Results

Adult barnacles were recognized by their rigid attachment to the substrate and their round or oval shape. Some adults were recognized by their cirri which extend and retract as they feed (Fig. 2). Sometimes, a molted cypris carapace was found adjacent to an adult. All metamorphosed barnacles were located at the edge of the well where the base meets the wall.

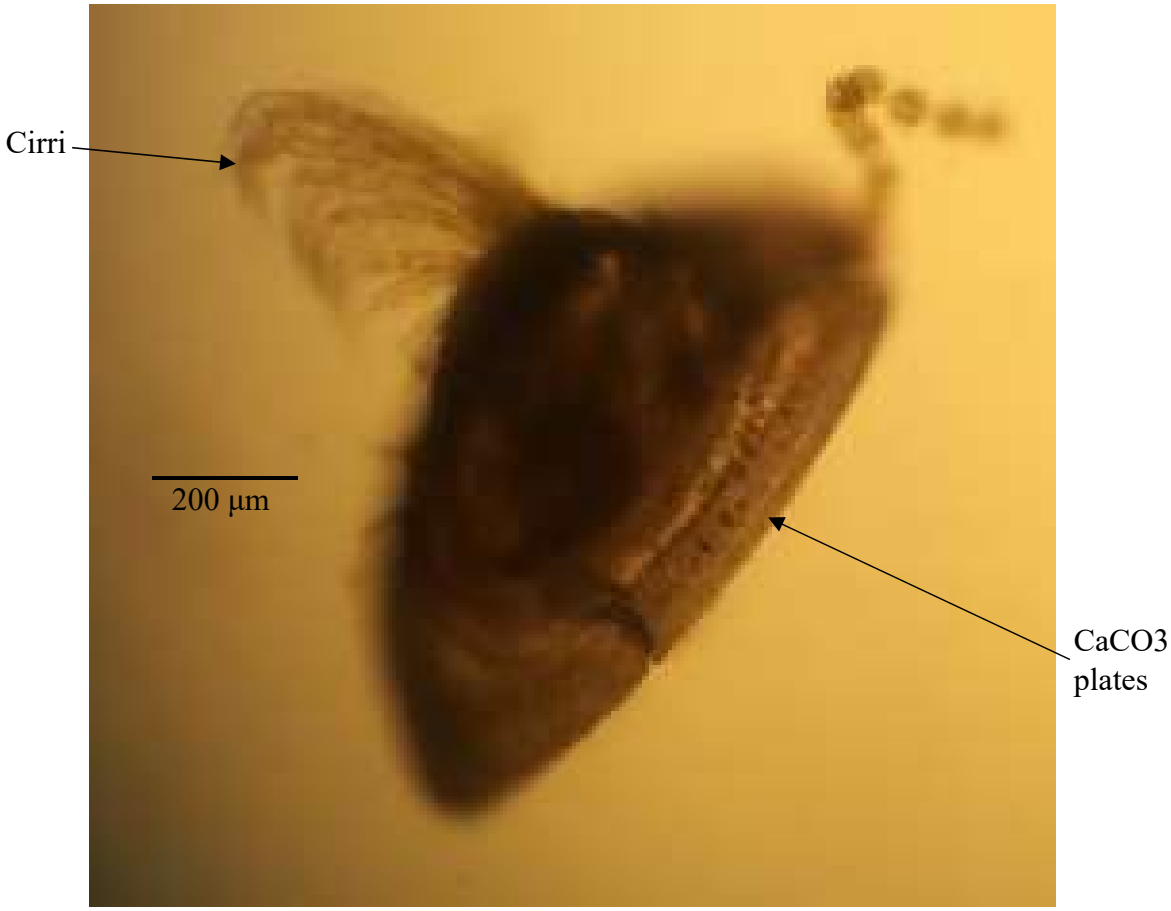

Fig. 2. An image of a newly metamorphosed adult barnacle with cirri extended.

The F value of the single factor ANOVA test comparing settlement percentage with cyprid density was 0.95 and the critical F value was 5.14. The P-value was 0.4381. The percent of cyprids settled in the low-density treatment of 0.5 cyprids/ mL water was 6.67% with a standard error of +/- 6.67%. The percent of cyprids settled in the intermediate-density treatment of 1 cyprid/ mL water was 26.67 +/- 17.64%. The percent of cyprid larvae settled in the high-density treatment of 2 cyprids/ mL water was 40 +/- 23.09% (Fig. 3).

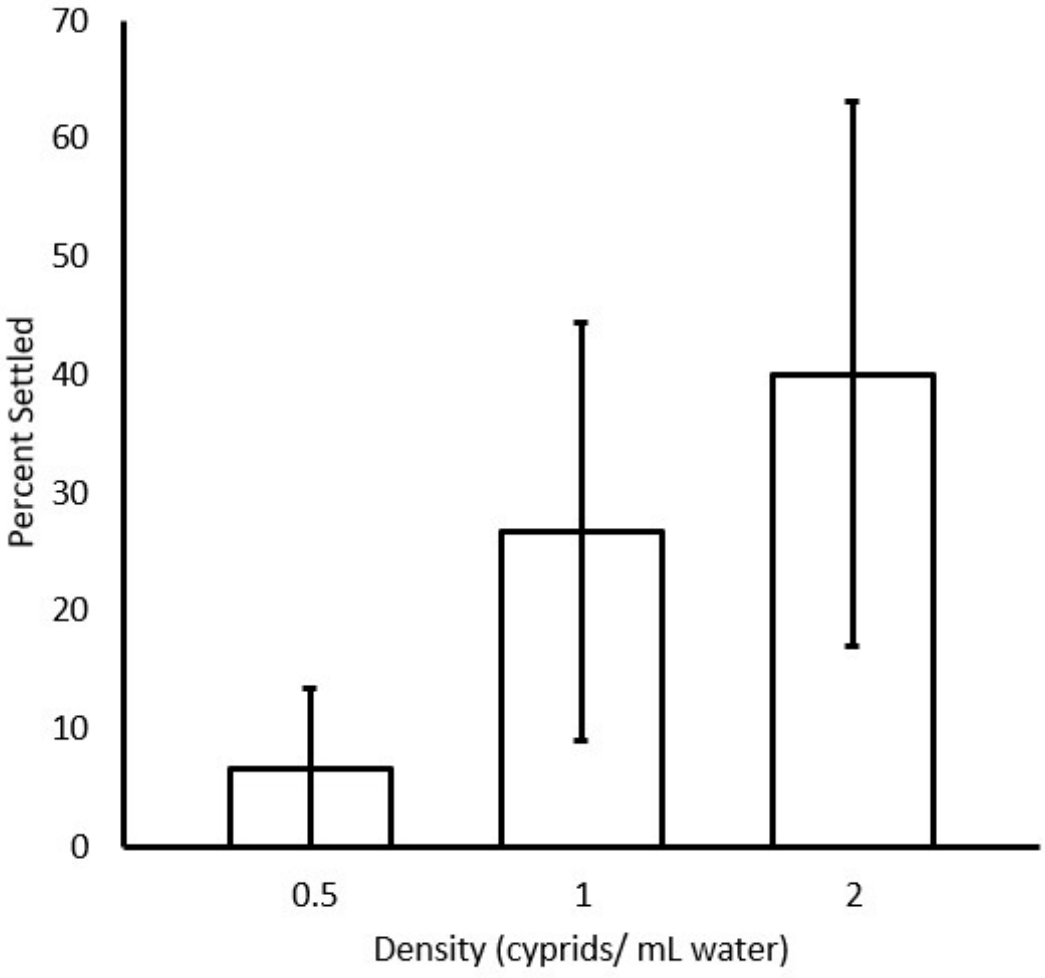

Fig. 3. A plot showing the average percent of cypris larvae settled for each treatment. The error bars show the standard error of the mean.

Discussion

These results suggest that settlement is not affected by cyprid density. This conclusion contradicts my hypothesis that settlement would be negatively impacted by both high and low cyprid densities. The value for the average settlement percentage did increase with increased density, but these average values have large error bars that overlap with each other. Increasing the number of replicates would likely reduce the standard error. The observation that all adult barnacles were located on the side of the well supports previous work showing that cyprid larvae prefer to settle in grooves (Crisp & Barnes 1954).

To improve this experiment, several changes could be made to control for potential confounding variables. Glass containers could be used instead of plastic containers because glass is an inert substance. Instead of setting up replicates on separate days, all the larvae needed for the entire experiment could be counted out from the plankton tow at once so that all larvae are given the same amount of time to settle and metamorphose. This would also reduce the amount of time needed to keep the well plates out of the sea table. Another way to investigate the impact of density on settlement would be to increase the number of treatments and increase the density of the highest density treatment, then analyze the data using a linear regression analysis.

Since this experiment did support a significant effect of density on cyprid settlement, future research on antifouling techniques may be more effective if it is directed toward other factors known to influence settlement such as those environmental factors listed in the introduction. If there was a clear relationship between density and settlement percentage, further research could seek to understand the mechanism behind this relationship in order to manipulate that mechanism for antifouling purposes.

Acknowledgements

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Appendix A

To catch cypris larvae, use a large (~500 mL) container and fill it with the contents of at least ten plankton tows conducted during the mid-afternoon around 3:00 or 4:00 PM. Place the container in a cold (10 degrees Celsius) place such as a sea table for at least ten minutes. The cypris larvae will concentrate on the side of the container as they explore the surface for a site to settle. Use a pipette to suck up the water and the larvae on the side of the container. Slowly scrape the pipette against the container to detach cyprid antennules without harming the larvae. To count out individual cyprids, empty the pipette into a bowl containing cold filtered sea water. Count out the desired number of larvae by sucking them up with the pipette one at a time. The adhesive on the antennules is very effective, so it is best to suck up the cyprids while they are swimming. Beware that they may become attached to the inside of the pipette. Double check your count once all the cyprids are in the treatment well.