

Effects of Flow Rate on *Balanus glandula* Feeding Rates

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ABSTRACT

Environmental changes can affect the abundance and community structure of various intertidal organisms. Water flow rate is a determining factor in food abundance and settling rate of food particles. For this study, we used the acorn barnacle, *Balanus glandula*, to study the effects of water flow on feeding rate. Many studies have been conducted involving beating rate, that is, how often barnacles extend their cirri. However little research has been done on how flow rates can affect the actual feeding rates of barnacles, meaning the rate at which they obtain food using their cirri. We predicted that in faster moving water barnacles would be able to obtain more food particles due to the flux of food around them. We also predicted that in slower moving water barnacles feeding rate would be lower because settling rate would be higher. These predictions led to the hypothesis that there must be an optimal flow rate at which feeding rate is highest. Our results showed that settling rates of food particles were highest in slower moving water, however, we were unable to obtain enough data about how feeding rates would vary with changing flow rates. In the future, more tests should be done using the improved methods we have developed in order to determine differences in barnacle feeding rates.

KEYWORDS

Balanus glandula, flow rate, Instant Algae, Brine Shrimp cysts, gut contents, feeding

INTRODUCTION

Organisms have physiological limitations that constrain their distribution and abundance (Kearney et al. 2009). A critical factor that affects the abundance and community structure of many marine organisms is water flow (Leonard et al. 1998). The flow velocity experienced by marine organisms impacts their abundance and distribution by affecting recruitment, predation, competition, and growth. For example, Leonard et al.

1998, showed that recruitment and growth of benthic suspension feeders, such as barnacles, was much greater in regions of high versus low water flow rates. They also showed that high flow sites were dominated by barnacles and mussels, whereas low flow sites had more bare space and less cover by barnacle and mussels (Leonard et al. 1998). These results suggest that flow rate should be included in any model that aims to predict shifts in species abundance and distribution. In spite of its importance, the physiological responses of marine organisms to changes in water flow are still not clearly understood.

Our project provides data about the effect of flow rate on feeding efficiency in *Balanus glandula*, the acorn barnacle (Anderson 1994). This species is common on intertidal substrates (rocks, mussel shells, pilings, etc.) along the North American Pacific Coast, from Alaska to Mexico. Barnacles filter feed on diatoms, invertebrate larvae, copepods, and organic debris (Barnes et al. 1959) using a fan formed from three pairs of biramous, setae-covered appendages called cirri. Other cirri clean particles from the feeding fan and transfer them to the organism's mouth (Anderson, 1994). Orienting the fan relative to the direction of the current and altering the pace and sweeping motions of the cirri achieve maximal particle capture. For example, at low current speeds, barnacles actively feed by periodically sweeping the water with their cirri and at higher current rates, they passively, and more efficiently, feed by holding the cirral fan in the current for prolonged periods (Trager et al. 1990). The curve of the fan is oriented away from the water flow during active feeding and toward the flow during passive feeding (Trager et al. 1990). In addition to delivering food to the sessile organisms, water flow is also important for waste removal and oxygen delivery. In fact, the cirral fan is the chief gas exchange organ for most of the sessile, gill-less adults (Personal communication, Megan Dethier, 2011).

While we understand how barnacles are feeding, there is still little understanding of the optimal flow rate and temperature at which barnacles can obtain the most food. We were able to locate only a few studies of the interrelationship between temperature and water flow rate on barnacle feeding (Southward 1957; Sanford et al. 1994; Marchinko 2007; Emler et al. 2009). In fact, most studies measure the beating rate of barnacles rather than the clearance rate (Sanford et al., 1994). The beating rate is the frequency at which a

barnacle extends its cirri into the water column, and does not necessarily equate to clearance rate. As a more accurate measure, feeding rates should be examined in order to determine how much influence water flow has on a barnacle's ability to obtain nutrients. If water flow increases, this could cause an increase in the flux of food over a barnacle, thus increasing the feeding rate. However, if the water flow is too high, it may cause a mechanical constraint on the cirri, that is, they could become deformed or detached. In this case, feeding would decrease at high flows. Another factor to consider is the impact of very low flow. Cirri may be able to easily extend into the water column and remain undamaged, but at a very low flow food particles may easily settle from the water column, out of reach of the cirri, thus decreasing the feeding rate. These types of tests have yet to be conducted so it is important to obtain this data to make localized predictions about the impact of environmental changes in various local communities.

As a first step in evaluating how environmental changes affect the physiology of barnacles, we will examine the feeding efficiency of *Balanus glandula*, on brine shrimp cysts and microalgae, at different flow rates (Sanford et al. 1994; Miller 2007; Marchinko 2007). Feeding efficiency will be quantified by comparing the concentration of cysts before and after barnacles have fed. An increased feeding rate at a particular flow rate may correlate to barnacles consuming more food, which could correlate with an increased growth or reproduction rate at this flow rate.

METHODS

Sample Collecting and Preparing:

Samples of *Balanus glandula* were collected off mussels (*Mytilus trossulus*) from the dock at Argyle Creek, Washington (N 48° 31.728' W 123° 00.802') on May 16th 2011. Organisms were brought back to the Friday Harbor Labs that same day and stored in a filtered, circulating seawater tank. We used a scalpel to remove the barnacles from the mussel shells, being careful to maintain basal plates intact. The removed barnacles were stored on a tray in the same seawater tank. We cut pieces of acrylic and covered them

with Z-spar (A-788 Splash Zone Epoxy, Kop-Coat Inc., Pittsburg, PA). The barnacles were then placed randomly on the Z-spar and the plate was left in the seawater tank to harden for a minimum of 24 hours. Barnacles could be used in experiments for up to a week after collecting.

Laboratory Experiments with Brine Shrimp Cysts:

To examine the feeding efficiency of barnacles, cysts were placed in water at different flow rates (Sanford et al. 1994; Miller 2007; Marchinko 2007). First, brine shrimp cysts from Sander's Brine Shrimp Company were hydrated in fresh, filtered seawater. We calculated the concentration of brine shrimp cysts needed to be about 20,000 cysts ml⁻¹ and hydrated them in filtered seawater for 30 minutes. Then floating cysts were decanted off and only the cysts that had settled to the bottom were used. Note: while the cysts were fractionated successfully, it would have been more appropriate to target neutrally buoyant particles.

We used a recirculating flow chamber made of PVC tubing and a clear acrylic rectangular test chamber (3 cm x 3 cm x 15 cm, H x W x L), where the barnacle plate was placed (Fig 1). A pump (Rule Model 25D) powered the flow chamber and was attached to a variable power source that was used to manipulate the flow rate between 0.7 and 150 cm s⁻¹. Water velocities along the centerline of the testing chamber were estimated by tracking the displacement of glass microbeads at each flow setting (mean particle diameter = 9 μm, density = 2.0 g cm⁻³, Potters Industries, Malvern, PA). The flow chamber was submerged in 10L of filtered seawater and filled until no air bubbles were present. The whole chamber was sealed and placed inside a cooler of fresh tap water. The cooler was connected to a circulating water bath (± 0.1°C; Ecoline RE 106, Lauda, Germany) that was used to regulate the temperature of the water surrounding the flow chamber, thus changing the temperature of the water inside the chamber.

We required cyst concentration inside the chamber to be about 50 cysts ml⁻¹ in order to be able to detect differences in cyst concentrations after feeding. The original cyst stock

solution (20,000 cysts ml⁻¹) was diluted to obtain the necessary concentration, which was inserted into the flow chamber using a syringe connected to the thin tubing and the hole in the side of the chamber.

For each trial the flow was set and samples of water from inside the chamber were taken every five minutes starting as soon as the cysts were placed in the chamber. Each sample was extracted using the syringe and placed into an Eppendorf tube. From this sample, three 400 µl subsamples were placed in a 24-well plate and the number of cysts in each well was obtained to calculate a concentration. The flow rate was then changed and the sampling procedure was repeated.

Control experiments were set up in order to determine if the decreases in cyst concentrations were due to barnacle feeding or due to cysts settling to the bottom of the flow chamber. For these experiments a plate of barnacles was boiled so no feeding could occur. This plate was placed into the flow chamber and the cyst concentration was sampled as in the experimental trials.

Lab Experiments with Dissected Barnacles:

To ensure that barnacles were feeding, we set up an experiment to examine the gut contents of barnacles after feeding for 24 hours. For this experiment, mussel shells with barnacles attached were selected from the original Argyle Creek collection and twelve shells were placed in a dish of unfiltered seawater with an air bubble pump. Twelve more shells were placed in the tank in the water outside the dish to act as a control. Excess hydrated brine shrimp cysts were added to this dish to feed the barnacles. After 24 hours, half the shells were kept in the dish with new unfiltered seawater and half the shells were frozen in order to notice changes in cyst digestion overnight. After a few hours, one shell with barnacles was removed from the freezer and soaked in bleach overnight to dissolve the gut tissue.

The barnacles were removed from the freezer and pulled from their shells, so that just the soft body of the animal remained. External cysts were pipetted away, and seawater was

used to wash over the organisms. Then the organisms were covered in bleach to allow for their tissue to dissolve. After a few hours, more seawater was added to the cysts to dilute the bleach. The next day the tissue had dissolved and only the cysts that had been in the stomach remained and were counted.

Laboratory Experiments with Instant Algae:

To test a second potential food source, a mixture of marine microalgae was tested in the flow chamber (Shellfish Diet 1800; Instant Algae, U.S.A.). Two ml of Instant Algae was added to 10 L of filtered seawater. The flow chamber was then submerged in this water completely filled. Experimental trials were run as described above and samples were taken every five minutes. Samples were analyzed using a hemocytometer to quantify the concentration of particles present in the samples.

For a control, the flow chamber was prepared as in the experimental trial, except using the dead barnacle plate.

RESULTS

We observed the feeding rates of barnacles when the water flow was at 12 cm s^{-1} and at 20 cm s^{-1} with the temperature kept constant at 10°C . One replicate was done for the flow rate of 20 cm s^{-1} (a), and two replicates were done for the flow rate of 12 cm s^{-1} (b and c). The results showed cyst concentration decreasing at a higher rate in both (b) and (c), than in (a) (Fig. 2). We used an exponential trendline and the resulting equation for (a) was $y = 49.754e^{-0.016x}$, while the equations for (b) and (c) were $y = 36.969e^{-0.078x}$ and $y = 28.179e^{-0.052x}$, respectively. The exponent values indicate that (b) and (c) both decreased more rapidly than (a).

Then another experimental trial and a control trial were done at a flow rate of 20 cm s^{-1} , to try and produce more data. The experimental trial showed a general decrease in cyst concentration over time, with an equation of $y = 86.096e^{-0.012x}$ (Fig. 3a). The control trial

had an equation of $y = 49.754e^{-0.016x}$, which represents a more rapid decrease in cyst concentration than in the experimental trial (Fig. 3b). The same experiment was done again, instead using a flow rate of 12 cm s^{-1} . The equation for the experimental trial was $y = 36.969e^{-0.078x}$ (Fig. 4a), while the equation for the control trial was $y = 32.548e^{-0.043x}$ (Fig. 4b).

Both the control and experimental trials resulted in a decrease in cyst concentration over time, so gut contents of the barnacles were analyzed after feeding overnight to determine if feeding was actually occurring. Cysts were in fact present in the guts and the number of cysts found in the each gut ranged from 0 to 207 (Fig. 5).

As an alternative to using cysts, we also performed experiments using Instant Algae as the food source. Instant Algae is a mixture of four microalgae: *Isochrysis*, *Pavlova*, *Tetraselmis*, and *Thalassiosira weissflogii*, and is more neutrally buoyant than brine shrimp cysts. For both the experimental and control trials the flow was set at 20 cm s^{-1} . The experimental trial resulted in a steep decrease in algae cell concentration with an equation of $y = 132959e^{-0.015x}$ (Fig. 6a). The equation shows the max rate between 10 and 30 min and levels out after 30 min. The control trial showed a more or less steady number of algal cells throughout the whole trial, with an equation of $y = 127455e^{0.0004x}$ (Fig. 6b). This slope is not negative meaning that the cyst concentration is not decreasing, but is staying about the same through the whole trial.

DISCUSSION AND CONCLUSIONS

The results from the first cyst experiment lead us to believe that the barnacles were feeding at a higher rate when the flow was lower (12 cm s^{-1} vs. 20 cm s^{-1}). However, upon more detailed observations we noticed that the barnacles were taking in the cysts with their cirri, but many times the cysts would expel the next time the barnacle extended its cirri. We considered the possibility that perhaps the barnacles were not feeding, or at least

not feeding at as high of a rate as expected. It is possible that the cysts were settling to the bottom of the chamber or getting caught in places inside the flow chamber causing the pattern seen in Fig. 2. These results could actually indicate that cysts settle faster in slower moving waters than in faster moving waters.

Based on the outcome of the previous experiment, we decided to run some control trials, using a plate of dead barnacles. We used a flow rate of 12 cm s^{-1} and the results demonstrated that the cyst concentration decreased rapidly in the control trial as well as in the experimental trial (Figs. 3a and 3b). This suggests that in the experimental trials we may have incorrectly assumed that barnacle feeding caused the decrease in cyst concentration over time, but really there may have been a different factor influencing this decrease. The same experiment was repeated, using a flow rate of 20 cm s^{-1} . In the absence and presence of feeding barnacles cyst concentration decreased over time (Figs. 4a and 4b). This also shows that cysts could be settling rather than being eaten. Insight can be gained by comparing the results of the control experiments. The control trial at 12 cm s^{-1} had an equation of $y = 32.548e^{-0.043x}$, while the control trial at 20 cm s^{-1} had an equation of $y = 49.754e^{-0.016x}$. This indicates that cysts are settling faster when the flow rate is slower. However, we predict that when the flow rate is too high barnacles will not feed because their cirri will not be able to withstand the impacts of the high water speed. More experiments will need to be conducted to test this hypothesis.

We conducted an experiment in which we dissected barnacles after allowing them to feed on cysts overnight, and then counted the number of cysts in their guts the next day to address the problem of whether or not barnacles were actually feeding or if cysts were simply settling. The results showed that barnacles ranged from having zero to 207 cysts present in their gut (Fig. 5). This indicates that the barnacles were in fact feeding on cysts and not just ejecting them back out every time they extended their cirri (Fig. 6). For these experiments we were using a very high concentration of cysts to begin with (around 10,000 cysts per barnacle). Due to the fact that the highest number of cysts found in a barnacle gut was 207, our starting concentration was likely too high to tell subtle differences in the cyst concentration. Other studies involving barnacle feeding have used

the beating rate of the barnacles, that is, how often they extend their cirri, as a measure of feeding (Sanford et al. 1994). However, our results indicate that this may not be the case. Many of our barnacles were beating their cirri very often but not necessarily obtaining any cysts. Analyzing how gut contents relate to beating rates may be a much better approach to measuring feeding rates in future research.

Because we had begun to think that barnacles might not have actually been feeding on cysts at all, we decided to set up an experiment using Instant Algae, a more neutrally buoyant food source. We ran an experimental and control trial at 20 cm s^{-1} . The experimental trial resulted in a rapid decrease between 10 and 30 minutes, and then leveled out after this time, indicating saturation in barnacle feeding (Fig. 6a). To test if this experiment was valid, we ran a control trial with a dead barnacle plate. This test resulted in a relatively level slope that did not decrease (Fig. 6b). These results indicate that algae particles were being removed from the water column by the barnacles rather than just by settling. Although more trials would need to be conducted to test the validity of these outcomes, this would still be a good approach to measuring barnacle feeding.

While the results of our studies using brine shrimp cysts do not provide information about whether or not barnacle-feeding rates vary with flow rate, they still give insight into how barnacles might be affected by changing environments. Comparing the results of the control trials seen in Figures 3b and 4b, shows that cysts settle at a higher rate in slower moving water. This means that in their natural environment, barnacles may be able to obtain more food if the water flow rate is faster because the food particles will not settle as quickly and more food particles would be available overall.

Time spent troubleshooting cost us several replicates of each experiment. In the future, I would make several changes to the original protocol. In our experiments we decanted the cysts that were neutrally buoyant or floating on the surface and used only the cysts that had sunk to the bottom. It may be better to use the neutrally buoyant cysts because these would be less likely to settle. Also, instead of measuring cyst concentration in the water, it would be more accurate to dissect each barnacle to see the exact number of cysts they

consumed. This would allow us to detect how much barnacles were actually feeding and the results would not be mistaken for settling. Another issue we encountered was cysts settling. Due to time constraints we were unable to examine how barnacle-feeding rates may change in response to changing temperatures, but in the future it would be relevant to conduct more experiments involving the correlation between water flow and temperature. This would be an important study because barnacles are exposed to a variety of temperatures and flows in the intertidal zone.

Our experiments provided insight into how to set up more effective similar experiments in the future, leading us to more accurate ways of measuring feeding rates.

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FIGURES

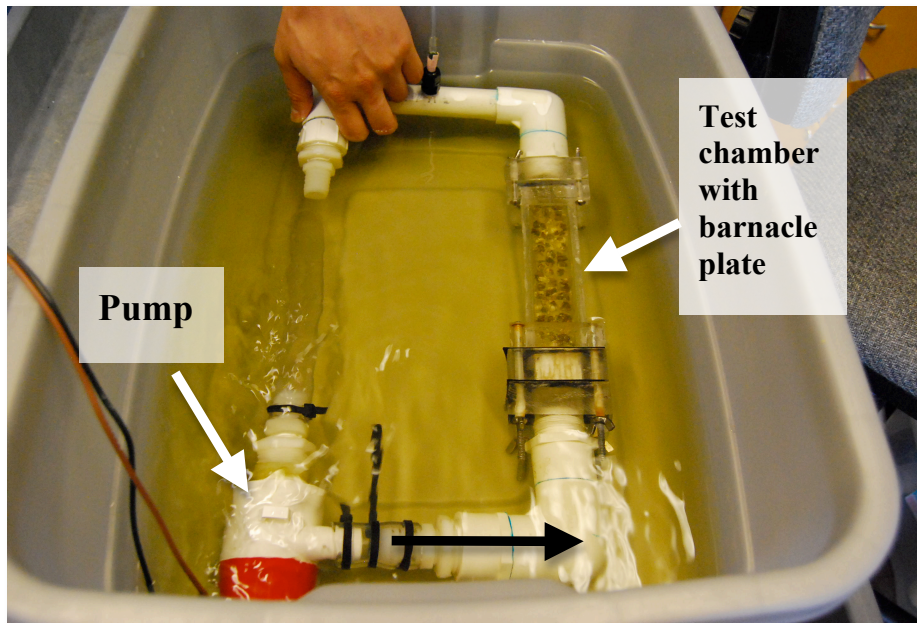


Figure 1. Recirculating flow chamber used in all experiments. Black arrow indicates direction of flow.

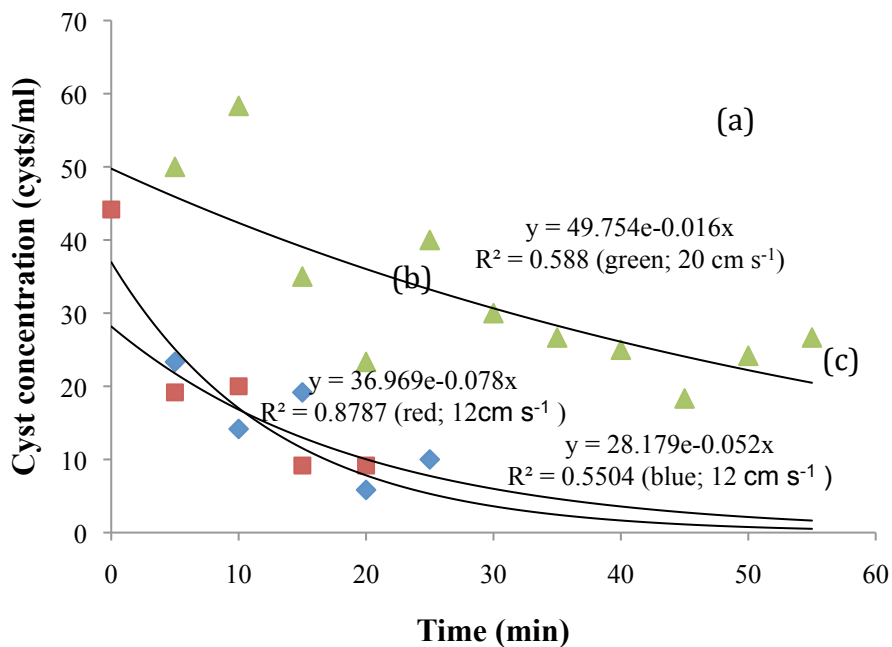


Figure 2. Cyst concentrations over time in feeding trials; Green triangles represent feeding trials conducted at 20 cm s⁻¹ (a); Red squares and blue diamonds represent feeding trials conducted at 12 cm s⁻¹ (b and c).

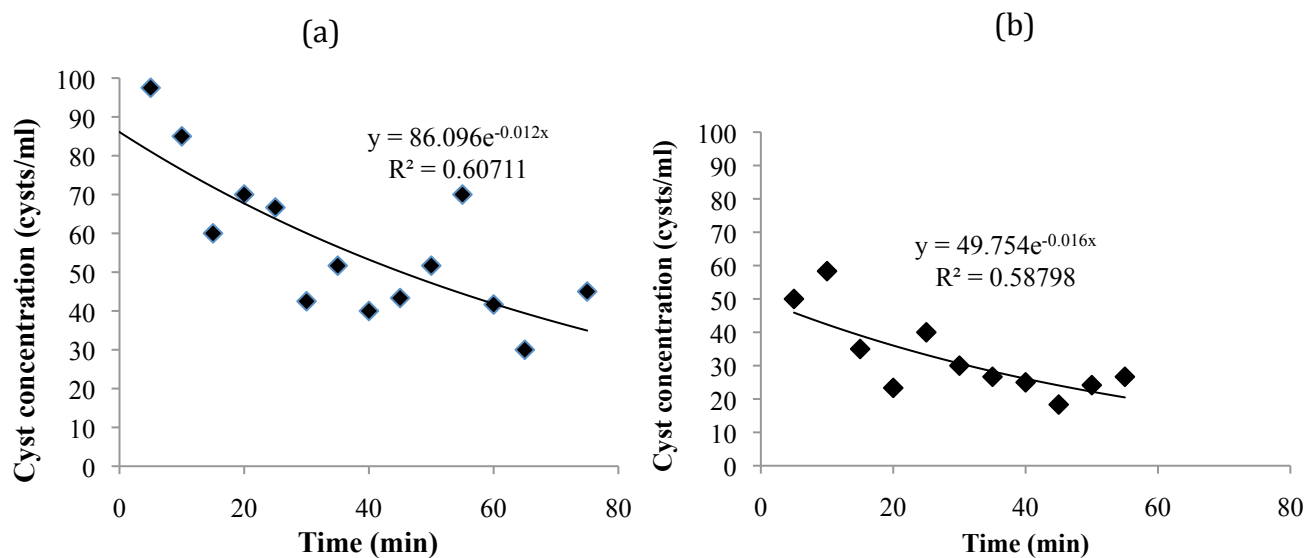


Figure 3. Cyst concentration trials with and without barnacles; (a) Experimental trial; (b) Control trial. Each point represents an estimate from three subsamples taken at a flow rate of 20 cm s^{-1} .

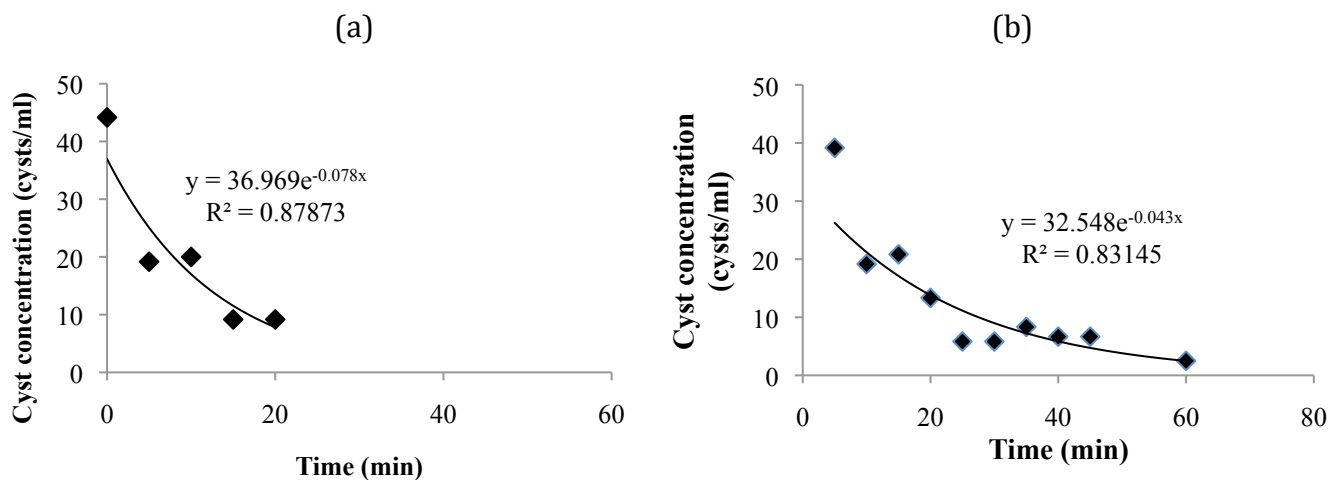


Figure 4. Cyst concentration trials with and without barnacles; (a) Experimental trial; (b) Control trial. Each point represents an estimate from three subsamples taken at a flow rate of 12 cm s^{-1} .

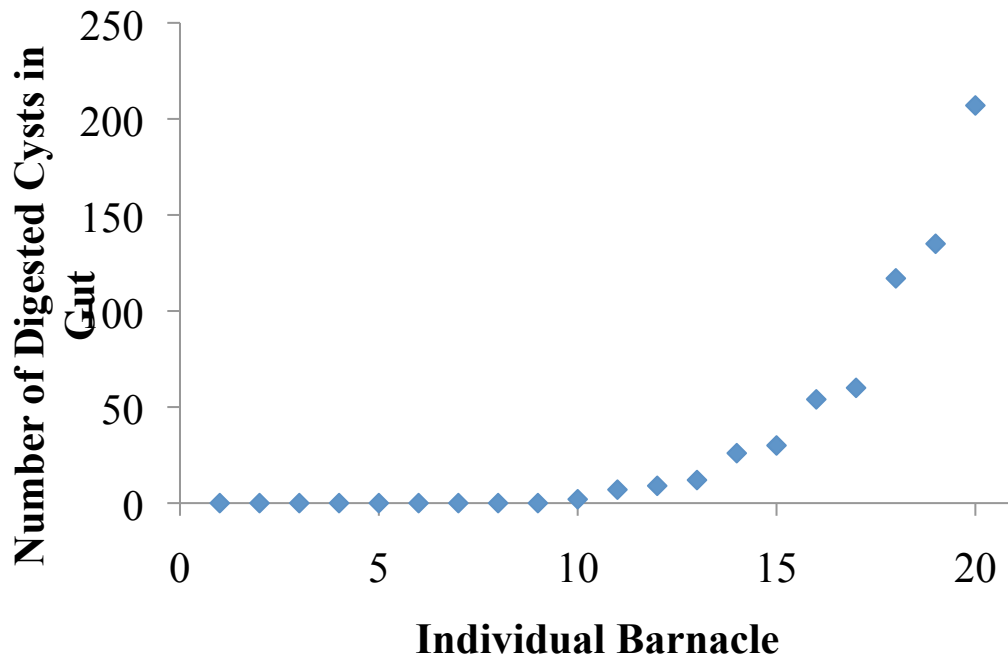


Figure 5. The number of cysts found in the guts of barnacles after barnacles had fed for 24 hours.

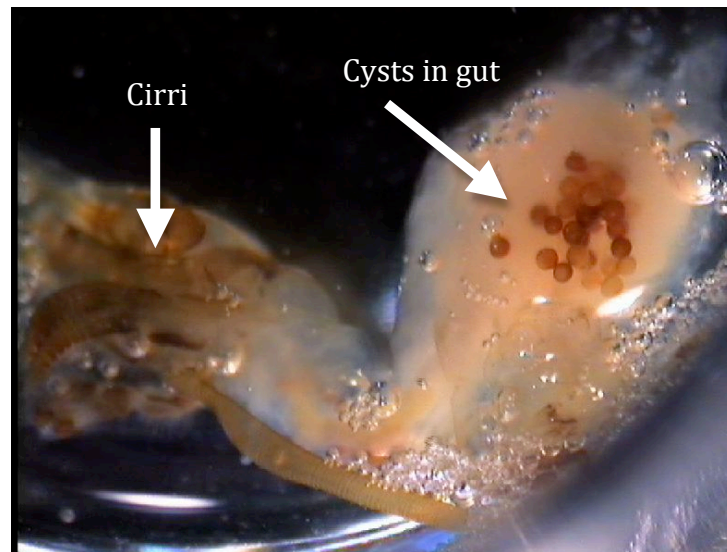


Figure 6. Cysts found in the gut of a barnacle after feeding for 24 hours.

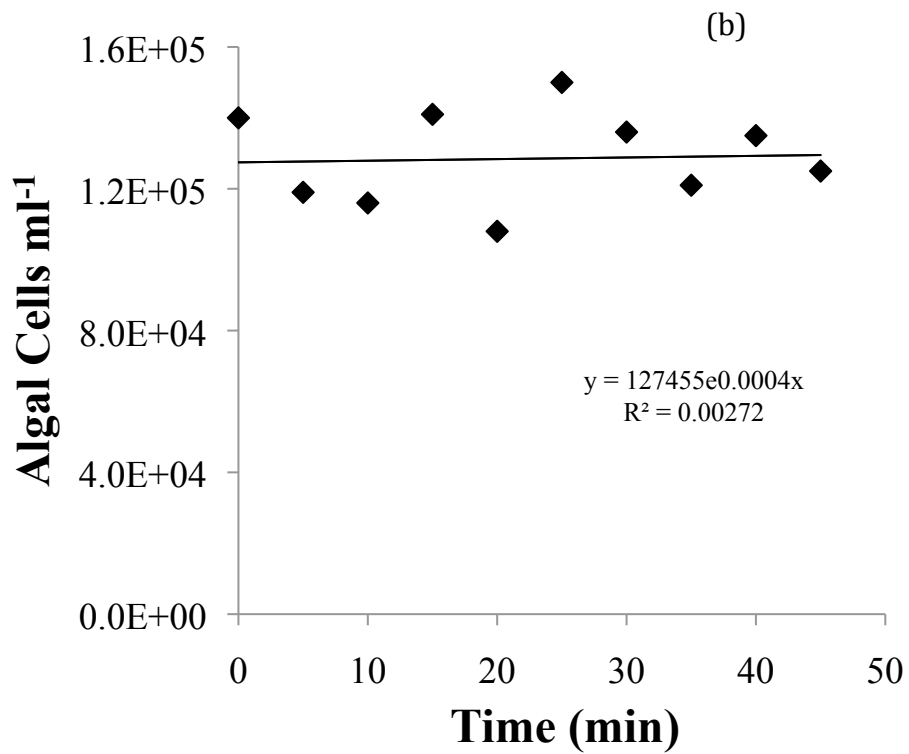
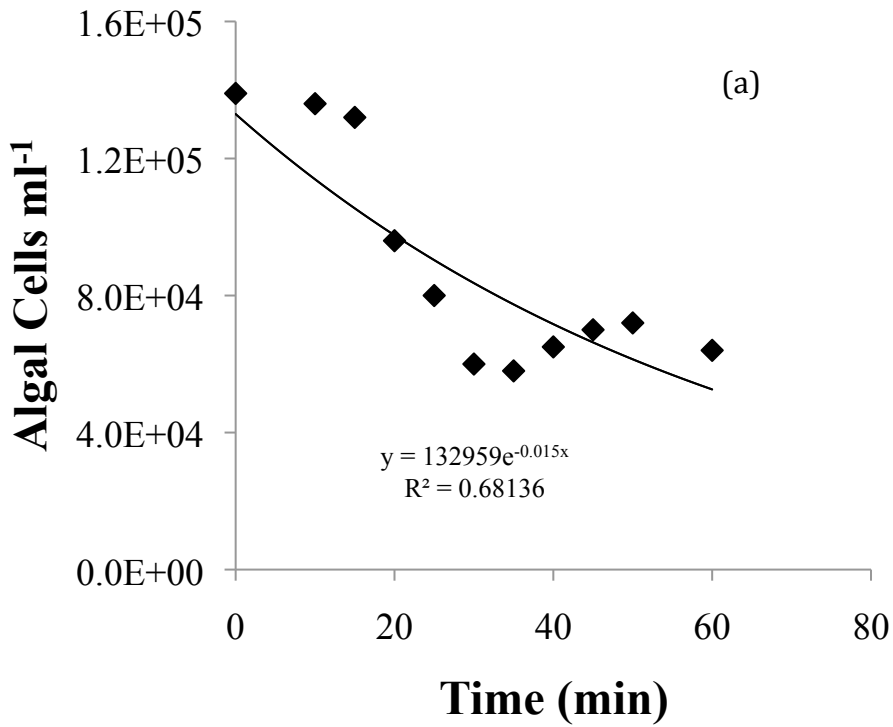


Figure 6. Algal cell concentrations over time, with and without barnacles; (a) Experimental trial; (b) Control Trial. Conducted at a flow rate of 20 cm s⁻¹.

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