BARNACLE FEEDING: COMPARING CIRRAL ANATOMY, FEEDING BEHAVIOR, REYNOLDS NUMBERS, AND CIRRAL FAN LEAKINESS ACROSS THREE SIZE CLASSES OF THREE SPECIES OF COMMON ACORN BARNACLES

by

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Title: BARNACLE FEEDING: COMPARING CIRRAL ANATOMY, FEEDING BEHAVIOR, REYNOLDS NUMBERS, AND CIRRAL FAN LEAKINESS ACROSS THREE SIZE CLASSES OF THREE SPECIES OF COMMON ACORN BARNACLES

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This thesis investigated feeding behavior, cirral anatomy, Reynolds numbers (Re's), and leakiness of the cirral fan for three barnacle species: *Balanus glandula*, *Semibalanus cariosus*, and *Balanus nubilus*. A study of the feeding of *B. glandula* revealed that newly-metamorphosed juveniles fed at Re's less than one and their cirral fan leakiness was <5%

at current speeds of 4 cm/s. At this speed, large and medium individuals fed at Re's near unity, and their cirral fans were 6.8±0.7% leaky. The experiments were repeated using *S. cariosus* and *B. nubilus* with the same tissue mass as *B. glandula.* No clear species-specific or size-specific trends were identified in these species. The observed switch from low to high Re feeding in *B. glandula* may indicate a lower limit to the size of this species, if further research confirms low Re feeding is less efficient and field current velocities are sufficiently low.

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CHAPTER I

GENERAL INTRODUCTION

Due to their ease of collection, sessile lifestyle, ubiquity, and ability to tolerate captivity, barnacles have become a favorite model organism of marine biologists. Many hypotheses that eventually became paradigms were originally tested using barnacles as models, such as Joseph Connell's work on zonation in the rocky intertidal. Connell (1961) found that the barnacle *Chthamalus stellatus* was able to occupy space in the upper intertidal due to its superior resistance to dessication, while it was out-competed in the mid-intertidal by the barnacle *Semibalanus balanoides.* It was using barnacles again that Connell (1985) demonstrated the importance of both variations in settlement and post-settlement mortality in the structuring of populations on the rocky shore. Menge (1992) used barnacles to demonstrate the importance of bottom-up processes, showing that areas along the Oregon coast with higher primary productivity and more phytoplankton also had higher barnacle recruitment, abundance, and growth. Recently, Leslie (2005) found that reproductive hotspots for *Balanus glandula* were linked to areas of higher primary productivity and proposed that these hotspots would make better marine reserves than less productive sites as they would be a greater source for larvae that would go on to seed other non-protected sites.

As a result of the popularity of barnacles as a model organism, more is known about barnacle recruitment than that of any other marine invertebrate. Larval retention in estuaries was first documented *Balanus improvisus* by Bousfield (1955). Gaines and Bertness (1992) linked barnacle larval retention in a bay to river run-off; years with high run-off had low recruitment. Cyprids, the final larval stage of barnacles, are known to explore potential settlement sites extensively before adhering to the substratum. The roughness of the rock face (Crisp & Barnes 1954), presence of conspecifics (Knight-Jones 1955), and chemical cues from conspecifics (Crisp & Meadows 1962, Crisp & Meadows 1963, Larman & Gabbott 1975, Raimondi 1990) are all known to enhance settlement.

With such a rich history of use as a model organism, barnacles were an obvious choice for use in experiments dealing with feeding. Barnacles have one strong advantage when it comes to feeding studies—they come pre-attached to the substratum. Work on the feeding of free-swimming marine invertebrates usually involves finding a way to restrain them for ease of videotaping. In the past, restraints used have included gluing organisms to rods with cyanoacrylate (Koehl & Strickler 1981) or even sucking one arm of a pluteus into a pipette to stabilize it (Podolsky

& Emlet 1993). The effect of natural and artificial tethers on feeding behavior has been examined by Emlet (1990) in bivalve (*Crassostrea gigas*) and gastropod (*Calliostoma ligatum*) veligers as well as in the larvae of the polychaete *Mesochaetopterus taylori.* While tethering was not believed to change the method of particle capture for these organisms, it did increase the shear around the cilia, the amount of water moved by the velum, and rate of ciliary beating. The effect of artificial tethers can be ignored completely in juvenile and adult barnacles, thus simplifying the experimental design and ensuring that observed behavior is as natural as possible.

This study compares barnacle feeding in three size classes of barnacle. In Chapter II, the feeding of juvenile, medium, and large *Balanus glandula* was investigated in-depth. The feeding behaviors of the barnacles in slow current were recorded, molts were collected and morphometric and meristic traits were measured, and the Reynolds numbers at which the cirri and the setae work were calculated. In addition, the barnacles were observed feeding with a stream of milk aimed at their upper cirri. Geometric models for the amount of water passing through the cirral fan were made using pictures of the milk interacting with the cirri. This study was expanded in Chapter IV to include two other species of acorn barnacle, *Semibalanus cariosus* and *Balanus nubilus*, of similar tissue weight to the *B. glandula* used in

Chapter II. Trends in feeding behavior, cirral anatomy, and Reynolds number were compared to determine the effects of species and size.

CHAPTER II

FEEDING BEHAVIOR, CIRRAL ANATOMY, CIRRAL REYNOLDS NUMBERS, AND CIRRAL FAN LEAKINESS OF *BALANUS GLANDULA*, FROM POST-METAMORPHIC JUVENILE TO THE ADULT

1. Introduction

Suspension feeding is a common mode of obtaining nutrition in the intertidal environment and provides a path for energy transfer from the plankton to the benthos (Gili & Coma 1998). Suspension feeding was once thought to be akin to holding a sieve in a current, catching only particles too large to pass through the holes. This view was amended by Jorgensen (1966) to include instances where water is passed along surfaces (usually covered in mucus) capable of retaining particles. Application of the aerosol filtration theory by Rubenstein and Koehl (1977) changed this view and identified five methods of extracting particles from a fluid. Predictions made by aerosol filtration theory were tested and confirmed empirically by La Barbera (1978) for the Pacific ophiuroid *Ophiopholis aculeata.* Aerosol filtration theory deals with tiny feeding structures operating at Re's less than one, and LaBarbera (1984) postulated that virtually all suspension feeding in marine invertebrates involves cylindrical filtering elements operating at low Re's. Later, Shimeta and Jumars (1991) showed that suspension feeding at intermediate Re's (e.g. 10) does occur and has been documented in at least four phyla.

The Re is a ratio that describes the relative importance of inertial and viscous forces for movement relative to a fluid (Vogel 1981). At Re < 1, the viscosity of seawater is more important than inertia and appendages that resemble sieves can behave like paddles (Cheer & Koehl 1987). For example, the copepod *Eucalanus pileatus* flaps its second maxillae, which superficially look like sieves but function as paddles, to drive water past itself (Koehl & Strickler 1981). The tendency of an appendage to function like a sieve or a paddle is determined by its leakiness, defined as the ratio of the volume of fluid that actually moves between a pair of cylinders of a unit length in a unit of time to the volume through which the cylinders sweep in that unit of time (Cheer & Koehl 1987).

As an organism gets older and its feeding structures enlarge, the structure may undergo a transition in function from a paddle to a sieve due to increasing Re (Koehl 2001). As the Re approaches one, the inertial and viscous forces become equal. Here streamline compression around a cylindrical appendage effectively increases the width of the stream of fluid encountered by the filter apparatus thus increasing encounter rate of food particles (Shimeta & Jumars 1991). The pedunculate barnacle *Lepas pectinata* is one animal that feeds at intermediate numbers. Using data from Anderson (1980), Shimeta and Jumars (1991) calculated that the cirrus of this barnacle reached a Re of one when feeding in a slow current of 0.4 cm/s.

Balanoid barnacles are another group of animals that, as adults, are sessile suspension feeders. Their feeding structure, known as a cirral fan, consists of three paired biramous appendages covered with setae (cirri IV-VI) and three smaller paired biramous appendages (cirri I-III) that function in cleaning the other cirri and transferring food to the mouthparts (Anderson 1994). Barnacles feed by orienting their cup-shaped cirral fans into the current and can even reorient their cirral fans to anticipate directional changes in oscillating flow (Trager *et al.* 1992). They use their cirri to directly capture food, filter food from the water column, and reject non-nutritious items (Anderson 1981, Hunt & Alexander 1991).

Barnacles are able to switch between active feeding (beating their cirri) and passive feeding (holding their cirri extended) and have been labeled facultatively active suspension feeders by LaBarbera (1977). Trager *et al.* (1990) demonstrated that current speed determines which feeding method is adopted in *Semibalanus balanoides*; they beat their cirri at low current speeds and hold them extended at higher current speeds. Trager *et al.* (1994) also found that capture rates were significantly higher for barnacles (*Nobia grandis)* feeding passively with cirri extended, compared to those feeding actively. This higher capture rate paired with reduced energy expenditure while feeding makes the passive feeding mode more efficient. In addition to these two modes of feeding, some species of balanoid barnacles can also generate a current by pumping their cirri and body up and down and trap smaller particles with their setous mouthparts (Barnes 1959). Based on gut content analysis, diatoms, invertebrate larvae, crustacean limbs, copepods, and organic debris are all common items in the diet of acorn barnacles (Barnes 1959).

Balanus glandula (Darwin, 1854) is an acorn barnacle common on the Pacific Coast. A recent series of studies have found that the cirri of adults of this barnacle exhibit phenotypic plasticity in response to local hydrodynamic conditions. Marchinko and Palmer (2003) found that four species of barnacle (*B. glandula, Chthamalus dalli, Semibalanus cariosus,* and *Pollicipes polymerus*) exhibit longer, thinner cirri with longer setae when found in areas with low wave exposure (where pumping or active feeding would predominate) compared to those found in areas with higher wave exposure where passive feeding would be the norm. Further work revealed that both juvenile and adult *B. glandula* will grow longer cirri when transplanted from high wave exposure to low wave exposure areas (Marchinko 2003) and that cirral length is precisely tuned to wave action (Arsenault *et al.* 2001). However, there is a limit to this phenotypic plasticity: no additional plastic response is detected in barnacles that live in areas exposed to water velocities over 2-4 m/s (Li and Denny 2004). These velocities correspond to those at which barnacles cease to feed in oscillatory flow environments (L. Miller, Stanford University, personal communication).

These studies focused on adult *Balanus glandula.* The newly-settled juveniles of this species have an aperture diameter smaller than the adult by as much as a factor of twenty (Geierman, pers. obsv.). The current study was conducted to address how the feeding of *B. glandula* differs among three size classes: newly-settled juveniles, large adults, and individuals intermediate in size. Specifically, we examined the behaviors exhibited by individuals feeding in a slow (4 cm/s) current, the morphology of the cirri, Re's experienced at different locations in the cirral fan, and the leakiness at one point in the cirral fan. We predicted that the barnacles would exhibit active feeding but not passive feeding at the relatively slow current speeds tested. Also, the barnacles of all sizes would feed with leaky cirral fans. This would mean that the barnacles would feed at Re > 1, where inertial forces dominate and water can pass between the cirri.

2. Materials and Methods

2.1 Barnacle Collection & Rearing

We conducted a haphazard survey of the local population of *Balanus glandula* in Coos Bay, Oregon, USA to determine the maximum adult size. This set the aperture diameter of the largest size class at 6.1 ± 0.1mm (mean ± standard error). Aperture diameter is defined here as the linear distance from the top inner edge of the rostrum to the top inner edge of the carina when viewed from above. Newly-settled individuals made up the small size class (aperture diameter 0.5 ± 0.02mm), and the medium size class was chosen to fit between these two extremes (average aperture diameter 3.6 ± 0.2mm).

Newly-settled individuals (small size class) were obtained by collecting plankton with a 73μm-mesh plankton net just inside the mouth of Coos Bay, Oregon, USA. The plankton was transported to the Oregon Institute of Marine Biology (OIMB), and the cyprids were separated manually from the rest of the plankton, and allowed to settle in fingerbowls containing 3M Safety Walk Tape treated with extract of adult *Balanus glandula* (Rittschof *et al.* 1984). The newly-settled barnacles and a portion of Safety Walk Tape surrounding them were removed using a scalpel and then attached with cyanoacrylate glue to small glass squares cut from microscope slides. Each recruit was held in an 8mL tissue culture well with filtered seawater and *Skeletonema costatum* until their first molt, after which fecal matter began to appear in the wells confirming that the juveniles were feeding. Scaled photographs were measured using ImageJ 1.34s (National Institutes of Health) to determine the aperture diameters.

Medium- and large-sized individuals were collected near the OIMB Boathouse Auditorium by chiseling them and their substrata off a sandstone shelf. Since large intra-specific variation in cirral length and setation has been described in *Balanus glandula* and attributed to differences in current speed (Marchinko 2003; Marchinko & Palmer, 2003; and Li & Denny, 2004), care was taken to collect the barnacles from the rock face within a 1m^2 area to control for variations in flow along the sandstone shelf. All barnacles were volcano-shaped and were not collected from hummocks. The aperture diameters of the medium and large barnacles were measured using Vernier calipers. Barnacles were taken back to OIMB, cleaned of epiphytes with a stiff brush, and housed in ice cube trays (17mL seawater per compartment). All barnacles in tissue culture wells or ice cube trays were placed on a rotor tray set at 38 rotations per minute inside an incubator set at 10 °C.

While in captivity, barnacles were fed *ad libitum* with the diatom *Skeletonema costatum* and the water was changed every three days.

2.2 Feeding Behavior

To quantify barnacle feeding behavior, barnacles were videotaped while feeding in a flow tank. Two recirculating flow tanks were used in these trials. Large barnacles were videotaped in the large flow tank (working area 20 X 6 X 13cm), which was designed according to Vogel & LaBarbera (1978) (Figure 1A). Small and medium barnacles were taped in a smaller flow tank (working area 7 X 5 X 3.5cm) positioned on the stage of a dissecting scope. The tank was made of plexiglass and powered by an aquarium water pump (Figure 1B). A velocity profile was generated for both tanks, and the height of free-stream velocity was determined. Rods were cut to these lengths (1/4in PVC pipe with cap for the large (1A); glass rods for the small flow tank (1C)) and mounted in the middle of tank. Thus, a barnacle attached to the top of a rod would experience free-stream velocity.

At the beginning of each trial, the flow tank was filled with cold (~12°) seawater filtered through a 0.45μm filter. The water velocity in the flow tank was determined by timing particles traversing a known distance. Mussel juice (see Appendix A for recipe) was added as a feeding stimulant (Allison & Dorsett 1977) at a concentration of 1mL juice per liter of seawater. A barnacle was attached with denture adhesive (Super Poligrip® Comfort Seal™ Strips) to the top of the rod with its cirri facing into the current and given five minutes to acclimate and begin feeding in the flow tank. If a barnacle extended its cirri from its shell during these five minutes, a trial was begun immediately and its behavior was videotaped (SVHS) for five minutes. A Pulnix (model TM-7CN) black and white camera was used. When used with the small flow tank, it was attached to the phototube of the dissecting scope. For use with the large flowtank, the camera was set on a tripod and was fitted with a Nikon AF Micro Nikkor 650mm macro lens with a 2X multiplier. If the barnacle did not extend its cirri during the five minute acclimation period, it was removed from the flow tank and exchanged for a new individual.

The temperature of the flow tank was monitored using a thermister (Fisher Scientific) and was recorded at the beginning and end of each trial. When the water temperature reached 16°C, the trial was ended; the tank was drained and filled again with cold seawater and mussel juice. This procedure was repeated for ten individuals in each size class.

While viewing the videotapes of the trials, we recorded the amount of time each barnacle spent exhibiting specific behaviors. The types of barnacle feeding behaviors observed were characterized based on the descriptions of Crisp and Southward (1961) and Anderson and

Southward (1987). A barnacle was recorded as "inactive" if its cirri were not extended for at least 3 seconds. Anderson (1981) found that the slowest beat observed by *Balanus perforatus* was 3s in duration. Thus, a pause of less than 3s may have been the retraction stage of a cirral beat cycle. A barnacle was characterized as "pumping" if its valves were open and the cirri were visible, but folded together with the cirral tips inside the shell. A barnacle was engaged in "active feeding" if the cirri were beating and the tips of the cirri were raised out of the shell. Passive feeding occurred when a barnacle was beating its cirri and held the cirri extended at the apex of the beat for longer than one second. The total amount of time a barnacle spent exhibiting each behavior was recorded and expressed as a percentage of time during each trial. Trends in active feeding were compared with two-way ANOVA followed by Tukey's test to compare the means.

The beat rate of each feeding barnacle was determined. We recorded the number of cirral extensions in four haphazardly selected, twenty-second portions of videotape showing the barnacle actively feeding. These values were averaged to calculate the number of beats per minute for each individual. As temperature is known to affect barnacle beat rate (Southward 1955, Anderson & Southward 1987), we analyzed the data using ANCOVA with temperature as a covariate, followed by orthogonal contrast statements which were used to make *a priori* comparisons of the means.

2.3 Cirral Anatomy

The barnacles used in this study were housed in individual chambers, either tissue wells or ice cube tray compartments and monitored daily for molting. Exuviae were collected and preserved in 70% ethanol on the day they were found. The aperture diameters of the barnacles were recorded using the methods mentioned in section 2.1. Each molt was viewed under a dissecting microscope and the ventral surface of the fan was photographed in the most natural posture possible. In cases where the fan was preserved in a clenched posture and could not be opened, one cirrus VI was cut away from the fan, straightened into a natural feeding posture, and its ventral surface photographed (Figure 2A). Finally, a cirrus VI was turned on its side and photographed in profile (Figure 2B). This study focused on the anatomy of the longest ramus of cirrus VI, as this was the longest ramus of the entire cirral fan and would determine the upper values for cirrus length, cirrus width, etc.

The photographs of exuvia were enhanced using Adobe Photoshop (version 6.0) and then analyzed with ImageJ (National Institutes of Health). The traits measured were similar to those measured in

Marchinko & Palmer (2003). The lower ramal diameter was measured at the second segment from the proximal end of the ramus (Figure 2A, a). The lower cirral gap was measured as the distance between the long and short rami of cirrus VI at the second segment from the proximal end of the short ramus (Figure 2A, b). The upper cirral gap was measured as the distance between the long and short rami of cirrus VI at the tip of the short ramus (Figure 2A, c). The upper ramal diameter was the diameter of the longest ramus at second segment from its distal end (Figure 2A, d). The ramus length was measured as the curvilinear distance along the dorsal margin from the base to the tip, excluding the terminal seta (from e to f in Figure 2B). The setal diameter was measured as the width of the longest seta on the longest ramus of cirrus VI (g in Figure 2C). The intersetal space was measured as the distance between the longest seta and its nearest neighbor (2C, h). The number of setae per segment and the number of segments on the longest ramus of cirrus VI were counted.

These measurements were used to calculate the Re's experienced at three different locations in the cirral fan: at the longest ramus of cirrus VI, both near the base and near the tip; and near the distal end of the longest seta. The Re was calculated using the following formula:

$$\mathrm{Re} = \frac{\rho l U}{\pi} \qquad (1)$$

where ρ is the density of seawater (1025 kg/m^3), ℓ is the diameter of the structure (cirrus or seta), U is the velocity of the water in the flow tank, and μ is the dynamic fluid viscosity of seawater (0.0011 kg/m*s). In addition, the number of setae per millimeter on the longest ramus of cirrus IV was calculated by multiplying the number of setae per segment by the number of segments and dividing that by the length of the ramus. Trends in Re and number of setae per millimeter were examined with a two-tailed ANOVA followed by a Tukeys HSD *post hoc* test when significant differences were detected.

2.4 Leakiness of the Cirral Fan

Ten barnacles of each size class were videotaped feeding in a flow tank according to the methods described in section 2.2. When an individual began active feeding, a stream of milk was aimed at its cirri. The milk was injected via a syringe held in a frame with a screw-type plunger and attached via tubing to a needle (27-gauge for the small and medium size classes, 21-gauge for the large size class) bent at a ninety-degree angle so as to create a milk stream parallel to the flow of water in the tank. Each barnacle was videotaped for a minimum of three minutes while actively feeding with the milk stream aimed at the top of its cirri.

The videotape of each trial was reviewed. The first section of tape representing one full cirral extension and retraction in which the milk stream hit the middle of the cirral fan as the cirri were extended was viewed frame by frame. The frame taken just before the cirri sweep forward showing milk billowing between the cirri (Figure 3E) was captured using a frame-grabber (Snappy, Play Inc.). It took an average of 0.14 ± 0.02 s for a billow to form. The number of frames in which the cirri were held extended at the apex of their sweep was recorded and used to calculate the duration of this pause.

A geometric model was used to calculate the leakiness between the two longest rami of cirri VI or the best visible billow between two other cirri during the pause at the apex of the feeding stroke. The cirri join together at their base and form a rough triangle. The volume of water that passes between the cirri while they are held extended would form a triangular prism (Figure 3A) and can be calculated by the formula:

$$V = b\, d\, h \tag{2}$$

where b is one half the distance between the tips of the rami, d is calculated by multiplying the speed of the water in the flow tank by the time the barnacle held its cirri extended, and h is calculated via the

Pythagorean theorem from the length of the ramus (l) as measured from the collected molts and b using the following formula:

$$h = \sqrt{l^2 - b^2} \tag{3}$$

The volume of water that actually passes through the cirri is approximated by one half of an elliptical cone (Figure 3B) as evidenced by the billows visible on the tape (Figure 3E). This volume is calculated by the formula:

$$V = \tfrac{1}{6}\pi a b h \tag{4}$$

where a is the radius of the arc described by the billow of milk at its midpoint, b is again one half the distance between the rami, and h is again the length of the ramus. The ratio of the observed volume to the expected volume yields the leakiness of the cirri, according to the definition of leakiness given by Cheer & Koehl (1987). Due to few (n=2) observations of milk billows in the tape of the small barnacles, statistical analysis was limited to the medium and large groups. The leakiness of the medium and large groups was compared using a two-tailed Student's t-test after Bartlett's Test confirmed that the variances were homogenous.

This geometric model makes several assumptions. The gap between the two cirri is assumed to be leaky all the way to the base, although this has not been tested. It is also assumed that the cirri are held straight in the xy-plane when they actually bend to form a concave cup in the z-direction. The images of the milk billowing between the cirri only reveal what is happening in a thin slice of water as deep as the milk stream is wide.

With this in mind, a second geometric model was made. This model makes fewer assumptions, but it is also less useful as it only calculates the leakiness of the cirri in the xz-plane with the depth in the y plane being the thickness of the milk stream aimed at the barnacle (estimated from the diameter of the syringe needle). The volume of water that passes through this thin plane through the cirri would form a rectangular prism (Figure 3C) and its volume was calculated using the formula:

$$V = b\,L\,d \qquad (5)$$

where L is the thickness of the milk stream. The volume of water that actually passes through the cirri is approximated by a thin slice through the top of the half of an elliptical cone we calculated earlier and can be calculated using the following:

$$V=\frac{\pi}{2}abL \qquad (6)$$

The ratio of the observed volume to the expected volume again gives us the leakiness. Again, the leakiness of the medium and large groups was compared using a two-tailed Student's t-test after Bartlett's Test confirmed that the variances were homogenous.

3. Results

3.1 Feeding Behavior

All size classes of barnacles spent a majority of their time either actively feeding or inactive at the relatively slow current speed of 4 cm/s (Figure 4). In general, the barnacles spent more time actively feeding and less time inactive with increasing size class. Large individuals spent significantly more time actively feeding (89.5 ± 5.1%) than small or medium individuals ($p < 0.05$). Only small individuals were observed passively feeding during these trials, and they spent an average of 7.1 ± 6.4% of the time engaged in this behavior.

As mentioned earlier, barnacle beat rate is known to increase with increasing temperature. The data confirmed this ($p = 0.02$). However, when the temperature was controlled for, there was still a significant difference in the beat rates exhibited by the different size classes (Table 1). The small barnacles beat their cirri faster (87 ± 6.1 beats per minute) than did the medium ($p < 0.001$) or large ($p = 0.01$) groups (Table 1). The mean beat rate of the medium and large groups was virtually identical and could not be differentiated statistically (49.6 ± 3.0 and 48.7 ± 3.0 beats per minute, respectively).

3.2 Cirral Anatomy

The Re's experienced by the barnacles at all measured points on their cirral fan increased significantly ($p<0.05$) with increasing size class. Small barnacles experienced Re's below one at the tips of their cirri and between their setae while the Re's near the base of the cirri were slightly above one (Figure 5). Large and medium barnacles experienced Re's greater than one at the base and tips of their cirri, while their setae were still operating at numbers below or near one.

All trials done in the lab subjected the barnacle to flow velocities of 4 cm/s. Water velocities encountered by barnacles in the field are likely to be higher and these higher velocities will cause the Re to increase. To predict how large the Re might get in the field, three graphs were made. Each graph showed Re as a function of velocity for one size class, with the maximum velocity set at 4 m/s (Figure 6). Li & Denny (2004) found that the highest current velocity that triggers a phenotypic change in leg morphology for *Balanus glandula* and *Semibalanus cariosus* is 2-4 m/s, which may indicate that they no longer feed at higher speeds. Chthamaloid barnacles are known to cease feeding at a current speed of around 4 m s^{-1} (L. Miller, Stanford University, personal communication). Thus we determined that 4 m s^{-1} is the best approximation of the maximum water velocity at which a west coast acorn barnacle may feed. Small barnacles at slow flows (1 cm/s) experience Re's on the order of 0.1 at their cirri and 0.01 at their setae. By the time a flow speed of 4 m/s is reached, the small barnacles experience Re's on the order of 100 at their cirri and 10 at their setae. Medium barnacles follow a similar trend, although their setae experience a Re an order of a magnitude higher (0.1) at slow flows (1cm/s) due to their increased setal diameter. Large barnacles only experience Re's less than one at any part of their cirral fan at extremely slow flows and, at 4 m/s, they experience a Re on the order of 100 at all parts of their cirral fan.

As the size classes of barnacle increased in diameter, the length of the longest ramus of cirrus VI increased significantly ($p<0.05$, Table 1). The number of setae per millimeter on cirrus VI decreased with increasing size class from a high of 84.1 ± 1.7 setae per millimeter for the small size class to a low of 46.9 ± 1.5 setae per millimeter for the large size class (Table 1). The large barnacles had significantly fewer setae per millimeter on their cirri than did the small or medium barnacles.

3.3 Leakiness of the Cirral Fan

The small barnacles were the least leaky, and the medium and large barnacles had similar leakiness (t-test, P=0.67) (Table 1). Only two observations were made where billows were visible with the small group (Figure 7A). In most instances where the milk stream encountered the tips of the cirri, the milk was blocked by the cirri and did not flow between them (Figure 7B). Thus, the leakiness of the small barnacles (n=2) was likely over-estimated.

4. Discussion

The aim of this study was to compare how different sized barnacles of the same species feed at a constant current speed. A transition in leakiness was observed between the small and the medium groups: the cirral fan of small barnacles behaved like a paddle while the cirral fans of the medium and large barnacles functioned like rakes. Changes in feeding behavior, beat rate, and cirral anatomy all contributed to this transition in leakiness.

4.1 Feeding Behavior

Small barnacles were observed to feed passively, which was not observed in larger groups feeding in the same 4 cm/s flow. Barnacles are known to passively feed in strong currents (Trager *et al.* 1990). Four cm/s may be fast for the newly-settled individual but slow to the larger groups. Although this could be a simple case of scaling the current down to match the small size of the cirral fan, it could also be due to local hydrodynamic conditions commonly experienced by juveniles. Smaller barnacles sit close to the substrate and deeper in the boundary layer, thus experiencing slower flows. Even barnacles that settle in wave-swept areas with extremely thin boundary layers may experience slower local flows due to their tendency to settle on rough surfaces in pits (Crisp & Barnes 1954, Wethey 1986, Walters & Wethey 1996) and in complex local hydrodynamic environments near or on adults (Knight-Jones 1953; Wethey 1984).

4.2 Cirral Beat Rate & Cirral Anatomy

The small barnacles had a higher cirral beat rate than did large or medium individuals. Unexpectedly, there was no significant difference between the beat rate of the large and medium groups, despite the fact the cirri of the large group were significantly longer than those of the medium group. This contradicts Newell & Northcroft (1965) and Anderson & Southward (1987), who both found a negative linear relationship between the log of cirral beat rate and the log of dry tissue weight (here approximated by aperture diameter). Decreasing beat rate with increasing size was attributed to depression of metabolic rate in larger individuals (Southward 1957), and was supported by evidence that the logarithm of oxygen uptake decreases as the logarithm of body weight increases (Barnes et al. 1963, Newell & Northcroft 1965). Anderson & Southward (1987) mention this difference in metabolic rate, but state that decreased beating with increasing size "probably represents a hydrodynamic effect". Although it is unclear what hydrodynamic effect Anderson & Southward meant, it likely refers to increased drag on the cirral fan, which is influenced by cirral length, cirral fan area, and number of setae per millimeter ramus length.

Thus, there are two possible reasons why the medium and large groups had the same beat rate. The slope of the regression of log cirral beat to log body weight for *Balanus glandula* is unknown, and it may be very shallow. Thus a large increase in body size would be needed to produce a noticeable decrease in beat rate. The difference in size in this study could have been too small to show a significant change in beat rate. This is unlikely, as the size classes were chosen to span the complete range of local body sizes and the medium group was approximately half as big as the large group. This leaves differences in hydrodynamics. The large group had significantly fewer setae per millimeter and significantly longer cirri than did the medium group. These trends probably apply to the entire cirral fan, not just the longest ramus on cirrus VI. Although no direct measurements were made, the area of the fan for the large group was observed to be bigger than the medium group and there were no visible differences in number of setae per millimeter within a fan. This decrease in setae per millimeter decreased the drag experienced by the cirral fan, thus allowing it to travel faster through the water and have a beat rate equivalent to that of the medium group with its shorter, more setous cirri.

4.3 Re and Leakiness

The Re's experienced by the cirral fan increased with increasing size of barnacles for all locations measured on the cirral fan. The Re calculated near the base of the cirri is likely an over-estimate, as direct observations of fluid passing through the lower cirri were not made and the importance of the bottom of the cirri in particle capture is not known. The small individuals experienced Re's less than one at the tips of the cirri and at the largest setae. Only the bases of the cirri experience Re's greater than one, and this may be an over-estimate. The region where particle capture was observed in feeding *Balanus eburneus* was near the tips of the cirri just above or behind the carina; the base of the cirri were not involved in particle capture (Young & Cameron 1989, Young 1990). When the barnacles in this study were observed feeding in a milk stream, the milk was often seen to stick to the cirri between beats. The estimates of leakiness from the geometric model were also low. The medium and large barnacles were observed occasionally to have milk sticking near the setae, but never to the same degree seen with the small individuals. The cirri of the medium and large groups of barnacles did have some water (visualized by the milk billows) traveling between them and their leakiness was an order of magnitude higher than the leakiness of the small group.

4.4 Conclusions & Future Directions

By necessity, this study took a very narrow view of the feeding of one species of barnacle in one current speed at three sizes. Future studies may be done to address feeding at faster current speeds more representative of what barnacles experience in the field. In addition, the mechanism of particle capture by the barnacles has not been examined by this study, although it is believed to occur when food particles become trapped in the boundary layers surrounding the setae. The large barnacles had fewer setae per millimeter than smaller groups. While it is unknown how this would affect feeding efficiency, the resulting reduction in drag per unit ramus length may be the reason that large and medium barnacles had the same beat rate despite the greater length of the large barnacle's cirri.

This study revealed that newly-settled *Balanus glandula* has cirral fans that can function like paddles. At some size between the small and medium size classes, the cirral fan begins to function more like a rake. However, this has only been demonstrated at a current speed of 4 cm/s. The predictions made of Re's experienced with increasing flow speed revealed that *B. glandula* feed at Re's greater than one except for newly-settled individuals feeding at currents slower than 5 cm/s. Direct measurements of the current speeds encountered by juveniles in the field have not been made, so it is unknown if the low Re feeding observed in the lab occurs in the field. This study did not address the efficiency of food capture and how feeding by juveniles at slow flows differs in efficiency from feeding at faster flows and higher Re's. This deserves future research. If feeding at Re<1 is inefficient and current speeds experienced by juveniles in the field are sufficiently slow, the minimum size of a newly-settled individual may be limited so that the cirri are large enough be slightly leaky. The observed non-leaky feeding in juvenile *Balanus glandula* may have implications on their diet as well. Future studies should address ontogenetic dietary changes, as this has not yet been investigated.

5. Bridge

The previous chapter took an in-depth look of the feeding of one species of barnacle at a specific flow speed and tried to answer some questions about how the biomechanics of barnacle feeding changed as the barnacle got bigger. This is an example of scaling, or how organisms adjust their structure and function to compensate for the geometric, physical, and biological consequences of being different sizes (Brown et al. 2000). Now that we have studied the scaling of one species of barnacle, the next logical step is to expand the study to compare similar size classes of different species of barnacles. The next chapter will address interspecific differences in cirral anatomy, feeding behavior, and Re experienced while feeding for three species of common acorn barnacle.

Table 1: A summary of some results from the behavior, cirral anatomy, and leakiness estimate analyses. Values in the table are means plus or minus standard error. Lines indicate groups statistically indistinguishable at the p=0.05 level. Cirral beat rate, ramus, length, and # setae data were analyzed using an ANOVA followed by a Tukeys HSD. Leakiness data for the small group could not be analyzed statistically due to low sample size (n=2). Medium and large groups were compared with a two-tailed Student's t-test.

	Small	**Medium**	**Large**
Cirral Beat Rate (beats/minute)	87.00 ± 6.11	49.60 ± 3.03	48.70 ± 2.97
Length of Longest Ramus of Cirrus VI (mm)	0.512 ± 0.051	3.641 ± 0.159	6.490 ± 0.08
# Setae / mm	84.13 ± 1.685	79.15 ± 3.26	6.490 ± 0.08
Leakiness- Elliptical Cone	0.31%*	4.05 ± 0.33%	4.56 ± 0.78%
Leakiness- Semiellipse	0.46%*	6.07 ± 0.49%	6.83 ± 1.16%

Figure 1: The two flow tanks used in this study. The large flow tank (1A) was constructed according to Vogel & LaBarbera (1978) and was videotaped from the side using a VHS camera mounted on a tripod. It had a working area of 20 X 6 X 13 cm. The small flow tank (1B & close up 1C), working area 7 X 5 X 3.5 cm) rested on the stage of a dissecting scope and was powered by a fish tank water pump (Rio 800 H P). The working area of both tanks had removable rods (labeled "r") attached to the bottom and side of the tank to facilitate taping in profile or from above (1A, 1C).

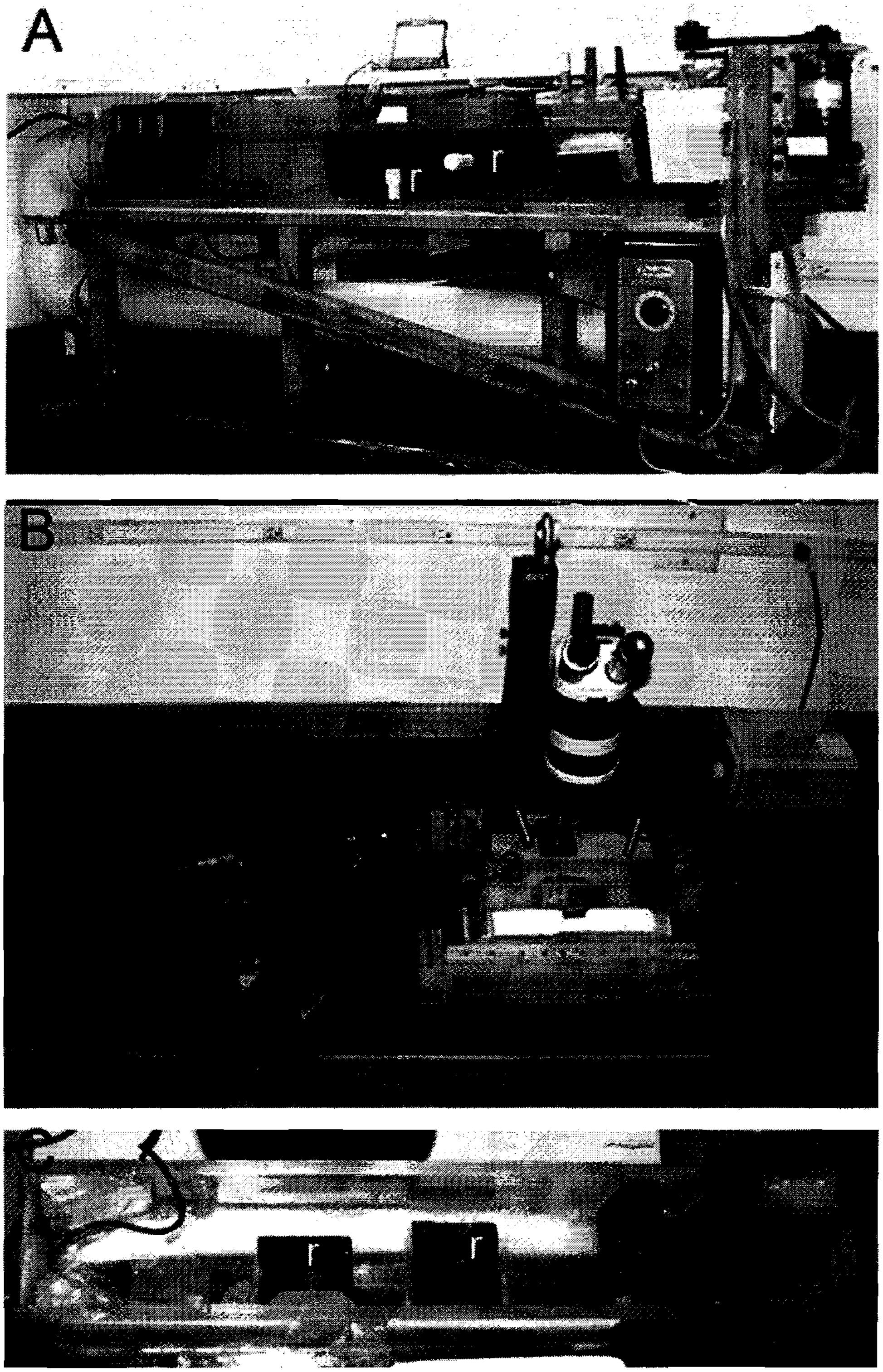
A
B
C

Figure 2: Cirral anatomy and traits measured. The ventral surface of cirrus VI was first photographed from above in as natural a position as possible (1A). The width of the longest ramus at the bottom (a), the distance between the rami at the bottom (b), the distance between the rami at the top (c), and the width of the longest ramus at the top (d) were measured. Then the longest ramus was cut at the base and photographed in lateral view (1B). The curvilinear distance from e to f was measured as the cirrus length. The number of segments and setae per segment of the longest ramus of cirrus VI were also counted. Under higher magnification (1C), the width of the longest seta (g) and the distance between this seta and its nearest neighbor (h) were measured.

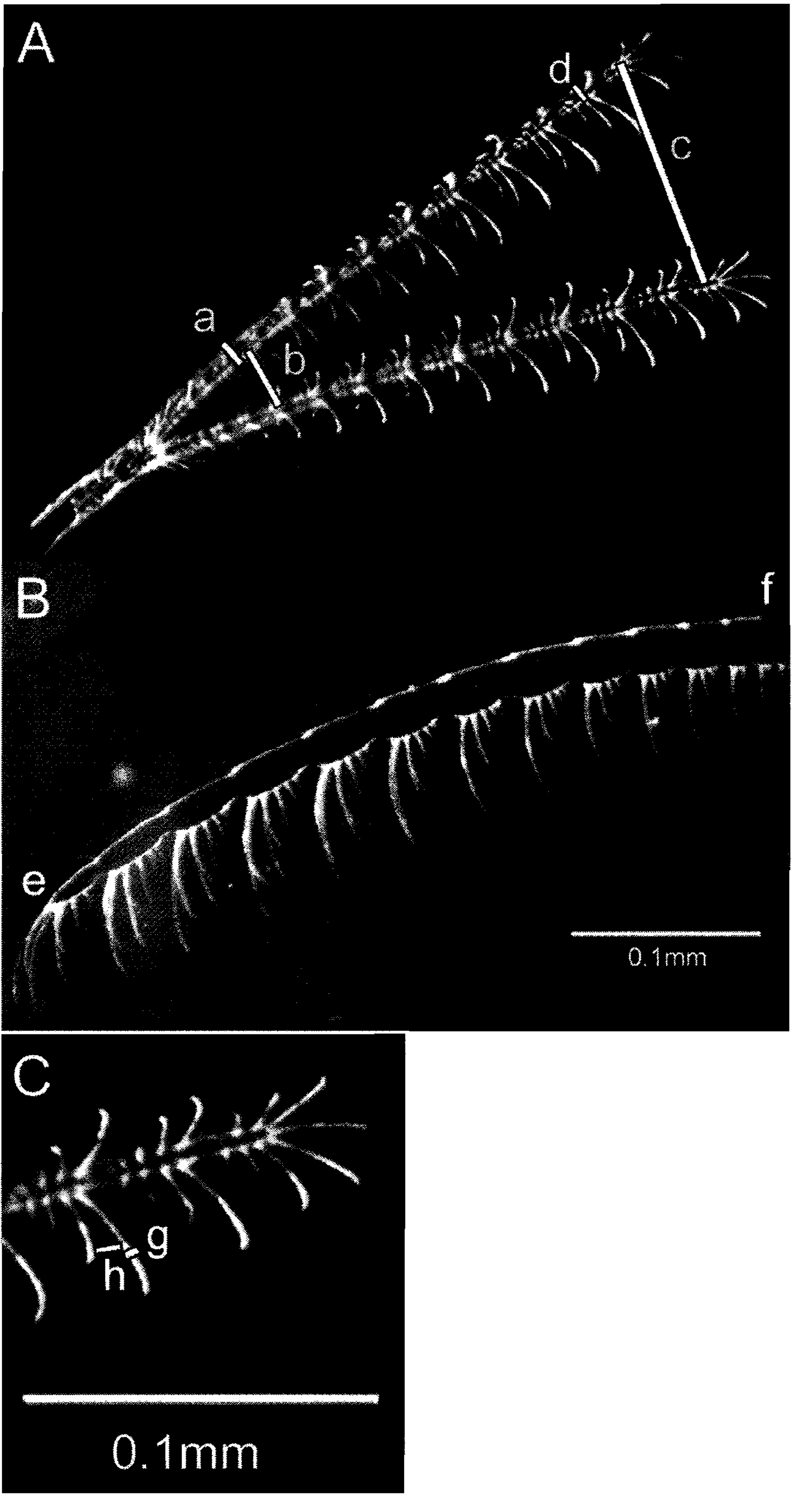
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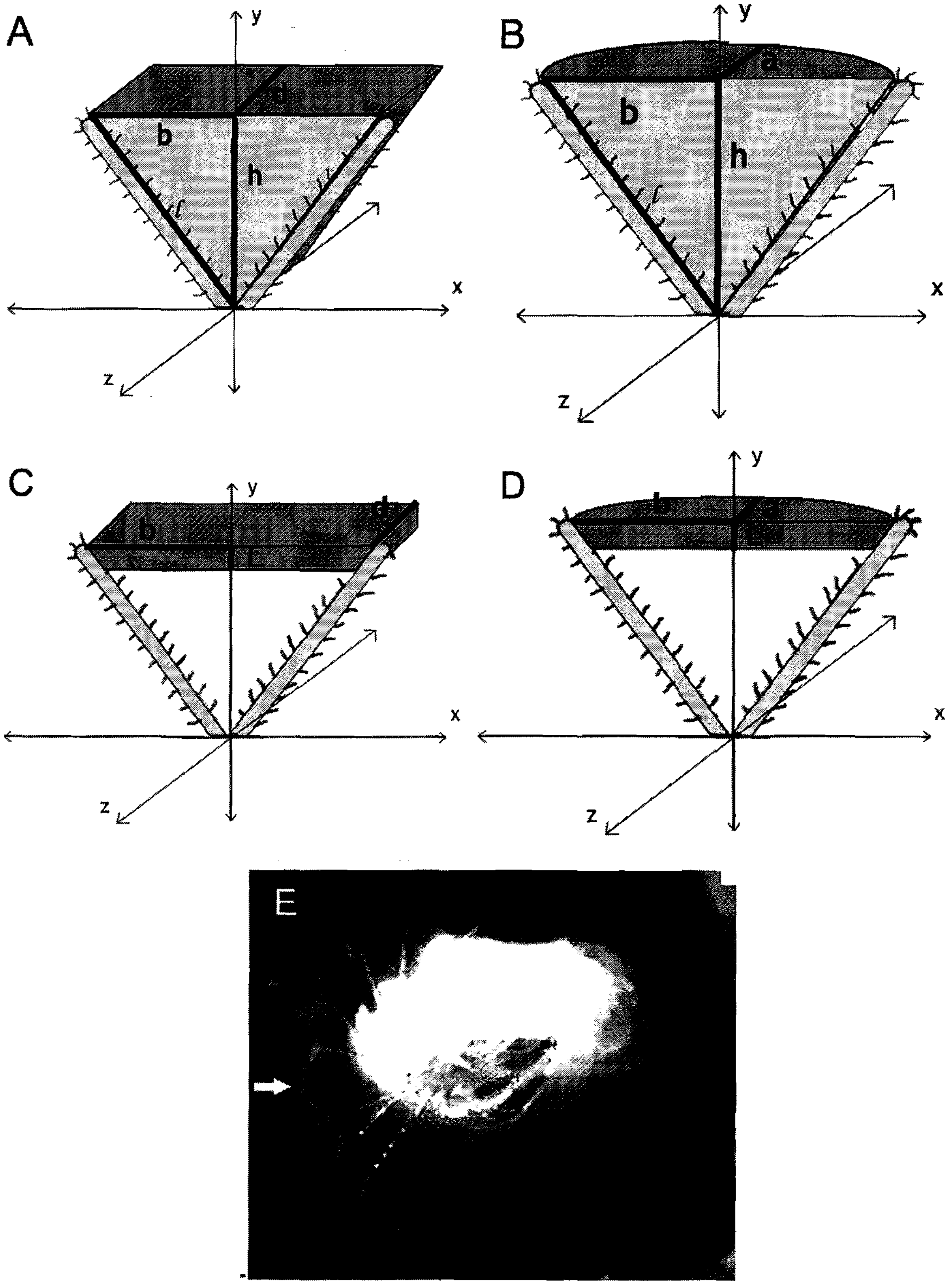
Figure 3: Geometric models for (A) the volume of water that is swept between the two rami of cirrus VI at the apex of the feeding stroke, (B) the actual volume of water that passes between the rami, (C) a slice of (A) taken at the top of the cirri the thickness of the milk stream, (D) a slice of (B) taken at the top of the cirri, and (E) a photograph of a barnacle feeding in a milk stream from above, with the billow indicated by an arrow.

a is the radius of the arc described by the billow of milk at its midpoint. b is one half the distance between the tips of the rami. d is calculated by multiplying the speed of the water in the flow tank by the time the barnacle held its cirri extended. h is calculated via the Pythagorean theorem from the length of the ramus (l) as measured from the collected molts and b using the formula $h = \sqrt{l^2 - b^2}$. L is the diameter of the milk stream estimated from the diameter of the needle.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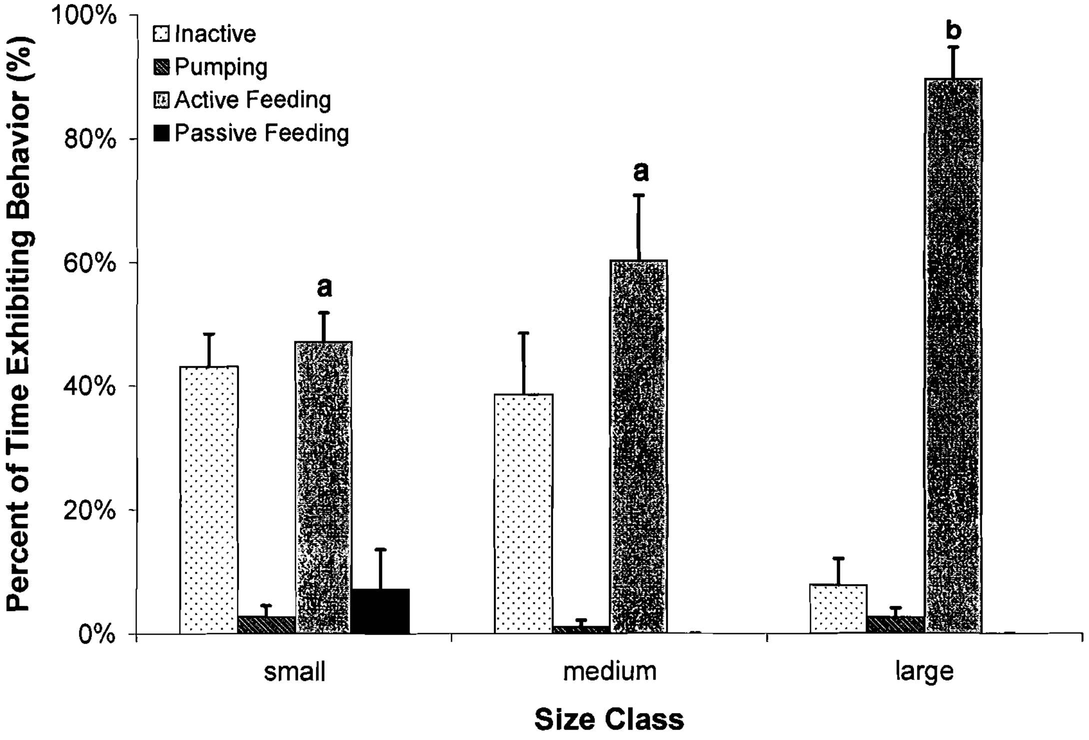


Figure 4: Behaviors exhibited by *B. glandula* individuals feeding in 4 cm/s current grouped by size class. Large individuals spent significantly more time actively feeding than medium or small individuals (p<0.05). Error bars indicate standard error. Letters above bars indicate Tukey's grouping (p<0.05).

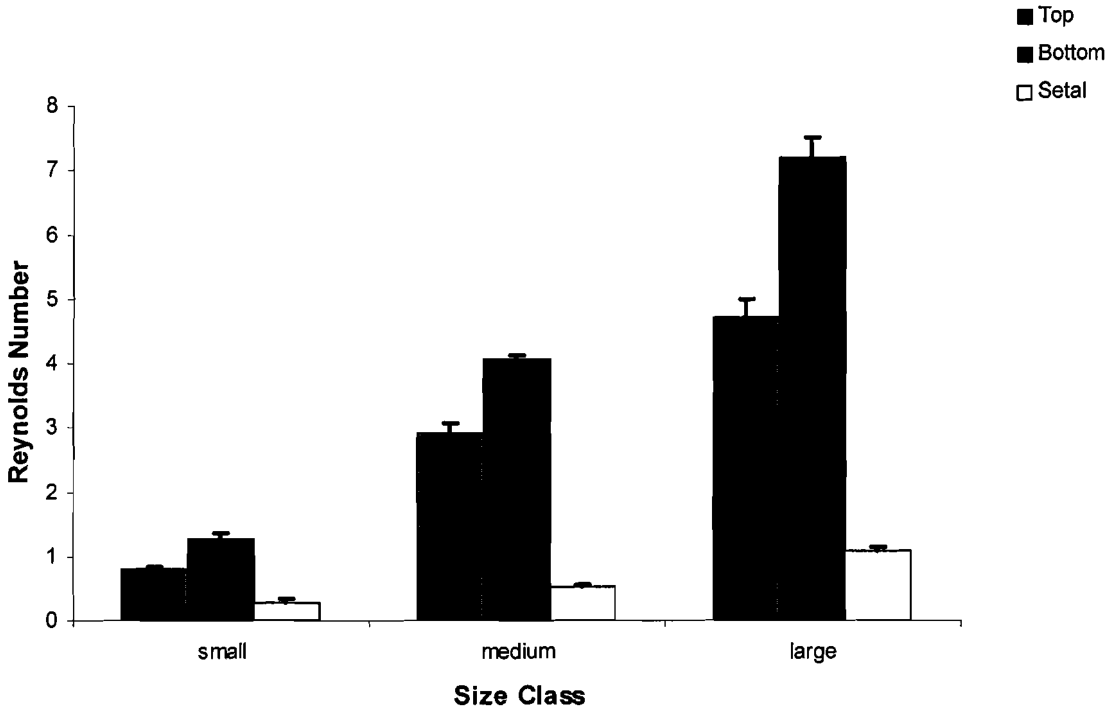
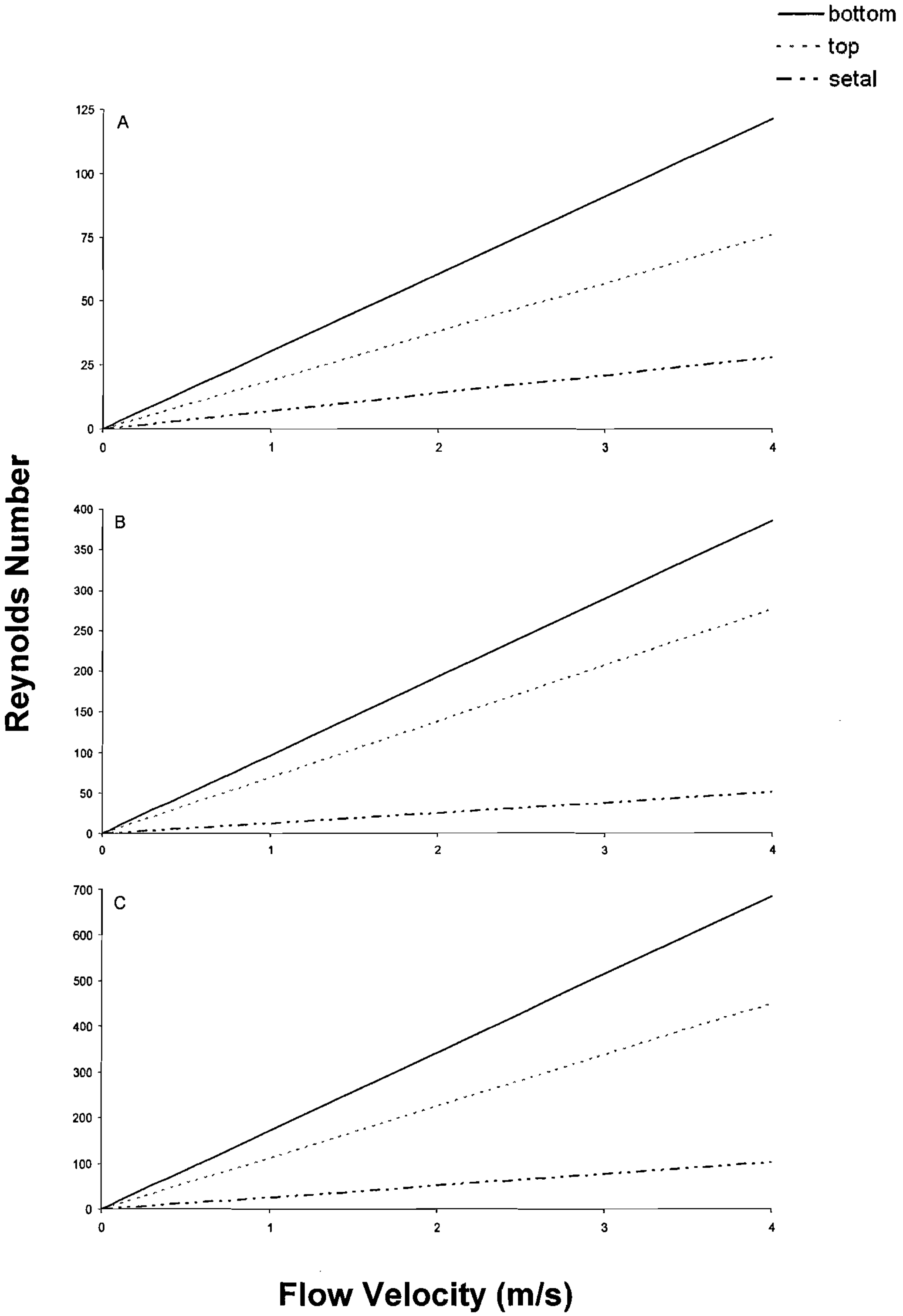


Figure 5: Re calculated at three different locations within the cirral fan (for longest ramus of cirrus VI near proximal and distal ends and for the longest setae) grouped by size class. Error bars indicate standard error. At each location where it was calculated, Re significantly increased with increasing aperture diameter of size class.

Figure 6: A graph of calculated Re as a function of flow velocity in meters per second for (A) small, (B) medium, and (C) large *B. glandula* measured at the bottom and top of cirrus VI as well as at the longest seta on cirrus VI. The small barnacles at flow velocities less than 5 cm/s experience Re's less on the order of 0.1 at the cirrus level and 0.01 at the setal level. At flow velocities higher than 5 cm/s, which may be more similar to what barnacles experience in the field, the small barnacles experience cirral Re's above one. Medium and large barnacles experience cirral Re's on the order of 100 at the velocities at which they are likely to feed in the field.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Reynolds Number
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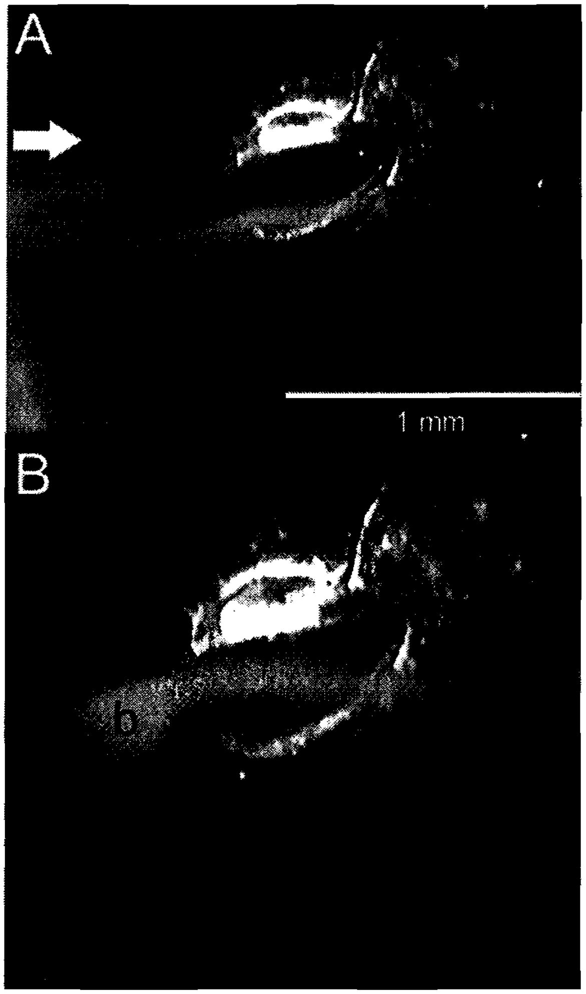


Figure 7: Screen captures of two small barnacles actively feeding in a milk stream. In (A), milk is passing between two of the cirri forming a billow (indicated by the arrow). The billow indicates the amount of water that is actually passing between those two cirri. In (B), the cirri are blocking the flow of the milk, causing the milk stream to back up and form a bolus (b). This indicates that the cirral fan is acting like a paddle and the leakiness is near zero.

CHAPTER III

FEEDING BEHAVIOR, CIRRAL ANATOMY, AND CIRRAL REYNOLDS NUMBERS OF THREE BARNACLES: *BALANUS NUBILUS, BALANUS GLANDULA,* AND *SEMIBALANUS CARIOSUS*

1. Introduction

Size is one of the most important descriptors of an organism. One consequence of size for organisms living in water is Reynolds number (Re). Re is the unitless ratio of inertial forces to viscose forces (Vogel 1981). For small organisms with Re < 1, viscosity dominates whereas organisms with Re > 1 live in worlds dominated by inertia. The mechanisms of particle capture for small suspension feeding organisms is especially impacted by Re (Koehl 1993; Koehl 1995; Koehl 2001; Koehl 2004). The copepod *Eucalanus pileatus* has feeding appendages covered in setae that operate at Re 10^{-2} to 10^{-1}. Although the second maxillae superficially resemble sieves, they function as solid paddles that push food towards their mouth as very little water passes between the setae (Koehl & Strickler 1981). Similarly, Rumrill (1987) calculated the Re of the feeding appendage (first maxilliped) of the crab zoea *Pugettia producta* to be on the scale of 10^{-2}. In contrast, a larger species of copepod (*Centropages furcatus*) has similar second maxillae, but their setae operate at Re=1 and thus the second maxillae functions like a leaky sieve (Koehl 1995).

Barnacles are another suspension feeder with setous appendages that operate at transitional Re's near one where small increases in size can result in a shift from a world dominated by viscosity to one dominated by inertia. As barnacles grow from recruits to adults, they increase in aperture diameter by as much as a factor of 100 (*Balanus nubilus*, Morris *et al.* 1980). Most studies dealing with changes in barnacle feeding over growth have focused primarily on changes in feeding behavior. In his senior thesis, Hart (2001) found that juvenile *Balanus nubilus* exhibit a faster beat rate while actively feeding than do adults. Juvenile whale barnacles (*Cryptolepas rhachianechi*) are known to re-orient their cirri in response to changes in direction of water flow, while adults show no such response (Achituv 1998). Likewise, the crab barnacle *Chelonibia patula* will reorient their cirral fans to align with current direction as juveniles, while adults will not feed if the new current direction is greater than 100° off their rostral-carinal axis (Pasternak et al. 2002)

Two studies addressed changes in feeding behavior between juveniles and adults in three related species of stalked barnacle. Lewis

(1981) found that juvenile stalked barnacles (*Pollicipes polymerus*) feed by actively beating their cirri, while larger adults only feed by passively holding their cirri in the current. Hui (1983) expanded this study and observed a similar switch in feeding behavior over growth in the congener *Pollicipes pollicipes*, but not in another stalked barnacle in the family Scalpellidae, *Capitulum mitella*. Thus the more closely related species, behaved similarly to each other.

The present study focuses on the feeding behavior, cirral anatomy, and Re of three sizes classes of three species of common acorn barnacle: *Balanus glandula* Darwin, 1854; *Balanus nubilus* Darwin, 1854; and *Semibalanus cariosus* (Pallas, 1788). *B. glandula* and B. *nubilus* are congeners in the family Balanidae, while *S. cariosus* is in the family Archaeobalanidae. We compared the observed trends in behavior, anatomy, and Re to determine if they were species- or size- specific. We predicted that the feeding behavior and cirral anatomy would be convergent within a size class, irrespective of species, as the three species live in similar environments and the viscous and inertial forces acting on them would be similar. We tested this by observing individual barnacles in flow tanks and recording their feeding behaviors. We collected molts from the barnacles to measure traits in cirral anatomy and calculated Re's for different points in the cirral fan.

2. Methods & Materials

2.1 Animal Collection & Rearing

Three size classes of each barnacle species were used in this study. The small size class consisted of newly-settled individuals (n=10 per species). The large size class was made of the largest *Balanus glandula* found locally (n=10) and ten individuals each of *Semibalanus cariosus* and *Balanus nubilus* with similar tissue mass. The medium group consisted of intermediate sized *B. glandula*, and *S. cariosus* and *B. nubilus* of similar tissue mass. As it is difficult to measure tissue mass of a barnacle without destroying the individual, we determined the relationship between tissue mass (ash-free dry weight, AFDW, in g) and aperture diameter (mm). Aperture diameter is defined here as the linear distance from the top inner edge of the rostrum to the top inner edge of the carina when viewed from above. Ten individuals each of medium and large *B. glandula* were collected, dried to constant weight in a drying oven, ashed in a muffle furnace at 500°C for six hours, and the average AFDW's were calculated. Twenty individuals each of *S. cariosus* and *B. nubilus* with varying aperture diameters were collected. They were dried to a constant weight, ashed as before, and their AFDW was plotted as a function of aperture diameter (mm) for the two species. Linear trend lines were fitted to these graphs. The regression equations (Table 1) were then solved for the average AFDW's calculated earlier for *B. glandula* to give the aperture diameters of *S. cariosus* and *B. nubilus* required for the experiments. Thus three size classes of three species of barnacles were collected with individuals of the same size class and having approximately the same AFDW (Table 2). AFDW was chosen instead of cirral length to standardize the size groups as it is easily measured (via aperture diameter).

The small size classes were either collected from the field as cyprids or raised from nauplii in the laboratory. For *Balanus glandula,* cyprids were collected with a 73-μm mesh plankton net inside the mouth of Coos Bay, Oregon, USA. The plankton samples were transported to the Oregon Institute of Marine Biology and cyprids were picked individually from the plankton with a glass pipette. *Semibalanus cariosus* and *Balanus nubilus* juveniles were obtained by collecting ripe egg lamellae from adult barnacles, transporting the lamellae to OIMB, shining a light on the lamellae to induce hatching, and rearing the larvae to cyprid stage. Larvae were reared in glass jars containing two liters of filtered seawater, nauplii at a concentration of one larva per two milliliters seawater, penicillin-G (0.016 mg mL^{-1}) and streptomycin sulfate (0.027 mg mL^{-1}) (Strathmann 1987). The jars were placed in a flowing seawater table and stirred with paddles. The barnacles were fed *Skeletonema costatum* at a concentration of 1.0×10^6 cells mL^{-1} for *S.*

cariosus and 2.5 X 10^5 cells mL^{-1} for *B. nubilus*. Cyprids began appearing in the jars after approximately two weeks.

Cyprids of all species were allowed to settle in finger bowls containing 3M Safety Walk Tape treated with extract of the adult of the respective species (Rittschof et al. 1984). The newly-settled barnacles and a portion of Safety Walk Tape surrounding them were removed using a scalpel, then attached with cyanoacrylate glue to small glass squares cut from microscope slides. Juveniles were held in 8-mL tissue culture wells with filtered seawater and *Skeletonema costatum* until their first molt, after which fecal matter began to appear, confirming that the juveniles were feeding. Scaled photographs were taken of the small barnacles and their aperture diameters were measured with ImageJ version 1.34s (National Institutes of Health).

Adult *Semibalanus cariosus* and *Balanus glandula* were chiseled off a sandstone shelf near the OIMB Boathouse Auditorium, Charleston, Oregon, USA. Since large intra-specific variation in cirral length and setation has been described in *B. glandula* and *S. cariosus* and attributed to differences in current speed (Marchinko 2003; Marchinko & Palmer, 2003; and Li & Denny, 2004), we took care to collect the barnacles from the rock face within a 4-m^2 area to control for variations in flow along the sandstone shelf. All barnacles were volcano-shaped and none were collected from hummocks. Adult *B. nubilus* were collected from "D" dock in the Charleston Boat Basin. Vernier calipers were used to measure the aperture diameters of the barnacles.

Medium and large barnacles were taken back to OIMB, cleaned of epiphytes, and housed individually in the compartments of ice cube trays (17mL of seawater in each compartment). All barnacles were placed on a rotor tray set at 38 rotations per minute inside an incubator set at 10 °C. Barnacles were fed *ad libitum* with the diatom *Skeletonema costatum* and the water was changed every three days.

2.2 Barnacle Feeding Behavior

To quantify feeding behavior, barnacles were videotaped while feeding in flow tanks. Two recirculating flow tanks were used in these trials. Large barnacles were taped in the large flow tank (working area 20 X 6 X 13cm), which was designed according to Vogel & LaBarbera (1978) (Figure 1A). The small and medium barnacles were taped in a smaller flow tank (working area 7 X 5 X 3.5cm) positioned on the stage of a dissecting microscope. The tank was made of plexiglass and powered by an aquarium water pump (Figure 1B). A velocity profile was generated for both tanks, and the height of free-stream velocity was determined. Rods were cut to these distances and mounted in the middle of each tank so that a barnacle attached to the top of a rod would experience free-stream velocity.

At the beginning of each trial, the flow tank was filled with cold (~12°C) seawater filtered with a 0.45µm filter. The water velocity in the flow tank was determined by timing particles traversing a known distance at the height of the top of the rod. Mussel juice (see recipe Appendix A) was added as a feeding stimulant (Allison & Dorsett 1977) at a concentration of 1mL per liter of seawater. Using denture adhesive (Super Poligrip® Comfort Seal™ Strips), a barnacle was affixed to the top of the rod with its cirri facing into the current and given five minutes to acclimate and begin feeding in the flow tank. If a barnacle extended its cirri during this time, a trial was begun immediately and its behavior was videotaped (SVHS) for five minutes. A Pulnix (model TM-7CN) black and white video camera was used. When used with the small flow tank, it was attached to the phototube of the dissecting scope. For use with the large flowtank, it was set on a tripod and fixed with a Nikon AF Micro Nikkor 650mm macro lens with a 2X multiplier for use with the large flow tank. If the barnacle did not extend its cirri within five minutes, it was removed from the flow tank and exchanged for a new individual. The temperature of the flow tank was measured using a thermister (Fisher Scientific) and recorded at the beginning and end of each trial. If the water temperature reached 16°C during a trial, the trial was ended, and the tank was drained and filled again with cold seawater and mussel juice before resuming trials. This procedure was repeated for ten individuals of each species in each size class. Small *S. cariosus* were observed in flow speeds of 5.6 cm/s, while the other two species were observed in flow speeds of 2 cm/s. Feeding behavior may be flow speed dependant, so no comparisons could be made including *S. cariosus* for this size class. All medium and large barnacles were videotaped feeding at flow speeds of 3.74 - 4.43 cm/s.

While viewing the videotapes of the trials, we recorded the amount of time each barnacle spent exhibiting specific behaviors. The categories of feeding behavior observed were based on the descriptions of Crisp & Southward (1961) and Anderson & Southward (1987). A barnacle was recorded as "inactive" if its cirri were not extended for at least 3 seconds, as Anderson (1981) found that the slowest beat observed by *Balanus perforatus* was 3s in duration. Thus, a pause of less than 3s may have been the retraction stage of a cirral beat cycle. A barnacle was characterized as "pumping" if its valves were open and the cirri were visible but folded together with the cirral tips inside the shell. A barnacle was engaged in "active feeding" if the cirri were beating, the tips of the cirri were raised out of the shell, and the cirri were held extended at the apex of their beat for less than one second. Passive feeding occurred when a barnacle was beating its cirri and held the cirri extended at the apex of the beat for longer than one second. The total amount of time a barnacle spent exhibiting each behavior was recorded and expressed as a percentage of the total time of each trial.

The cirral beat rate of each feeding barnacle was determined. We recorded the number of cirral extensions in four haphazardly selected, twenty-second portions of videotape showing the barnacle actively feeding. These values were averaged to determine the number of beats per minute for each individual.

2.3 Cirral Anatomy & Re's

The barnacles used in this study were housed in individual chambers and monitored daily for molting. Exuviae were collected and preserved in 70% ethanol on the same day they were found. The aperture diameters of the barnacles were recorded using the methods described previously. Each molt was viewed under a dissecting microscope and the ventral side of the fan was photographed in the most natural posture possible. In cases where the fan was preserved in a clenched posture and could not be opened, one cirrus VI was cut away from the fan, straightened into a natural feeding posture, and its ventral surface photographed (Figure 2A). Finally, a cirrus VI was turned on its side and photographed in profile (Figure 2B).

The photographs of exuvia were enhanced using Adobe Photoshop version 6.0 and then analyzed with ImageJ 1.34s (National Institutes of

Health). The traits measured were similar to those measured by Marchinko & Palmer (2003). The lower ramal diameter was measured at the second segment from the proximal end of the ramus (Figure 2A, a). The upper ramal diameter was the diameter of the longest ramus at second segment from its distal end (Figure 2A, b). The ramus length was measured as the curvilinear distance along the dorsal margin from the base to the tip, excluding the terminal seta (from c to d in Figure 2B). The setal diameter was measured as the width of the longest seta on the longest ramus of cirrus VI (e in Figure 2C). The number of setae per segment and the number of segments were counted on the longest ramus of cirrus VI.

These measurements were used to calculate the Re experienced at three different locations in the cirral fan: at the longest ramus of cirrus VI, both near the base and near the tip; and near the distal end of the longest seta. The Re's calculated at the base of the cirral fan are probably an overestimate, as it is not clear if or how much water passes through that portion of the fan during feeding. The Re's were calculated using the following formula:

$$\mathrm{Re} = \frac{\rho l U}{\pi} \qquad (1)$$

Where ρ is the density of seawater (1025 kg $(m^{3})^{-1}$), ℓ is the diameter of the structure (cirrus or seta), U is the velocity of the water in the flow tank, and μ is the dynamic fluid viscosity of seawater (0.0011 kg $(m*s)^{-1}$). In addition, the number of setae per millimeter on the longest ramus of cirrus VI was calculated by multiplying the number of setae per segment by the number of segments and dividing that by the length of the ramus. The Re calculated near the base of the cirri is likely an over-estimate, as direct observations of fluid passing through the lower cirri were not made and the importance of the bottom of the cirri in particle capture is not known.

2.4 Statistics

As a result of a problem with flow tank calibration, the three size classes of barnacle were tested at statistically different current speeds (one-way ANOVA performed on current speed, followed by Tukey's HSD p=0.05; Table 3). Feeding behavior, cirral beat rate, and Reynolds number are all dependent on current speed. Thus, no statistical comparisons could be made between the size classes for these traits. The small *Semibalanus cariosus* were videotaped feeding in a current speed statistically higher than the small individuals of the other two species. By the time this error was realized, the reproductive season of *S. cariosus* was passed and no new small individuals could be obtained. Thus, only the cirral anatomy data for small *S. cariosus* is included in this paper.

Of the behavioral data, only the time spent active feeding was compared statistically between species. The data were analyzed separately for each size class, with the data for the small *Semibalanus cariosus* omitted. Within the medium and large size class, the percent of time spent actively feeding was analyzed with a one-way ANOVA followed by Tukey's HSD to compare the means of the species (Table 4). The two species in the small size class were compared with a two-tailed Student's t-test after homogeneity of variance was confirmed with Bartlett's Test. Likewise the cirral beat rate data were analyzed separately for each size class, with the data for the small *S. cariosus* omitted. As temperature is known to affect beat rate (Southward 1955, Anderson & Southward 1987), we analyzed the data using ANCOVA with temperature (thermister readings) as a covariate, followed by orthogonal contrast statements that were used to make *a priori* comparisons of the means (Table 5). Similarly, the data for the Re calculated at different points in the cirral fan were analyzed separately for each size class with the data for the small *S. cariosus* omitted. Three separate two-way ANOVA's were run (Table 6).

Trends in cirral anatomy are not dependent on the current speed used during the flow tank behavior trials. Thus, data for all sizes of all species were included in the analysis and comparisons could be made across size classes. Trends in number of setae per millimeter were examined using two-way ANOVA (Table 5).

3. Results

3.1 Feeding Behavior

All species and size classes spent most of the observed time actively feeding (Figure 3). *Balanus nubilus* spent the most time actively feeding in all size classes (small- 73.22 ± 9.83%, medium- 91.90 ± 3.6%, large 91.77 ± 5.21%), but this trend was not significant. In large size class, *B. nubilus* spent more time actively feeding than did *Semibalanus cariosus* ($p < 0.05$). Passive feeding was not observed at any size in any species. *B. nubilus* was not observed pumping as a medium or large size class barnacle, while medium and large *Balanus glandula* and *S. cariosus* did pump for small periods of time.

In the small and medium size classes, the beat rates of the different species of barnacle were statistically indistinguishable (Figure 4A). In the large group, *Balanus nubilus* had a significantly faster beat rate than did *Semibalanus cariosus* ($p < 0.05$) when the effects of temperature were accounted for.

3.2 Cirral Anatomy & Re

The number of setae per millimeter on cirrus VI in *Balanus glandula* and *Semibalanus cariosus* significantly decreased as the size class got larger (Figure 4B). However, medium *Balanus nubilus* had the largest number of setae per millimeter for that species.

At the slow flow speeds used in this study, small *Balanus glandula* and *Balanus nubilus* both fed at Re's lower than one at all points in their cirral fan (Figure 5). Large *B. glandula* experienced higher Re's at all points in their cirral fan than the other two species. Data used to calculate Re as well as other cirral morphology data are shown in Table 7.

All trials done in the lab subjected the barnacle to relatively slow flow velocities (2 cm s^{-1} for small, 3.74 - 4.43 cm s^{-1} for medium and large). Barnacles in the field are likely to encounter higher water velocities, perhaps up to 4m s^{-1}. Li & Denny (2004) found that the highest current velocity that triggers a phenotypic change in leg morphology for *Balanus glandula* and *Semibalanus cariosus* is 2-4 m s^{-1}, which may indicate that they no longer feed at higher speeds. Chthamaloid barnacles are known to cease feeding at a current speed of around 4 m s^{-1} (Lucas Miller, personal communication). Thus 4 m s^{-1} is the best approximation of maximum water velocity at which a west coast acorn barnacle may feed. These higher current speeds also mean higher Re. To predict what Re's might be experienced by a barnacle in the field, Re was plotted as a function of velocity for the three species, with one graph being devoted to each size class and the maximum velocity set at 4 m s^{-1}. Figure 6 shows Re as a function of current velocity for the three size classes of barnacle at the top of their cirri. Figure 7 shows the same, except the Re was calculated at the setal level.

According to the predictions made of Re experienced at the top of the cirrus (Figure 6), all sizes of all species of barnacles in this study make the transition from Re <1 to Re >1 at flow speeds of about 5 cm*s^{-1}. Re = 1 is a transitional Re where inertial and viscous forces are equal. The large *Balanus glandula* feed at much higher Re's than similar sized *Balanus nubilus* and *Semibalanus cariosus*; the top of its cirri reach an Re of 100 at a current velocity of about 90 cm s^{-1}. *B. nubilus* and *S. cariosus* reach a cirral top Re of 100 at about 2 m s^{-1}.

The Re's the barnacles are predicted to encounter at the setal level (Figure 7) are about an order of magnitude smaller than the cirral Re's for all size classes. At slow currents less than 20 cm s^{-1}, the small barnacles operate at setal Re's less than one. Most of the barnacles of all size classes experience setal Re's on the order of 10 for most velocities, indicating that there is some flow of water between the setae.

4. Discussion

All barnacles spent the majority of their time actively feeding. Passive feeding was not observed at all, although passive feeding is commonly observed in strong flows (Crisp & Southward 1961), and the current speeds used in this study were likely not fast enough to induce a shift to this behavior. *Semibalanus cariosus*, like other archaeobalanids, was thought to rely heavily on passive feeding (Southward & Crisp 1965). This study demonstrates *S. cariosus* does not exhibit passive feeding at slow current speeds and instead feeds like a balanid.

One trend of interest is the higher Re's experienced by large *Balanus glandula* compared to the other two species, due to their relatively wider cirri and setae. It is interesting that *Semibalanus cariosus* of similar AFDW collected within a 4-m2 area on the same sandstone shelf had much thinner cirri. It is possible that the differences in current velocity on that shelf occur at scales less than $4m^2$ and that the *B. glandula* lived in locally faster flow. Cirri of both *B. glandula* and *S. cariosus* are known to become shorter, thicker, and more setous in regions of faster flow (Marchinko 2003; Marchinko & Palmer, 2003). This is further supported by the fact that *S. cariosus* had significantly more setae per millimeter on the longest ramus of its cirrus VI than did *B. glandula*. Alternatively, this difference in cirral thickness could be a species-specific trait. A more comprehensive survey of the cirral widths of local populations of these two species across different hydrodynamic regimes would determine if this was the case.

Semibalanus cariosus and *Balanus glandula* showed a significant decrease in number of setae per millimeter on cirrus VI as size class diameter increased. *Balanus nubilus* did not show this trend. In *B.*

nubilus, the medium size class had the largest number of setae per millimeter, followed by the small and large size classes, respectively. Difficulty in positively identifying small individuals of B. nubilus in the field and the relative local scarcity of the species caused the authors to rear the small group of that species in the lab until they reached the diameter of the medium size class. *B. nubilus* was the only species so reared. It is possible that the large number of setae per millimeter for this species and size class may be due to the unique hydrodynamic conditions caused by rearing them while isolated in an individual tissue culture well set on a rotary tray or on their restricted diet of only the diatom *Skeletonema costatum.* Thus, the trend of decreasing number of setae per millimeter with increasing size class diameter may hold across the species. This may represent a mechanism of improving particle capture at small sizes. Small barnacles feed at lower Re and would have less leaky cirral fans than larger individuals. Increased number of setae in small individuals would increase the surface area that food particles might contact and adhere to, facilitating their transfer to the maxillae and ultimate ingestion. In contrast, larger individuals would feed with leakier cirral fans where sieving would become a more important method of suspension feeding than direct interception. Large numbers of setae per millimeter would also increase the drag on cirral fans. As the size of the cirral fan increases, the increased drag caused by the setae would reduce the maximum current speed at which the barnacle could feed. Thus, lower numbers of setae per millimeter would be beneficial for larger individuals.

The observed non-leaky feeding by small (juvenile) barnacles at current speeds less than 5 cm/s may have important ecological implications for these species. Future experimentation is needed to determine at which current velocities juvenile acorn barnacles feed in the field, thus confirming if this low Re feeding occurs. If juvenile acorn barnacles do feed at cirral Re<1 in the field, their cirral fans would function more like paddles than rakes, changing their method of particle capture and influencing their diet. Only one study to date has examined cirripede diets, and it focused solely on adults. Barnes (1959) found that *Semibalanus cariosus* (then *Balanus cariosus*) consumed mainly crustaceans, with 73.9% of their stomach contents particles 4-8 μm in diameter, while *Balanus nubilus* consumed mostly diatoms and algal filaments, with 40% of their stomach contents <2 μm in diameter. *Balanus glandula* was not included in this study. The size of the adults used in that study was not mentioned. It is assumed that juveniles of these species probably have different diets than adults, perhaps consisting of small algal cells that would stick to the setae of their cirral fans. Ontogenetic changes in barnacle diet have not yet been studied.

In general, the similarities between the feeding of the three species of barnacles are more striking than the differences between them. Ideally, all the barnacles in this study would have been tested at the same current speed to allow us to test the interaction of species and size class with ANOVA. Nevertheless, significant trends in feeding behavior, beat rate, and setation, when present, were not consistent across size classes. This confirms our original prediction that acorn barnacles of the same size behave similarly, regardless of species.

Table 1: Species-specific formulas for ash-free dry weight (AFDW, in grams) as a function of aperture diameter (A.D., in mm).

Species	**AFDW as a function of A.D.**
Semibalanus cariosus	$y = 0.220\,x - 1.01$ $R^2 = 0.874$ $n = 20$
Balanus nubilus	$y = 0.259x - 3.10$ $R^2 = 0.620$ $n = 20$

Table 2: Aperture diameter (A.D.) and ash-free dry weight (AFDW) of three size classes of three species of barnacle used in this study. AFDW's of *Semibalanus cariosus* and *Balanus nubilus* are estimated from the regression equations in Table 1.

	Small	**Medium**	**Large**
Balanus glandula	AD: 0.52 ± 0.02mm	AD: 3.59 ± 0.19 mm AFDW: 0.021g	AD: 6.09 ± 0.13 mm AFDW: 0.072g
Semibalanus cariosus	AD: 0.64 ± 0.03mm	AD: 1.59 ± 0.07mm AFDW: 0.021g	AD: 3.1 ± 0.26 mm AFDW : 0.072g
Balanus nubilus	AD: 0.98 ± 0.13mm	AD: 3.23 ± 0.14 mm AFDW: 0.021g	AD: 6.42 ± 0.15 mm AFDW: 0.072g

Table 3: Flow tank water velocity used in the behavior trials. Velocity given as mean plus or minus standard error. Data were analyzed by one-way ANOVA followed by Tukey's HSD, p=0.05.

Species	Size	Mean Current Velocity (cm/s)	Tukey's Grouping
S. cariosus	small	5.63 ± 0.13	A
B. nubilus	medium	4.43 ± 0.71	A B
B. glandula	medium	4.40± 0.07	A B
B. nubilus	large	4.24 ± 0.22	A B
B. glandula	large	4.23 ± 0.08	A B
S. cariosus	large	3.99 ± 0.21	B
S. cariosus	medium	3.74 ± 1.01	B
B. nubilus	small	2.20 ± 0.17	C
B. glandula	small	2.07 ± 0.16	C

Table 4: One-way ANOVA tables for time spent actively feeding as a function of species. (A) Analysis of medium size class. (B) Analysis of large size class. For data, see Figure 3.

A) Medium

Effect	df	MS	F	p
Species	2	17541.1250	3.95	0.035
Error	21	93207.75		

B) Large

Effect	df	MS	F	p
Species	2	10659.1	4.08	0.028
Error	27	2609.578		

Table 5: ANCOVA tables for beat rate as a function of species and ANOVA table for number of setae per millimeter as a function of species and size. (A) ANCOVA table for small barnacle beat rate. (B) ANCOVA table for medium barnacle beat rate. (C) ANCOVA table for large barnacle beat rate. (D) two-way ANOVA table for number of setae per millimeter.

A) Small Size Class Beat Rate ANCOVA

Effect	df	MS	F	p
Species	1	68.056	0.44	0.52
Temperature	1	1604.701	10.29	0.006
Error	15	155.983		

B) Medium Size Class Beat Rate ANCOVA

Effect	df	MS	F	p
Species	2	514.500	2.43	0.113
Temperature	1	329.294	1.56	0.226
Error	20	211.417		

C) Large Size Class Beat Rate ANCOVA

Effect	df	MS	F	p
Species	2	608.633	5.32	0.012
Temperature	1	22.227	0.19	0.663
Error	15	114.322		

D) Number of setae per millimeter ANOVA

Effect	df	MS	F	p
Size	2	4519.319	19.59	<0.0001
Species	2	836.912	3.63	0.034
Size*species	4	990.330	6.07	0.0005
Error	45	163.190		

Table 6: Two-way ANOVA tables for Reynolds number as a function of species. (A) Analysis of small size class. (B) Analysis of medium size class. (C) Analysis of large size class. For data, see Figure 5.

A. Small Re

Effect	df	MS	F	p
Species	1	0.362	19.21	<0.0001
Site Re is calculated	2	1.357	71.91	<0.0001
Species * Size	2	0.055	2.90	0.0662
Error	42	0.019		

B. Medium Re

Effect	df	MS	F	p
Species	2	0.427	0.24	0.79
Site Re is calculated	2	62.707	34.73	<0.001
Species * Size	4	1.164	0.64	0.633
Error	54	1.806		

C. Large Re

Effect	df	MS	F	p
Species	2	32.626	77.61	<0.0001
Site Re is calculated	2	97.653	232.30	<0.0001
Species * Size	4	6.160	14.65	<0.0001
Error	54	22.700		

Table 7: Some cirral anatomy traits measured in this study. Values represent mean plus or minus standard error.

Species	Size	Width of Top of Cirrus VI (mm)	Width of Bottom of Cirrus VI (mm)	Width of Longest Seta on Cirrus VI (mm)	Length of Longest Ramus of Cirrus VI (mm)	Setal Length (mm)
Balanus glandula	small	0.019 ± 0.002	0.030 ± 0.005	0.007 ± 0.002	0.512 ± 0.145	0.118 ± 0.035
Semibalanus cariosus	small	0.023 ± 0.003	0.035 ± 0.006	0.007 ± 0.002	0.813 ± 0.289	0.146 ± 0.035
Balanus nubilus	small	0.029 ± 0.009	0.038 ± 0.010	0.008 ± 0.003	1.324 ± 0.613	0.192 ± 0.056
Balanus glandula	medium	0.070 ± 0.013	0.093 ± 0.006	0.012 ± 0.002	3.614 ± 0.503	0.422 ± 0.070
Semibalanus cariosus	medium	0.049 ± 0.018	0.114 ± 0.097	0.020 ± 0.014	2.732 ± 0.608	0.352 ± 0.065
Balanus nubilus	medium	0.051 ± 0.018	0.086 ± 0.015	0.019 ± 0.006	4.602 ± 1.056	0.485 ± 0.077
Balanus glandula	large	0.110 ± 0.020	0.167 ± 0.023	0.025 ± 0.006	6.490 ± 0.254	0.770 ± 0.063
Semibalanus cariosus	large	0.052 ± 0.013	0.076 ± 0.018	0.013 ± 0.003	3.386 ± 0.872	0.391 ± 0.102
Balanus nubilus	large	0.053 ± 0.017	0.106 ± 0.015	0.017 ± 0.006	5.315 ± 0.872	0.513 ± 0.065

Figure 1: The two flow tanks used in this study. The large flow tank (1A) was constructed according to Vogel & LaBarbera (1978). It had a working area of 20 X 6 X 13 cm. The small flow tank (1B & close up 1C), working area 7 X 5 X 3.5 cm) Rested on the stage of a dissecting scope and was powered by a fish tank pump (Rio 800 H P). The working area of both tanks had removable rods (labeled "r") attached to the bottom and side of the tank to facilitate taping of individual barnacles in profile or from above (1A, 1C).

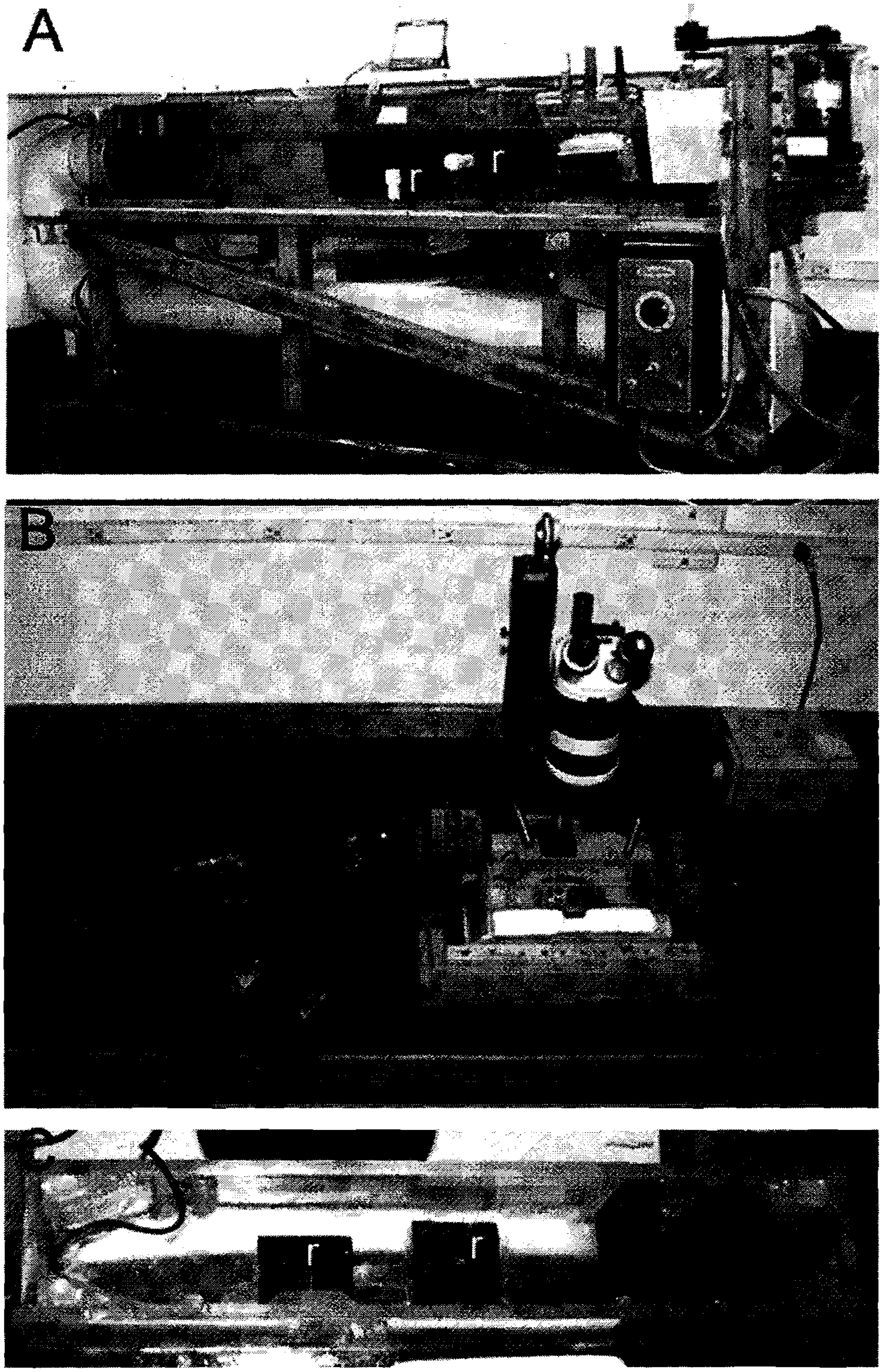

A
r
r
B
C
r
r

Figure 2: Cirral anatomy and traits measured. The ventral surface of cirrus VI was first photographed from above in as natural a position as possible (1A). The width of the longest ramus at the bottom (a) and the width of the longest ramus at the top (b) were measured. Then the longest ramus was cut at the base and photographed in lateral view (1B). The curvilinear distance from c to d was measured as the cirrus length. The number of segments and setae per segment on the longest ramus of cirrus VI were also counted. Under higher magnification (1C), the width of the longest seta (e) was measured.

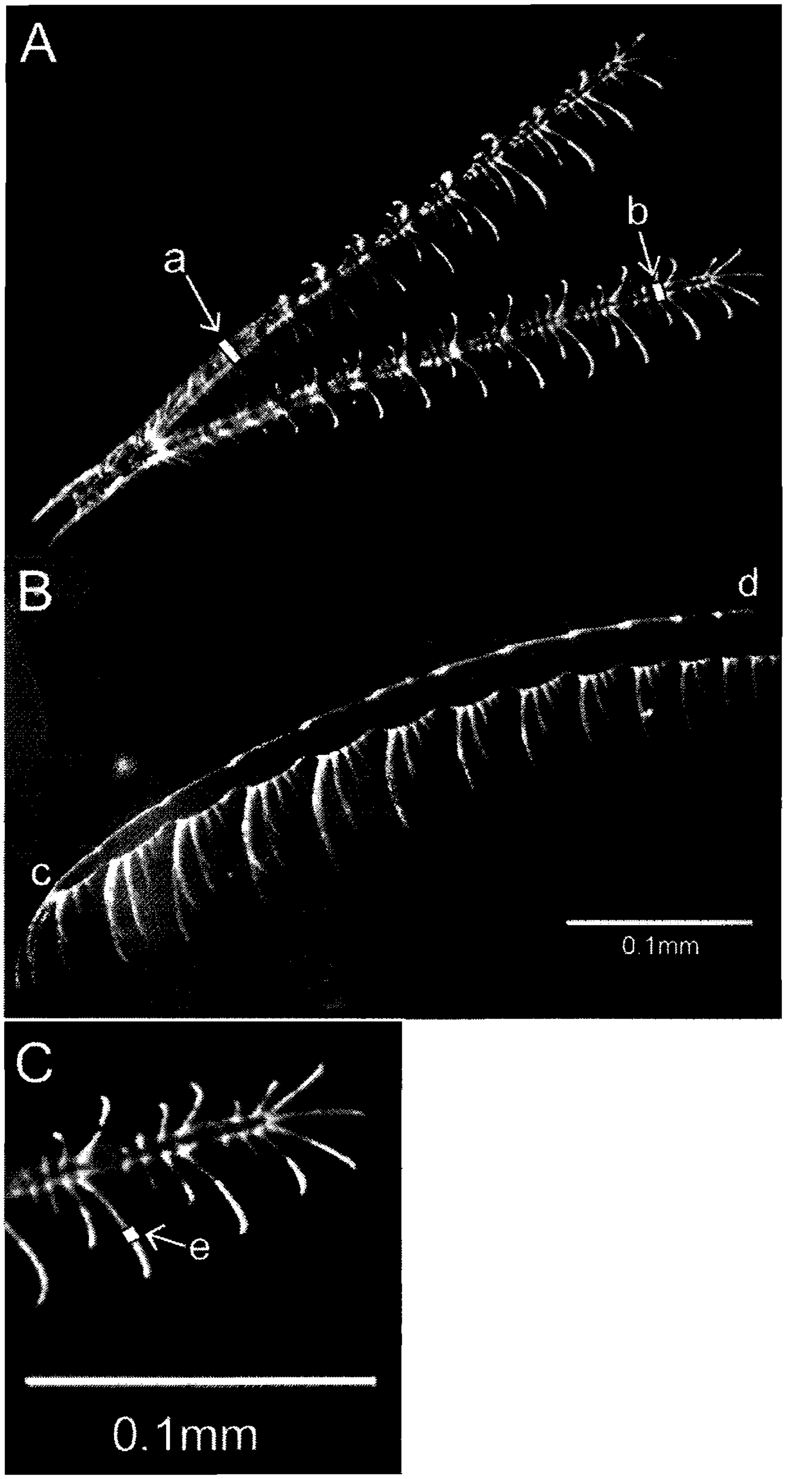

A
a
b
B
d
c
0.1mm
C
e
0.1mm

Figure 3: Behaviors exhibited by *B. glandula, S. cariosus,* and *B. nubilus* while feeding in a flow tank grouped by size class: (A) small size class, (B) medium, and (C) large. Letters above active feeding bars indicate Tukey's HSD grouping ($p<0.05$) within that size class. Error bars indicate standard error. Data for small *S. cariosus* are unavailable due to a difference in current speed.

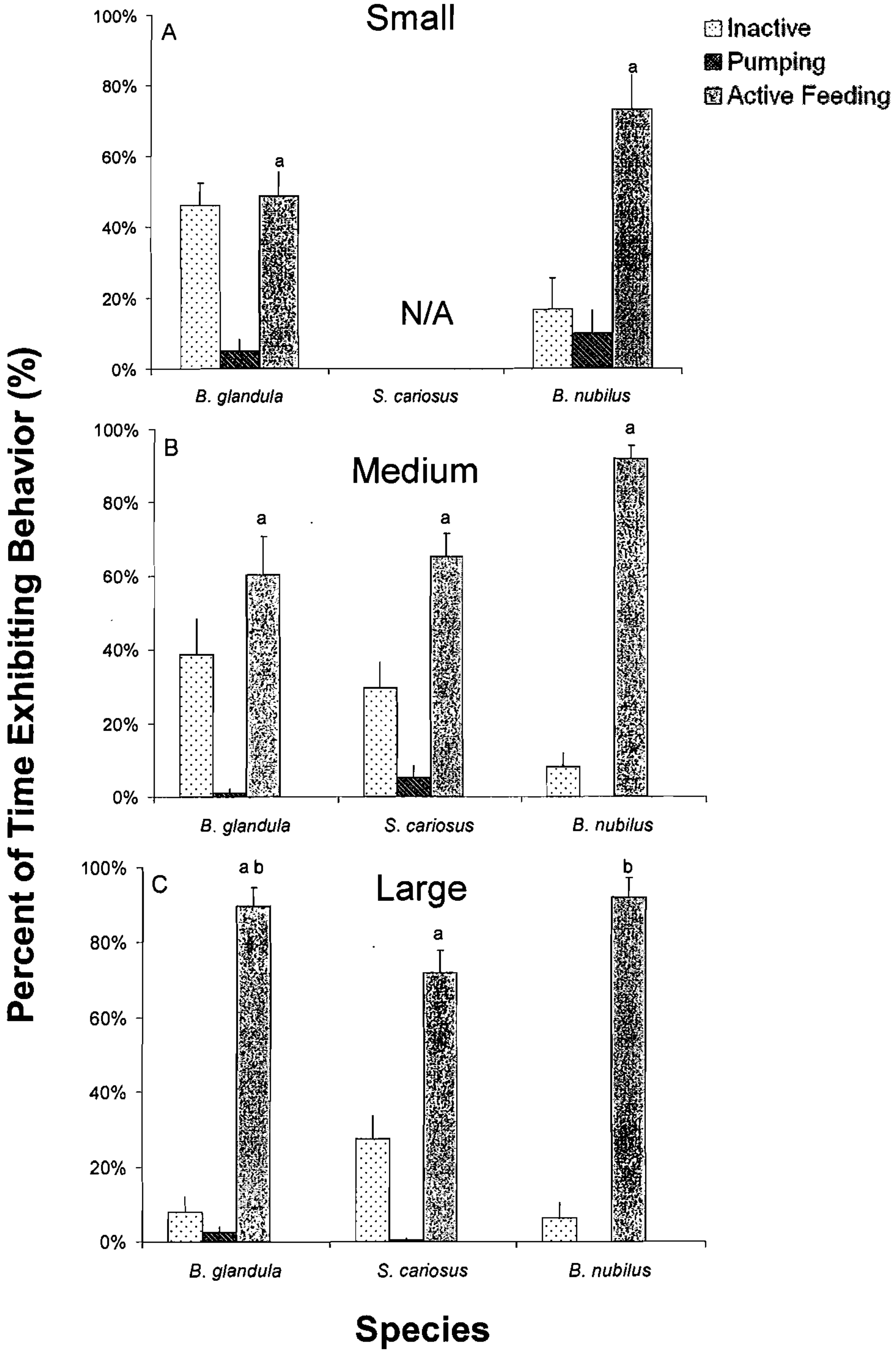

Percent of Time Exhibiting Behavior (%)
Inactive
Pumping
Active Feeding
A
Small
100%
80%
60%
40%
20%
0%
a
a
N/A
B. glandula
S. cariosus
B. nubilus
B
Medium
100%
80%
60%
40%
20%
0%
a
a
a
B. glandula
S. cariosus
B. nubilus
C
Large
100%
80%
60%
40%
20%
0%
a b
a
b
B. glandula
S. cariosus
B. nubilus
Species

Figure 4: (A) Cirral beat rates exhibited by *B. glandula, S. cariosus,* and *B. nubilus* while actively feeding, grouped by size class. Data for small *S. cariosus* are unavailable due to a difference in current speed. Data analyzed separately for each size class. Letters above bars indicate Tukey's HSD grouping ($p<0.05$). (B) Number of setae per millimeter on the longest ramus of cirrus VI, grouped by species and size class. Error bars indicate standard error.

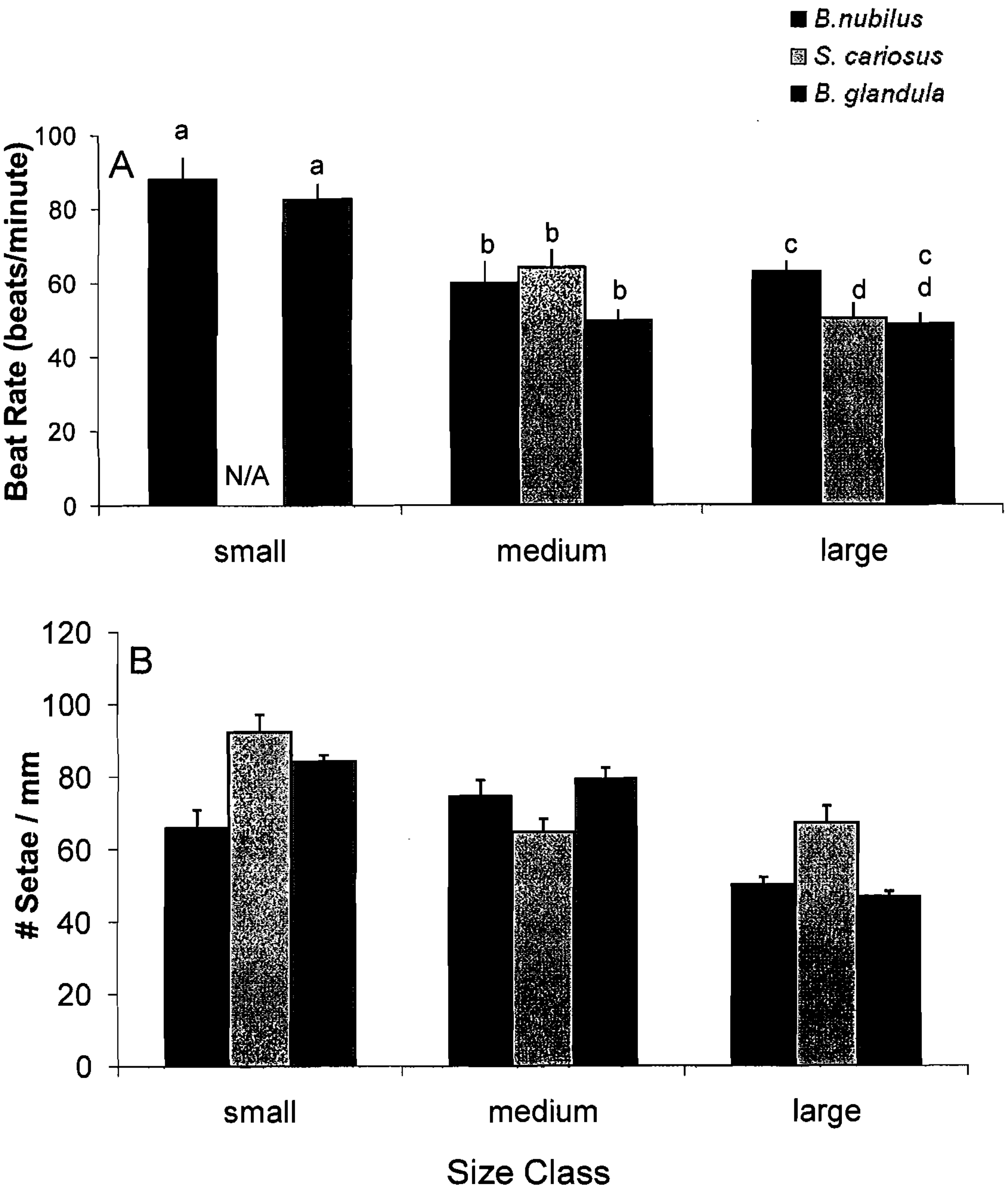

■ B.nubilus
▨ S. cariosus
■ B. glandula
A
Beat Rate (beats/minute)
0
20
40
60
80
100
a
a
b
b
b
c
d
c
d
N/A
small
medium
large
B
Setae / mm
0
20
40
60
80
100
120
small
medium
large
Size Class

Figure 5: Re's experienced at the top and bottom of cirrus VI as well as at the longest seta of cirrus VI during behavior trials for (A) small, (B) medium, and (C) large barnacles. Note the reduced scale for 5A. Data for small *S. cariosus* are unavailable due to a difference in current speed. Data for each size class analyzed separately. Error bars denote standard error.

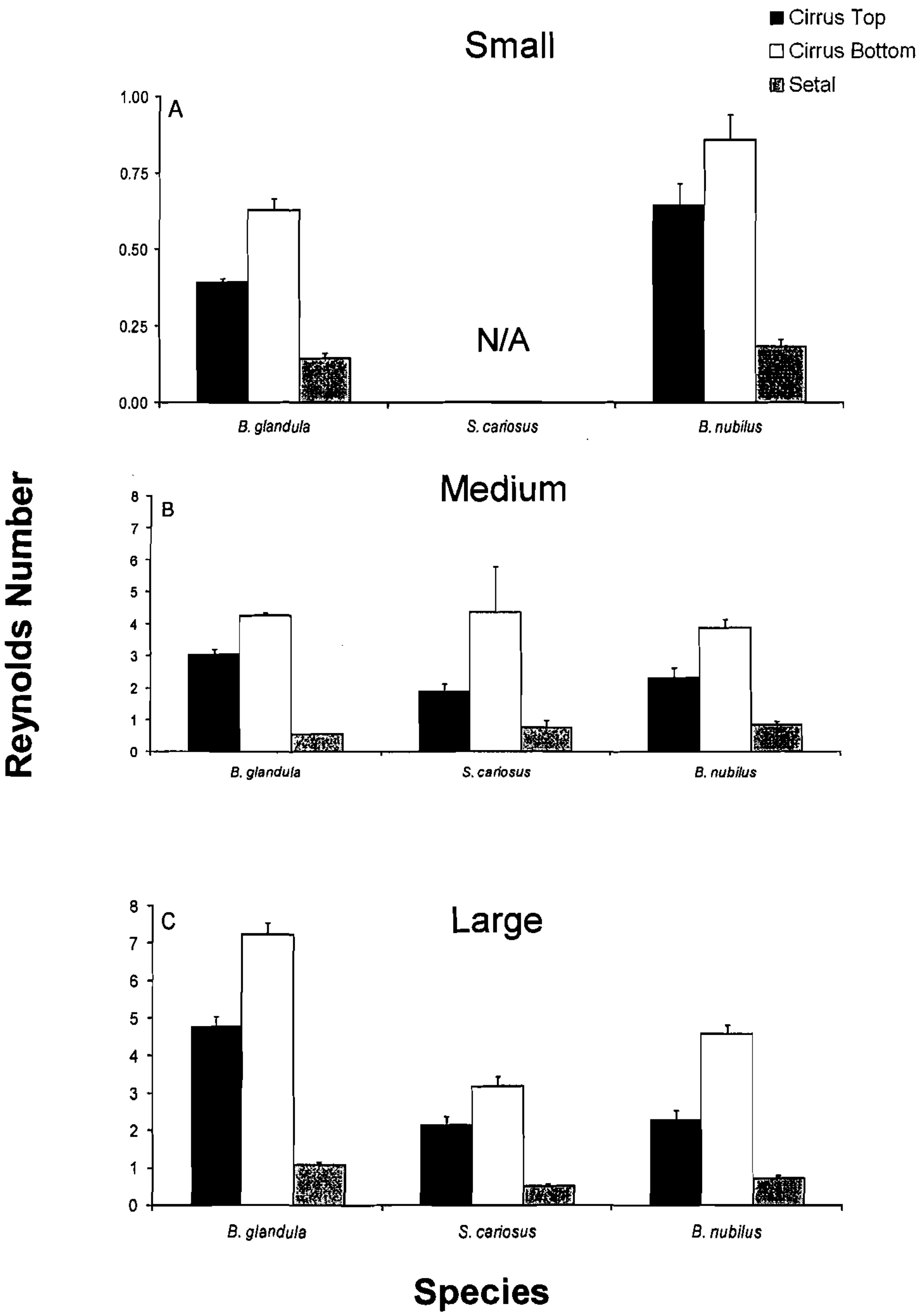

■ Cirrus Top
☐ Cirrus Bottom
▨ Setal
Small
A
1.00
0.75
0.50
0.25
0.00
N/A
B. glandula
S. cariosus
B. nubilus
Medium
B
8
7
6
5
4
3
2
1
0
B. glandula
S. cariosus
B. nubilus
Large
C
8
7
6
5
4
3
2
1
0
B. glandula
S. cariosus
B. nubilus
Reynolds Number
Species

Figure 6: Projected Re's as a function of flow velocity in centimeters per second for (A) small, (B) medium, and (C) large barnacles calculated at the top of cirrus VI. Note the reduced scale for 6A.

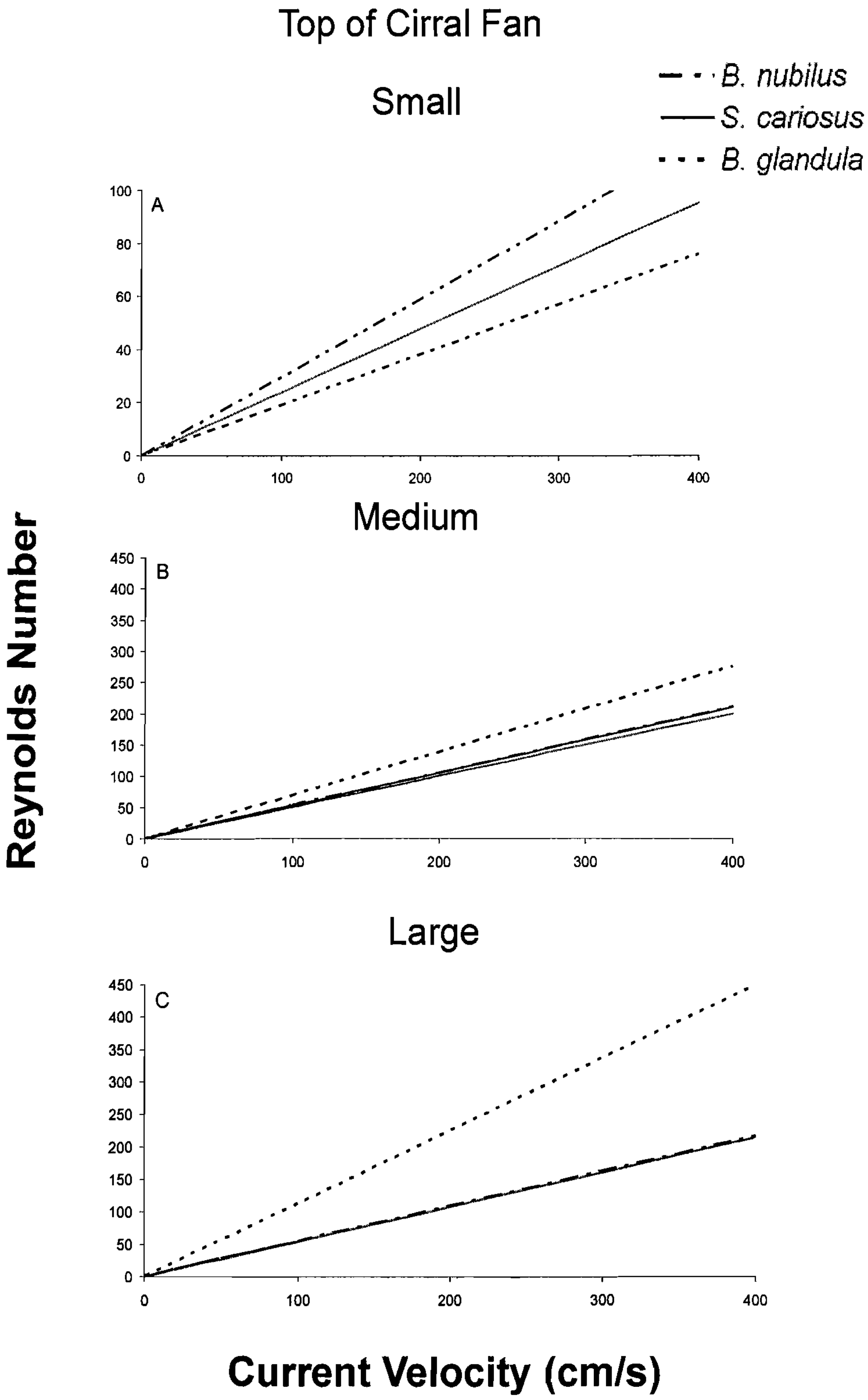
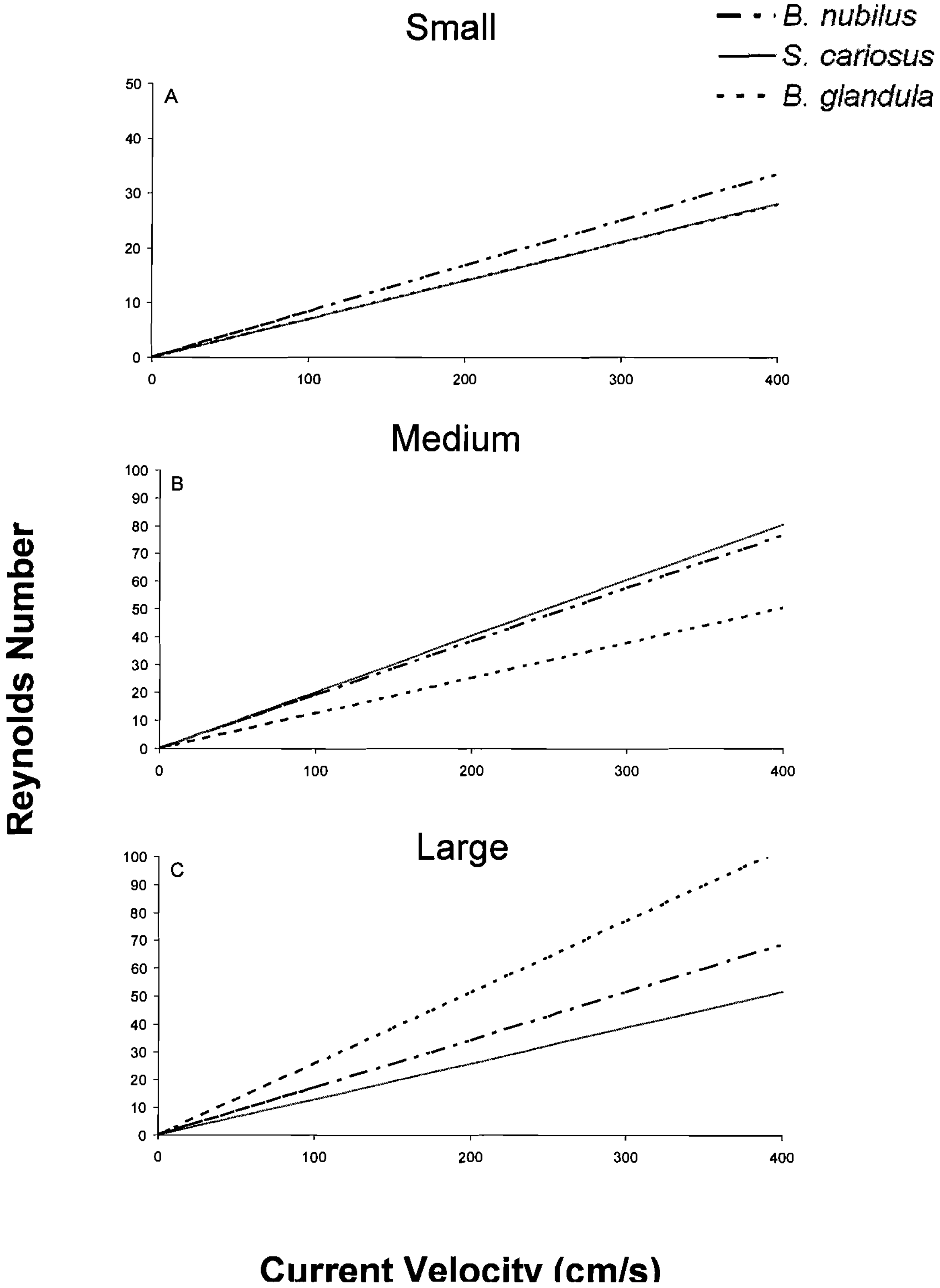

Top of Cirral Fan
B. nubilus
S. cariosus
B. glandula
Small
A
100
80
60
40
20
0
0
100
200
300
400
Medium
B
450
400
350
300
250
200
150
100
50
0
0
100
200
300
400
Large
C
450
400
350
300
250
200
150
100
50
0
0
100
200
300
400
Reynolds Number
Current Velocity (cm/s)

Figure 7: Projected Re as a function of flow velocity in centimeters per second for (A) small, (B) medium, and (C) large barnacles calculated at the longest seta on cirrus VI. Note the reduced scale for 7A.

Setae


Small
— · · B. nubilus
—— S. cariosus
- - - B. glandula
A
50
40
30
20
10
0
0
100
200
300
400
Medium
B
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
Large
C
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
Reynolds Number
Current Velocity (cm/s)

CHAPTER IV

CONCLUDING SUMMARY

We demonstrated that the cirral fans of juvenile *Balanus glandula, Semibalanus cariosus,* and *Balanus nubilus* operate at cirral Reynolds numbers less than one at current speeds below 5 cm/s. We also showed that the juvenile cirral fan functions like a non-leaky paddle during active feeding for *B. glandula.* It is not known if these low flow conditions also occur in the field, but it is likely that juveniles experience locally slower flows than adults. Their cirri are closer to the substratum and so they may feed deeper in the boundary layer. Even barnacles that settle in wave-swept areas with extremely thin boundary layers may experience slower local flows due to their tendency to settle on rough surfaces in pits (Crisp & Barnes 1954, Wethey 1986, Walters & Wethey 1996) and in complex local hydrodynamic environments near or on adults (Knight-Jones 1953; Wethey 1984). Some (n=2 out of 10) juveniles exhibited passive feeding. This may be because 4 cm/s is a relatively fast current speed for them.

Small *Balanus glandula* beat their cirri faster while actively feeding than did medium and large *B. glandula*. Medium and large *B. glandula* exhibited statistically indistinguishable beat rates. Previous studies have found that as log tissue mass increases, log beat rate decreases (Newell & Northcroft 1965; Anderson & Southward 1987). Although large *B. glandula* had longer sixth cirri than did medium *B. glandula*, they also had fewer setae per millimeter on the longest ramus of their sixth cirri. These trends in length of cirrus and number of setae per millimeter probably apply to the entire cirral fan. The relative reduction in setal number likely reduced the drag on the cirral fan of the large individuals, allowing them to beat as fast as the shorter and more setous medium individuals.

In chapter IV, we expanded our analysis to include two other species of common acorn barnacle, *Semibalanus cariosus* and *Balanus nubilus*. In general, barnacles of the same size behaved similarly, regardless of their species. All the species relied primarily on active feeding, with pumping only occasionally observed and passive feeding never observed at the relatively slow current speeds used. *S. cariosus* is thought to rely heavily on passive feeding (Southward & Crisp 1965), but relatively small *S. cariosus* feeding at slow flow speeds exhibited the active feeding characteristic of balanids.

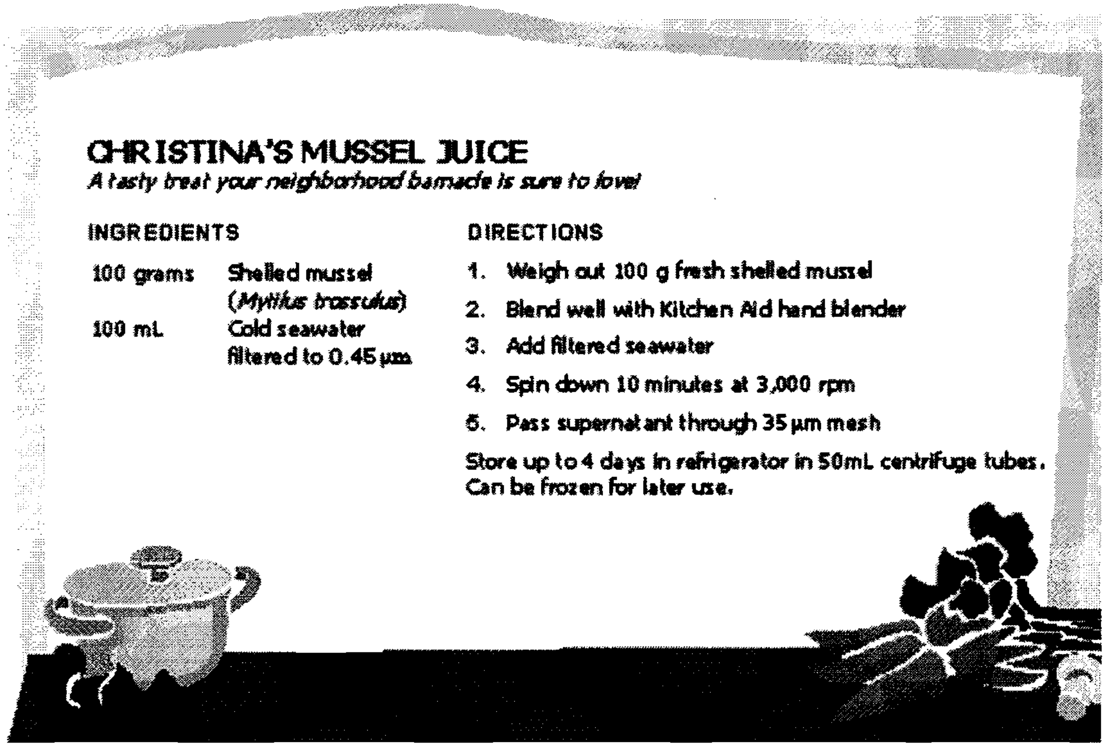
Future work should address what flow speeds are biologically relevant to the size classes of barnacle in this study, especially for juveniles. Once we determine if juvenile acorn barnacles feed at cirral tip Reynolds numbers less than one in the field, we can further determine the ecological significance of this feeding.

In chapter IV, *Balanus glandula* and *Semibalanus cariosus* were collected within a $4m^2$ area to attempt to control for current-dependent phenotypic plasticity in cirral anatomy. Large *B. glandula* had significantly wider cirri than large *Semibalanus cariosus.* This suggests that the $4m^2$ collection area was too big, and that local hydrodynamics may have been sufficiently different within this area to cause phenotypic variation. To control for this, future researchers may desire to obtain juveniles of each species and raise them to desired aperture diameter in the lab. Alternatively, wider cirri in large *B. glandula* may be a species-specific trait. This could be resolved by a more extensive survey of cirral widths in the local populations to see if the trend in cirral width holds over different hydrodynamic regimes.

In summary, juvenile acorn barnacles were found to feed with non-leaky cirral fans at current speeds less than 5 cm/s, although confirmation of field velocities experienced by juveniles must occur before the ecological relevance of this result is known. Some juveniles *Balanus glandula* were observed to feed passively, perhaps indicating that 4cm/s flow may be relatively fast for them. Medium and large *B. glandula* exhibited the same beat rate while actively feeding, which was perhaps made possible by a lower number of setae per millimeter cirrus length in the large group. When the feeding of *B. glandula, Balanus nubilus*, and *Semibalanus cariosus* were compared, similar sized (ash-free dry weight, as approximated by aperture diameter) barnacles fed similarly, regardless of species.

APPENDIX

A RECIPE FOR MUSSEL JUICE USED TO STIMULATE BARNACLE FEEDING



CHRISTINA'S MUSSEL JUICE

A tasty treat your neighborhood barnacle is sure to love!

INGREDIENTS

100 grams	Shelled mussel (*Mytilus trossulus*)
100 mL	Cold seawater filtered to 0.45 µm

DIRECTIONS

1. Weigh out 100 g fresh shelled mussel
2. Blend well with Kitchen Aid hand blender
3. Add filtered seawater
4. Spin down 10 minutes at 3,000 rpm
5. Pass supernatant through 35 µm mesh

Store up to 4 days in refrigerator in 50mL centrifuge tubes.
Can be frozen for later use.

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