Phenotypic and behavioral plasticity in the feeding of *Balanus glandula*

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Phenotypic plasticity is one mechanism by intertidal organisms to change and survive in a variety of local flow environments. One such species is the barnacle *Balanus glandula*, which exhibits plasticity in both its feeding behavior and the morphology of its feeding cirri. Barnacles feed actively at low flow speeds, but can feed passively at higher flow speeds. Additionally, individuals in habitually low flow environments have longer and thinner cirri than barnacles in high flow environments. This study explores how behavioral and morphological plasticity interact to give barnacles from low flow environments an advantage while feeding at slower water velocities. Using a flow tank to observe feeding behavior, barnacles were placed in flows ranging from 0 to 10 cm/sec. Barnacles from a low flow site employed passive feeding at lower water velocities than barnacles from a high flow site. Passive feeding, when possible, is thought to increase the energetic efficiency of feeding compared to active feeding. In slower water velocities, the increased ability to feed passively benefits low flow barnacles compared to high flow barnacles.

Key words: *Balanus glandula*, plasticity, feeding, cirri, Argyle Lagoon

INTRODUCTION

Phenotypic plasticity is the ability of an organism to change a trait in response to its immediate environment. This mechanism is especially useful in intertidal areas that contain a wide range of local flow regimes. Successful species are able to survive in both areas with low water velocities and those with high flow rates. One intertidal species known to exhibit plasticity is the barnacle *Balanus glandula*, which inhabits areas of both fast and slow water velocities (Marchinko and Palmer 2003). Larval barnacles can disperse, but adults are sessile (Anderson 1994). Consequently, offspring of a single barnacle can settle in areas with unlike flow conditions. Once settled, adults are then unable to actively relocate if their local flow environment should change. Faced with these challenges, phenotypic plasticity allows barnacles to continually adjust and survive in a variety of flow conditions.

B. glandula is a suspension feeder that uses cirri to capture particles of food from surrounding water (Anderson 1994). Plasticity of two types has been observed in the feeding of barnacles- both behavioral and morphological plasticity are known (Crisp and Southward 1961, Arsenault *et al.* 2001). Behavioral plasticity occurs as barnacles use different modes of feeding depending on the surrounding water velocity. Barnacles actively sweep their cirri to feed in slower flows, and passively feed in faster flows by holding cirri extended into ambient currents (Crisp and Southward 1961). Passive feeding, when flows permit, requires less metabolic energy to capture food than active feeding (LaBarbera 1984).

Plasticity is also seen in the morphology of barnacle feeding cirri (Arsenault *et al.* 2001). *B. glandula* from environments with low flows have longer and thinner cirri compared to barnacles from regions with high flows (Arsenault *et al.* 2001). When faced with a new flow regime, barnacles are able to alter their cirral morphology in the course of one to two molts (Marchinko 2003). This difference in morphology affects the forces that a barnacle will encounter in flow. The low flow cirral form, which extends farther out of the boundary layer, will experience more drag and intercept greater volumes of water. The shorter and thicker cirri of high flow barnacles will experience less drag, but at the expense of filtering lower volumes of water.

Behavioral and morphological plasticity interact to affect overall feeding behavior at different flow speeds. Barnacles from high flow areas are known to have an advantage when feeding at higher speeds, where low flow barnacles are unable to feed as fast velocities deform their long and thin cirri (Marchinko 2007). This study explores how the cirri of barnacles from low flow areas allow feeding to be more effective at slower water velocities. I hypothesized that the cirri of barnacles from low flow environments allow for passive feeding at slower velocities.

Presumably, barnacles switch to a passive mode of feeding at a point where ambient flow is great enough that it is not necessary to force water through their cirri with active beating. I expected that this point occurs at slower flow speeds for low flow barnacles because a greater volume of water passes through their cirri in a given amount of time. If longer cirri allow *B. glandula* to passively feed at lower water velocities, then individuals habituated to low flow can use passive feeding when barnacles from high flow areas must employ active feeding. Low flow individuals would be at an advantage in this velocity range because as they use a more energetically efficient mode of feeding than high flow barnacles.

MATERIALS AND METHODS

Study site. All *B. glandula* specimens were collected from two sites within Argyle Lagoon on San Juan Island, Washington (Figure 1). I chose the two locations for their proximity to one another and their different flow conditions. The closeness of the two sites ensures that all specimens have been exposed to the same water mass, and therefore the same food supply and larval pool. One site is situated along the edge of the outer bay and experiences relatively slow water flow with little wave action. The other site is located in the tidal channel connecting the outer bay and inner lagoon, where water flows are faster as incoming and outgoing tides are funneled through a small area. Additionally, barnacles at both sites are found on small cobbles that are easily collected.

I monitored water velocity, temperature, and salinity at each site during one day of a spring tidal cycle in early summer. Measurements were taken approximately every half hour while the water above the barnacles was accessible with a Marsh-McBirney flow meter (up to approximately 70 cm deep). Water temperatures ranged from 13° to 20° C and salinity ranged from 29 to 33 parts per thousand. Temperature and salinity spans were similar at both sites. Water velocities were measured between 0.6 and 6.1 cm/sec at the slow flow site and were comparable for incoming and outgoing tides. Water velocities at the fast flow site ranged from 0.3 to 79.2 cm/sec, with faster flow speeds occurring during incoming tide. More barnacles appeared to feed during the faster speeds of incoming tide. For both sites, water flow was largely unidirectional.

***B. glandula* collection and measurement.** Barnacles were collected during low tide by removing entire cobbles with barnacles attached. Specimens were kept out water so that they only encountered their natural local flow environment before laboratory observations. All observations of feeding behavior were made within 32 hours of collection.

Body size measurements were taken after observations of feeding in flow. Overall body size was determined by measuring the aperture diameter. Phenotypic plasticity was characterized by measuring the ramus length of cirrus IV, as ramus length was found to be the most plastic cirral dimension (Marchinko and Palmer 2003). I determined ramus length by dissecting the cirrus, mounting the cirrus in seawater on a slide, and photographing the cirrus through a dissecting microscope (Marchinko and Palmer 2003). Length was measured in the resulting photograph using ImageJ.

Initially, 10 barnacles from each site were collected to confirm that flow velocities at the collection sites are different enough to demonstrate plasticity in cirral morphology. Barnacles collected for initial cirrial measurements were chosen to represent a range of sizes, while barnacles collected for behavioral observation were chosen to have similar aperture diameters.

A two-way ANOVA was used to determine if cirral length is dependent on both aperture diameter and flow site. The ANOVA was performed on combined data from initial cirral measurements and from measurements of barnacles observed feeding in flow. A t-test was used to compare the average aperture diameters of sample groups used in behavioral observations. Average cirral lengths of groups were also compared with a t-test.

Observation of feeding in flow. Ten barnacles from the fast flow site and twelve from the slow flow site were observed in flow using a recirculating flow tank, which should accurately represent unidirectional flows observed at the collection site (Figure 2). Seawater from the sea table system at Friday Harbor Laboratories was used to fill the flow tank. The tank was refilled between trials to maintain a consistent temperature (12-14.5 ˚C) and food concentration. Barnacles remained attached to their original cobbles, which rested in a notch between Plexiglas ® plates at the top of the flow tank such that only the barnacles were suspended into flow. Barnacles were oriented so that the opercular opening was parallel to flow and cirri were facing flow. Barnacles were lit from below with a fiber optic lamp and recorded from the side with a Samsung 4.7 Mega Pixel Full HD Memory Cam.

Before each trial, feeding behavior was induced by subjecting the barnacle to a 7 cm/sec flow. If no movement was observed after 7 minutes, the barnacle was removed and replaced with a new barnacle. Trials were begun 90 seconds after feeding behavior was first observed. Each barnacle was exposed to a set of water velocities ranging from 0 to 10 cm/sec in 1 cm/sec increments. The water velocity was changed every 90 seconds, and a random number generator determined the order of velocities for each individual.

I allowed the first 60 seconds of each 90-second flow period for barnacles to adjust to a new flow speed, and I analyzed the last 30 seconds of video footage. First, I determined the amount of time a barnacle spent feeding with its cirri extended. I then categorized feeding behavior as active, passive, transitional, or deflected. Active behavior was characterized by an extension, sweeping, and withdrawal of cirri without pause (Crisp and Southward 1961). Passive behavior was characterized by a pause and prolonged extension that was long compared to the sweep of cirri (Crisp and Southward 1961). Transitional behavior, an intermediate between active and passive feeding, was characterized by a shorter pause compared to the cirral sweep (Crisp and Southward 1961). Deflected behavior was also characterized by a shorter pause, but in this case the cirri are bowed backwards with flow and do not sweep forward.

Distributions of the total time (summed over all barnacles) spent feeding by a sample group over the range of flow velocities were compared for each mode of feeding with one-sided two-sample Kolmogorov-Smirnov tests. For each flow speed and behavior, the average feeding time for barnacles in a group was calculated by dividing the total time the behavior was observed by the number of subjects in a group.

RESULTS

Study groups. Cirral length was correlated with aperture diameter for *B. glandula* collected from both the fast and slow sites ($P < 0.0001$, $F=22.73$) (Figure 3). However, barnacles collected from the fast flow site had significantly shorter cirri than barnacles collected from the slow flow site ($P < 0.0001$, $F = 61.14$).

The average aperture diameters from the slow flow site (4.74 ± 0.24 mm) and the fast flow site (4.75 ± 0.20 mm) were not significantly different ($P = 0.931$, $t = -0.0882$) (Figure 4). The average cirral length for the slow flow site (5.51 ± 0.69 mm) was significantly longer than for the fast flow site (3.70 ± 0.89 mm) ($P < 0.001$, $t = 5.403$).

Feeding behavior. For active, transitional, and passive feeding, the distribution of time spent feeding was shifted significantly towards slower flow speeds for the low flow group compared to the high flow group ($P_{\text{active, transitional, passive}} < 0.005$; $D^+_{\text{active}} = 0.094$, $D^+_{\text{transitional}} = 0.612$, $D^+_{\text{passive}} = 0.429$) (Figure 5).

Deflected behavior was observed only in individuals from the low flow site (Figure 5). The distribution of deflected feeding was shifted significantly toward higher flow speeds compared to a uniform distribution ($P < 0.005$, $D^- = 0.731$).

DISCUSSION

B. glandula from both the low flow and the high flow site showed a relationship between aperture diameter and cirral length (Figure 3). This relationship confirms that aperture diameter, used to choose sample groups for behavioral observations, is a valid indication of overall body size. Additionally, low flow barnacles had consistently longer cirri than high flow barnacles. The difference in cirral length verifies that the low flow and high flow sites are unlike enough to demonstrate morphological plasticity.

For barnacles used in observations of feeding behavior, the average aperture diameter was not significantly different between the low flow group and the high flow group (Figure 4). As a result, the two sample groups were of similar overall body size and differences in behavior can be attributed to flow environment and cirral morphology. The average cirral length was significantly different between the two groups of barnacles, again confirming morphological plasticity.

Overall, low flow barnacles fed passively at slower water velocities than high flow barnacles (Figure 5). Passive feeding was first observed at slower flow speeds for the low flow group, and the distribution of time spent feeding passively was shifted towards lower velocities compared to the high flow group. The low flow morphology has an advantage over the high flow morphology when feeding in the range of water velocities for which longer cirri allow an increase in passive feeding. While some daily variation in flow speed is expected, field data suggest that the low flow barnacle group feed in this velocity range. As active feeders in this region of flow speeds, high flow barnacles must expend energy sweeping their cirri to push water through their feeding sieve (LaBarbera 1984). Passively feeding low flow barnacles can instead rely on ambient currents to deliver water through their cirri, and must only expend energy to position their cirri in flow (LaBarbera 1984).

At a flow speed of ten cm/sec, low flow barnacles spent less time feeding passively and more time exhibiting deflected behavior than at a flow speed of nine cm/sec. High flow barnacles, which did not show deflected behavior, continued to spend more time feeding passively at the highest flow speed. This pattern is consistent with previous research and indicates that low flow barnacles are unable to feed effectively at faster speeds, as higher drag forces prevent extension of their long, thin cirri (Marchinko 2007). While ten cm/sec might not appear to be a fast flow, it could be higher than speeds in the natural environment of these low flow barnacles. Further observations of barnacles in faster flow speeds are necessary to confirm trends of decreased passive feeding in the low flow group and increased passive feeding in the high flow group.

It is apparent that differences exist in the feeding behavior of the low flow and high flow groups observed in this study. However, multiple factors could be responsible for these disparities. Mechanical differences between the longer low flow cirri and the shorter high flow cirri might necessitate a certain mode of feeding for a given flow speed. Alternately, it is possible that barnacles that are habitually exposed to a flow condition adjust feeding behavior independent of morphology. The relative importance of feeding mechanics and behavior could be explored by observing an additional group of barnacles from the low flow population. Low flow barnacles with a smaller aperture diameter could be chosen such that their cirral length is similar to that of high flow barnacles observed in this study.

Balanus glandula has the ability to adapt to its flow environment both by changing its cirral morphology and by changing its feeding behavior (Arsenault et al. 2001, Crisp and Southward 1961). This study demonstrates that these measures of plasticity allow barnacles to feed in a wide range of flow speeds. Even barnacles exposed to flows outside the scope of their optimum feeding velocities, for instance low flow barnacles in faster speeds or high flow barnacles in slower speeds, can modify their behavior to achieve some amount of feeding. If a barnacle is relocated to a new flow environment, this ability could allow barnacles to survive long enough to modify their cirral morphology by molting.

Understanding the flow conditions in which an organism can feed gives us insight into the habitats in which it can live. In general, active suspension feeders are able to inhabit areas with lower flow speeds while passive suspension feeders must live in areas with higher flows (LaBarbera 1984). Plasticity in the feeding of *B. glandula* allows the species to be widely distributed in the intertidal (Marchinko and Palmer 2003).

This information is useful for considering the distribution of intertidal species and understanding how this zonation will be affected by changing environmental conditions.

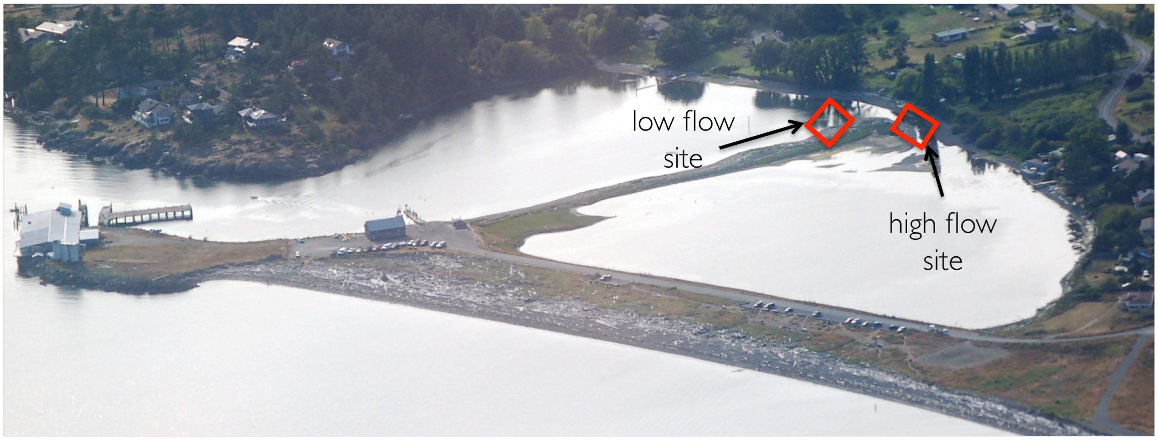

Figure 1. *B. glandula* collection sites at Argyle Lagoon on San Juan Island, Washington. Photograph by Rachel Merz.

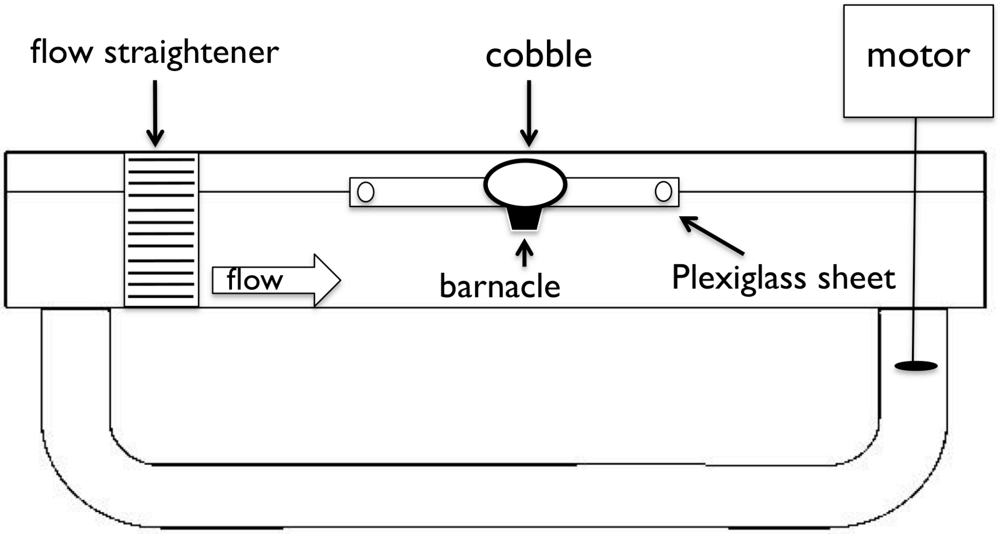

Figure 2. Schematic of flow tank used for observations of feeding behavior in flow. The flow tank used had a working area of 16 (width) x 66 (length) x 14 (height) cm. Barnacles on cobbles were suspended in flow through a notch in Plexiglas sheets 25 cm downstream of the flow straighteners. Barnacles were oriented such that their opercular openings were parallel to flow and their cirri were facing flow.

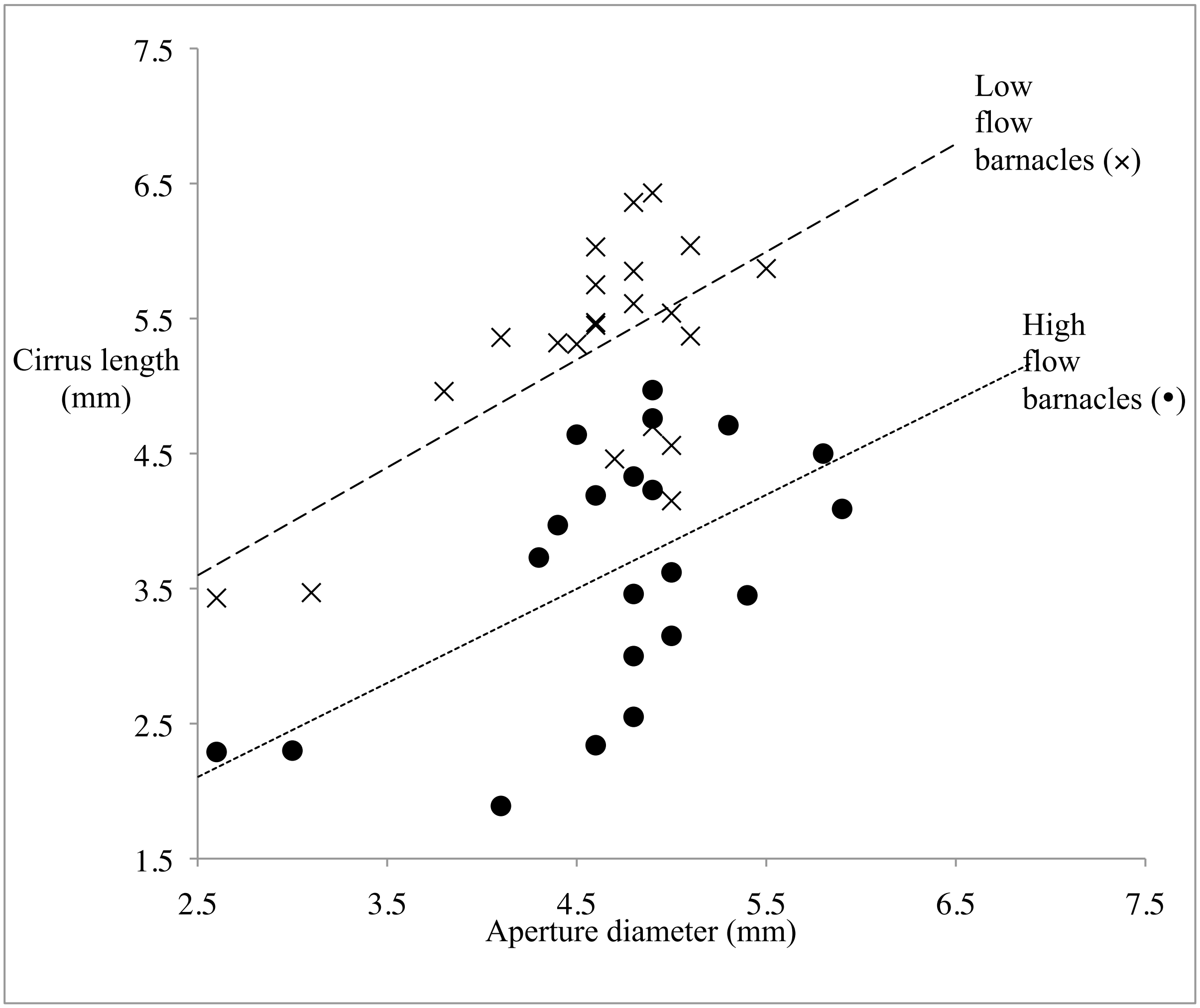

Figure 3. Relationship between aperture diameter and the cirral length (ramus length of cirrus IV) for *B. glandula*. There was a correlation between cirral length and aperture diameter ($P < 0.0001$, F=22.73) and between cirral length and flow site ($P < 0.0001$, F = 61.14).

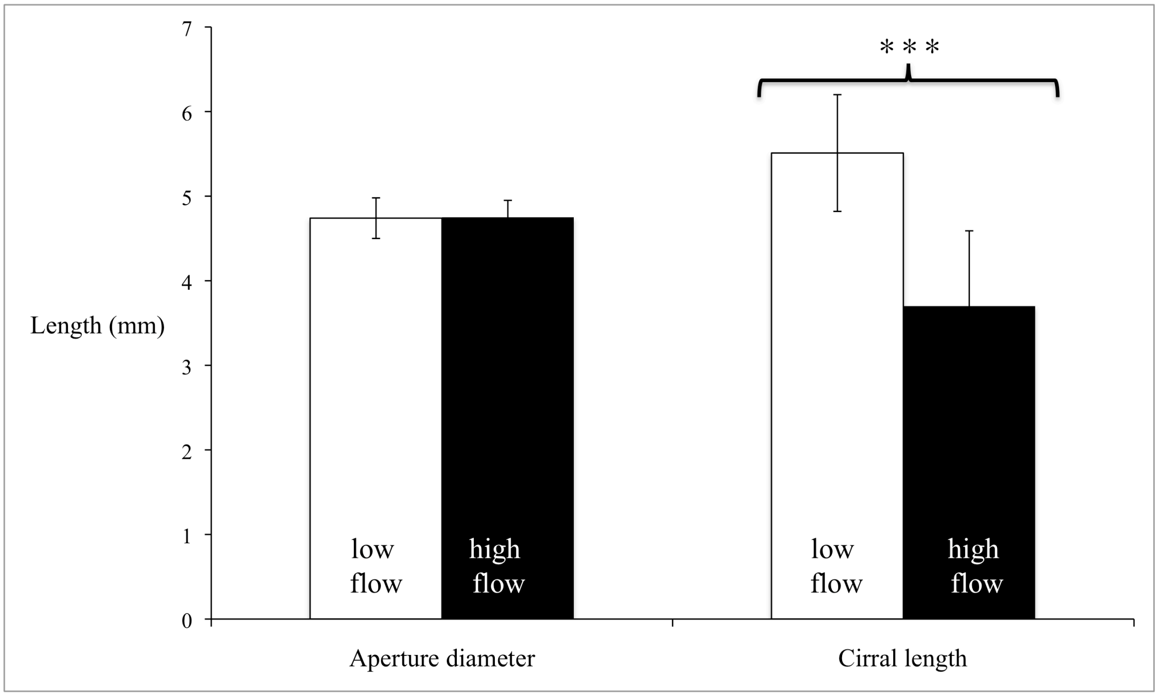

Figure 4. Average aperture diameter and cirral length of low flow (N=12) and high flow (N=10) barnacles. The average aperture diameter of *B. glandula* collected from the slow flow site (4.74 ± 0.24 mm) was not significantly different from barnacles collected from the fast flow site (4.75 ± 0.20 mm) (P = 0.931, t=-0.0882). Barnacles from the slow flow site had a significantly longer average cirral length (5.51 ± 0.69 mm) than barnacles from the fast flow site (3.70 ± 0.89 mm) ($P < 0.001$, t=5.403).

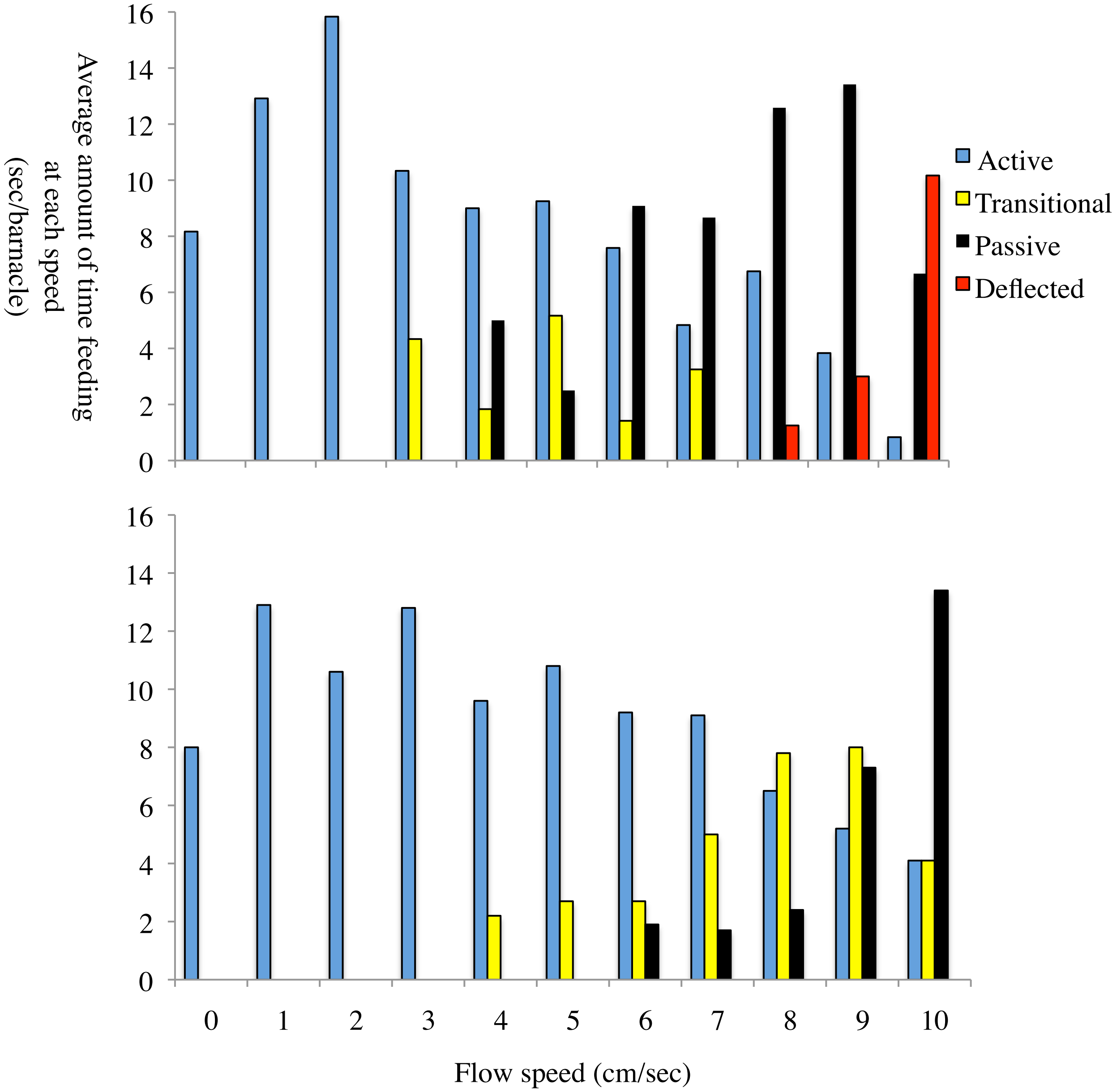

Figure 5. Average amount of time feeding behaviors were observed at each flow speed. Distributions of time spent feeding in the active, transitional, and passive modes were shifted significantly towards lower flow speeds in the low flow barnacles compared to high flow barnacles ($P_{\text{active, transitional, passive}} < 0.005$; $D^{+}_{\text{active}} = 0.094$, $D^{+}_{\text{transitional}} = 0.612$, $D^{+}_{\text{passive}} = 0.429$). The distribution of deflected behavior in low flow barnacles was shifted significantly toward higher flow speeds compared to a uniform distribution ($P < 0.005$, $D^{-} = 0.731$).

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LITERATURE CITED

Anderson, D. T. *Barnacles: Structure, Function, Development, and Evolution*. London: Chapman and Hall, 1994.

Arsenault, D.J., Marchinko, K.B., Palmer, A.R. "Precise tuning of barnacle leg length to coastal wave action." *Proc. R. Soc. Lond. B* 268 (2001): 2149-2154.

Crisp, D.J., Southward, A.J. "Different types of cirral activity of barnacles." *Phil. Trans. R. Soc. Lond. B* 243 (1961): 271-307.

LaBarbera, M. "Feeding currents and particle capture mechanisms in suspension feeding animals." *Amer. Zool.* 24 (1984): 71-84.

Marchinko, K.B., Palmer, A.R. "Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species." *Zoology* 106 (2003): 127-141.

Marchinko, K.B. "Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): magnitude, age dependence, and speed of response." *Evolution*. 57 (2003). 1281-1290.

Marchinko, K.B. "Feeding behavior reveals the adaptive nature of plasticity in barnacle feeding limbs." *Biol. Bull.* 231 (2007): 12-15.