

# Temperature and water flow influence feeding behavior and success in the barnacle *Balanus glandula*

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**ABSTRACT:** Suspension feeding is a common energy acquisition strategy for many marine organisms. Conditions in the fluid environment (e.g. flow, temperature) can influence both the flux of particles past an organism and the efficiency with which organisms retain those particles. We investigated feeding behavior in the barnacle *Balanus glandula* under a range of water velocities and temperatures using gut dissections to directly quantify capture rates of food particles. Overall, the percentage of barnacles observed beating was typically high ( $68 \pm 3\%$ ), yet gut dissections confirmed that a far lower percentage had actually ingested food particles (hydrated *Artemia* cysts;  $22 \pm 3\%$ ). This discrepancy suggests that cirral activity may serve other functions and that simple behavioral descriptions provide a poor proxy for barnacle feeding rate. Although the delivery of cysts to the cirral net and cyst capture rates peaked at intermediate water velocities ( $7.5$  to  $20 \text{ cm s}^{-1}$ ), capture efficiency (the ratio of cysts captured to cysts encountered) was highest under the slowest flow ( $1 \text{ cm s}^{-1}$ ). Model analysis demonstrated that detailed characterization of cirral beating behavior is required to accurately predict patterns of flow-dependent cyst capture. Barnacles also showed a clear thermal optimum between  $10$  and  $15^\circ\text{C}$  in both capture rate and efficiency. At high temperatures ( $25^\circ\text{C}$ ), feeding was reduced due to an increase in abbreviated beating behavior, whereas at low temperatures ( $5^\circ\text{C}$ ) reduced capture was likely a consequence of slower beating rate. Again, only when beating behavior was incorporated into models were patterns of temperature-dependent cyst capture accurately predicted. These results suggest that the limits to feeding success are not simply biophysical, but also behavioral in nature.

**KEY WORDS:** Suspension feeding · Barnacles · Water flow · Thermal tolerance

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## INTRODUCTION

Barnacles are numerically dominant suspension feeders found on rocky shores around the world. Due to their abundance, these benthic invertebrates dominate large portions of the intertidal zone and are important prey for other invertebrates (Connell 1961, Navarrete et al. 2000), fish (Hueckel & Stayton 1982) and birds (Vermeer 1982). They are ecosystem engineers that increase habitat complexity and provide important microhabitat for other invertebrate fauna (Barnes 2000, Harley 2006, Sueiro et al. 2011). As suspension feeders, barnacles represent an important link between benthic and planktonic communi-

ties through their procurement of food particles from surrounding waters.

Barnacles feed using a cirral fan that consists of 6 pairs of legs or 'cirri' (feeding appendages), each with 4 to 7 pairs of evenly spaced setae. The extension and withdrawal of these appendages allows for feeding on planktonic prey (see Riisgård & Larsen 2010). Suspension feeding activity can be quantified as a capture rate (defined as the total number of particles caught per unit time by a standard mass weight of a suspension feeder; Wildish & Kristmanson 2005) and capture efficiency (the ratio of particles captured to those encountered by feeding structures; Rubenstein & Koehl 1977). Both measures of feeding are

likely influenced by 2 potentially important environmental factors: water motion and temperature (Jørgensen 1983, Labarbera 1984, Wildish & Kristmanson 2005).

Water motion can enhance capture rate by increasing the flux of particles past feeding structures (Helmut & Sebens 1993, Sanford et al. 1994, Ackerman & Nishizaki 2004). However, the proportion of water that passes through a feeding structure, or 'leakiness', also increases with increasing flow (Loudon & Alstad 1990, Geierman & Emlet 2009), so capture efficiencies can decrease at higher velocities. At excessively fast flows, capture rates may decrease through mechanical deformation and damage to exposed feeding appendages or behaviors to avoid such damage (Marchinko 2007, Miller 2007).

Water temperature can affect feeding rate through changes in physiology and fluid dynamic conditions. Barnacles deploy their cirri into the water column to capture seston. Many barnacle species show a thermal optimum in maximum beating rates of these cirri, which may influence particle capture rates (see review by Anderson & Southward 1987). From a physical perspective, temperature may also affect suspension feeding as a consequence of changing water density and viscosity (Podolsky 1994).

For suspension feeders, sieving has been assumed to be the primary particle capture mechanism, with particles larger than the space between filter elements unable to pass (see Riisgård & Larsen 2010). However, Jørgensen (1966) noted that limited pressure differentials across such filter elements suggests sieving may not always be the dominant capture mechanism. An alternate approach based on aerosol-filtration theory (Rubenstein & Koehl 1977) provides 4 non-dimensional indices that describe capture efficiencies related to each of 4 non-sieving mechanisms: (1) direct interception ( $N_R$ ), (2) inertial impaction ( $N_I$ ), (3) gravitational deposition ( $N_G$ ) and (4) diffusional deposition ( $N_D$ ). A fifth mechanism, electrostatic attraction, is of minor importance in an electrolyte such as seawater (Shimeta & Jumars 1991, but see Labarbera 1978) (Table 1). Together, the 4 models allow for predictions about the effect

of water motion on particle capture. Shimeta & Jumars (1991) extended this approach by introducing 4 similar indices describing particle encounter rate, rather than efficiency, for each of the 4 mechanisms. Together, these capture efficiency and capture rate indices provide a powerful framework to predict patterns of feeding under various environmental conditions.

Studies linking environmental conditions to barnacle feeding activity are based largely on work correlating cirral beating rate to feeding rate (Southward 1955b, Sanford et al. 1994). Although beating rate has been related to both temperature (Southward 1957) and flow (Marchinko 2007, Miller 2007), surprisingly little data exists measuring actual capture rates of suspended food particles (but see Trager et al. 1994, Bertness et al. 1998). In addition, describing the effects of multiple environmental stressors on organismal performance provides a mechanistic explanation of how organisms may respond to environmental uncertainty (Kearney & Porter 2004, Németh et al. 2013, Todgham & Stillman 2013). Here, we present results from experiments directly measuring feeding rates of *Balanus glandula* under a range of water temperatures and velocities.

In this study, we (1) conducted feeding experiments to explore the influence of water temperature and velocity on particle capture rate and particle capture efficiency; (2) made detailed measurements of cirral activity under different water temperatures and velocities to explore the interactions between

Table 1. Reported mechanisms of particle capture for various aquatic organisms. (-) not available

Capture mechanism	Organism	Velocity (cm s <sup>-1</sup> )	Particle diameter (µm)	Source
Sieving	Brachiopods	-	~4.5	Strathmann (2005)
	Mussels	-	0.2–1	Wright et al. (1982)
Direct interception	Corals	2–40	200	Sebens et al. (1997)
	Corals	3–20	200	Patterson (1991)
	Brittlestars	4	30–360	Labarbera (1978)
	Seagrasses	20	7.5–2700	Ackerman (1997)
	Blackfly larvae	80	0.091–30	Ross & Craig (1980)
	Tunicates	2–4	0.5–3	Sutherland et al. (2010)
Inertial impaction	Brittlestars	-	20–340	Labarbera (1984)
	Octocorals	50	256–1000	Sebens & Koehl (1984)
	Bryozoans	1–12	11.9 ± 1.9	Okamura (1984)
Gravitational deposition	Corals	30–50	<6000	Koehl (1977)
	Corals	<10	200	Sebens & Johnson (1991)
Diffusional deposition	Blackfly larvae	4–45	0.5–100	Braimah (1987)
	Glass fibers	0.1–1	5	Schrijver et al. (1981)
Electrostatic attraction	Brittlestars	4	30–360	Labarbera (1978)

environment, feeding and behavior, and (3) employed models to predict patterns of particle capture rate and particle capture efficiency under a range of water velocities and temperatures.

## MATERIALS AND METHODS

### Organism collection

Barnacles *Balanus glandula* attached to shells of the mussel *Mytilus trossulus* were collected from Argyle Creek (48° 31.728' N, 123° 00.802' W) on San Juan Island, WA, USA, in May and June of 2012. The site was characterized by unidirectional tidal flow. Barnacles were gently excised from mussel shells with a razor blade and maintained unfed at 8°C in a recirculating seawater system for 1 wk before use in experiments. Shell height and basal diameter (along the rostro-carinal axis) of each barnacle were measured with Mitutoyo 500-196-20 digital calipers (Mitutoyo America; ±0.01 mm). Cirri were dissected and photographed under a dissecting microscope with a CoolPix 995 digital camera (Nikon), and the length of the sixth cirrus was measured from the tip to the base of the ramus as described in Arsenault et al. (2001) using ImageJ software (NIH; ±0.001 mm).

To characterize the fluid conditions that barnacles were exposed to during feeding, the Reynolds number ( $Re$ ) was calculated as:

$$Re = \frac{\rho_s d_{ci} U}{\mu} \quad (1)$$

where  $\rho_s$  is the density of seawater ( $\text{kg m}^{-3}$ ),  $d_{ci}$  is the diameter of the sixth cirrus (m),  $U$  is water velocity ( $\text{m s}^{-1}$ ), and  $\mu$  is the dynamic viscosity of seawater ( $\text{kg m}^{-1} \text{s}^{-1}$ ).

In our experiments,  $U$  at the level of the cirri was not directly quantified, so we approximated its value as 10% of free-stream (Carrington et al. 2008). These velocities were then used for Eq. (1) (also Eq. 4).

### Experimental design

Before each trial, barnacles were attached to an acrylic plate (10 × 3 cm) using modeling clay. Ten barnacles were placed on each side of the centerline of the plate, forming 2 columns that extended along the primary axis of water flow (see Fig. A1 in the Appendix; 20 barnacles total). The plate was placed in the flow chamber such that the long axis was parallel to the direction of flow. An additional row of bar-

nacles was placed on both the upstream and downstream ends of the test barnacles to avoid effects that might be associated with leading and trailing edges (Sebens et al. 1997). Individual barnacles were used as replicates in all experiments ( $N = 20$ ).

### Measuring feeding rates

Feeding trials were conducted in a closed, recirculating flow chamber of 600 ml volume as described by Nishizaki & Carrington (2014). Barnacles were fed hydrated *Artemia* cysts (hydrated diameter =  $228.1 \pm 4.1 \mu\text{m}$ ,  $N = 30$  cysts subsampled from experiment), that were within the size distribution of barnacle prey (Trager et al. 1994, Wang et al. 1999). Before each trial, *Artemia* were hydrated in 1  $\mu\text{m}$  filtered seawater for 30 min and then fractionated to isolate near neutrally buoyant (slight negative buoyancy) cysts at a concentration of  $27 \pm 1$  cysts  $\text{ml}^{-1}$ . Cysts were used as we were unable to quantify plankton prey and found no published identification of *B. glandula* diet. *Artemia* cysts are within the size range of prey for other barnacle species (Barnes 1959, Wang et al. 1999) and provide a means of quantifying barnacle ingestion rates as demonstrated by Bertness et al. (1998). Cysts ( $27 \pm 1$  cysts  $\text{ml}^{-1}$ ) were added to the flow chamber at the beginning of each run, and 1 ml water samples were taken from the flow chamber at the beginning of each trial and again at their conclusion to quantify any decrease in cyst concentration due to sedimentation. Cyst diameters did not change significantly during the course of each trial ( $t_{28} = 1.33$ ,  $p > 0.05$ ).

After a 5 min acclimation period in the flow chamber, barnacles were exposed to experimental conditions for 45 min. This test duration was based on preliminary observations to provide sufficient time for barnacles to commence feeding, while avoiding feeding saturation (data not shown). The plate was exposed to one combination of water temperature (5, 10, 15, 20, and 25°C) and velocity (1, 7.5, 12, 20, 30, 40, and 60  $\text{cm s}^{-1}$ ), and individual barnacles were used as replicates in all experiments ( $N = 20$ ). Water velocities along the centerline of the testing chamber were estimated by tracking the displacement of glass microbeads (Potters Industries; mean particle diameter =  $9 \mu\text{m}$ , density =  $2.0 \text{ g cm}^{-3}$ ) at each flow setting (for details, see Nishizaki & Carrington 2014). Water temperatures varied by less than 0.1°C during the course of each trial. These experimental conditions conform to the range of conditions that barnacles experience in the field. Under natural conditions,

barnacles experience water velocities ranging from 0.96 to 98 cm s<sup>-1</sup> (Marchinko & Palmer 2003, Miller 2007) and water temperatures from 2.5 to 26.7°C (Nishizaki & Carrington 2014).

After removal from the flow chamber, all 20 barnacles (excluding those in the first and last rows) were dissected and the number of cysts present in the gut was quantified. Under a dissecting microscope, cysts showed no signs of digestion and remained identifiable after 45 min. Cyst capture rates were standardized by calculating the number of cysts captured per min per barnacle. Capture efficiency was calculated as cyst capture rate (cysts s<sup>-1</sup>) divided by the encounter rate of cysts to the cirral net (cysts s<sup>-1</sup>):

$$\text{Capture efficiency} = \frac{\text{Cyst capture rate}}{\text{Cyst encounter rate}} \quad (2)$$

Encounter rate (cysts s<sup>-1</sup>) was calculated as the delivery of cysts passing through the area projected by the cirral net:

$$\text{Capture encounter rate} = C_{\text{cy}} UA_{\text{cirral net}} \quad (3)$$

where  $C_{\text{cy}}$  is the concentration of cysts in the water column (cysts m<sup>-3</sup>),  $U$  is free-stream water velocity (m s<sup>-1</sup>) and  $A_{\text{cirral net}}$  is the projected area of the cirral net (m<sup>2</sup>). The projected area of the cirral net was calculated assuming a triangular shape ( $\frac{1}{2}bh$ ), where base ( $b$ ) is cirral net width (m) and height ( $h$ ) is measured as cirral length (m). Video analysis was used to measure cirral height during different cirral beating behaviors ( $N = 5$  barnacles per behavior; random numbers generated to select individuals subsampled), which varied with flow and temperature. Cirral net widths were also measured from video recordings ( $N = 30$  barnacles). Cirral behaviors were then categorized (see next section) every 2 s for 10 min ( $N = 10$  barnacles per trial; random numbers generated to select individuals subsampled) to calculate average cirral height over the course of each trial. In addition, the length of time that the cirral net remained exposed to the water column during the various types of beating was measured ( $N = 10$  barnacles per behavior; random numbers generated to select individuals subsampled). The length of cirral exposure during each trial was calculated as the product of the time exposed during each beat type and the total number of beats (calculated from observed beat rate for each behavior). Cirral heights were then standardized to the proportion of time cirri were exposed before calculation of projected area.

## Cirral behavior

Cirral beating behavior was recorded during each feeding trial using a PowerShot SX20 IS digital camera (Canon). For each trial, 10 min of cirral behavior was assessed using an open-source processing software package (Avidemux 2.6.0). Cirral behaviors were classified according to Nishizaki & Carrington (2014), and the proportion of time barnacles spent engaged in each behavior was measured. These behaviors were then categorized as either extended (i.e. normal beat, fast beat, extension) or abbreviated beating (i.e. pumping, testing, gaping). Both extended and abbreviated behaviors were considered as potentially capable of capturing food in our analysis. Cirral beat rate was also measured from 10 barnacles for each trial.

A separate analysis was undertaken to determine the efficacy of using cirral beating rate as a proxy for barnacle feeding rate. A 10 min section of feeding experiment video was analyzed to determine the proportion of barnacles actively beating their cirri (i.e. both extended and abbreviated). These numbers were compared to the number of barnacles with cysts found in their gut by dissection.

## Statistical analysis

Kruskal-Wallis tests were employed to analyze the effect of velocity and temperature on (1) cyst capture rate, (2) cyst capture efficiency, (3) percentage of barnacles actively beating cirri (total beating), (4) percentage of barnacles with extended beating and (5) percentage of barnacles with abbreviated beating.

Differences in the percentage of barnacles beating and percentage with cysts in their gut were tested for with a Z-test (Zar 1999). The relationship between cirral beating rate and feeding rate was assessed with linear regression. All statistical tests were conducted with MATLAB v.R2011a (Mathworks) and SPSS v.19 (IBM).

## Predicted cyst capture

For each of the 4 particle capture mechanisms (Fig. 1), 2 non-dimensional indices were calculated: one that predicts cyst encounter rate by a cirrus and one that predicts cyst capture efficiency. The efficiency indices assume capture by a single cirrus in a moving fluid. This assumption does not consider the contribution of fine cirral setae to feeding (South-

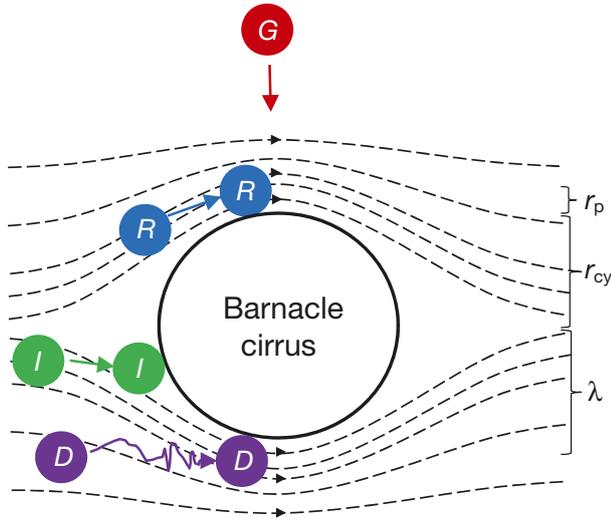


Fig. 1. Mechanisms of aerosol particle capture (after Shimeta & Jumars 1991). Direct Interception ( $R$ ) of a cyst from flowing seawater as it moves along a streamline around the barnacle cirrus. Inertial impaction ( $I$ ) occurs when the momentum of a cyst causes it to deviate from the path of a streamline and contacts the cirrus. Gravitational deposition ( $G$ ) can occur when sedimenting cysts contact the cirrus. Diffusional deposition ( $D$ ) applies when cysts exhibiting random paths collide with water molecules and are collected when they contact the cirrus.  $\lambda$  is the effectively swept distance (m),  $r_c$  is the radius of the cylinder (m) and  $r_p$  is the particle radius (m)

ward 1955b). The indices were calculated across a range of velocities (1, 7.5, 12, 20, 30, 40 and 60  $\text{cm s}^{-1}$ ) and temperatures (5, 10, 15, 20 and 25°C), as described in the following subsections.

#### Direct interception

At low to intermediate  $Re$ , neutrally buoyant cysts move along streamlines, and a proportion approach within 1 cyst radius from a single cirrus ( $r_{cy} = 1.28 \times 10^{-4}$  m). Capture rate ( $F_R$ ) due to direct interception can be calculated for intermediate  $Re$  (Shimeta & Jumars 1991):

$$F_R = 2CU_{I_{ci}}\lambda + 2C_vU_v r_{cy}I_{ci} \quad (4)$$

where  $C$  is cyst concentration ( $\text{cells ml}^{-1}$ ),  $U$  is water velocity around the fixed cirrus ( $\text{m s}^{-1}$ ),  $I_{ci}$  is exposed cirrus length (m) and  $\lambda$  is half the cross-streamline distance of fluid for which particles encounter the cirrus.  $C_v$  and  $U_v$  are cyst concentration ( $\text{cells ml}^{-1}$ ) and velocity ( $\text{m s}^{-1}$ ) in the trailing vortex. No depletion of cysts behind the cirrus is assumed since 'fresh' water, which has not contacted the front of the cirrus, is entrained into the vortex (Shimeta & Jumars 1991).

$\lambda$  at intermediate  $Re$  is calculated according to Humphries (2009):

$$\lambda = a_i \times Re_{ci}^{b_i} + c_i \quad (5)$$

where  $a_i = 0.216 \times r_p^{1.954}$ ,  $b_i = 0.652 - 0.186 \times r_p$ ,  $c_i = 0.160 \times r_p^{1.911}$ , and where  $r_p$  is the absolute particle radius (m; Fig. 1), estimated according to Humphries (2009).

Capture efficiency ( $N_R$ ) of cysts due to direct interception can be calculated as:

$$N_R = \frac{d_{cy}}{d_{ci}} \quad (6)$$

$d_{cy}$  is cyst diameter (m) and  $d_{ci}$  is cirrus diameter (m). The length of exposed cirrus varied among the various beating behaviors (described below) employed at each velocity.

#### Inertial impaction

At fast water velocities, the momentum of a cyst causes it to deviate from the path of a streamline, and it can be intercepted by the cirrus. Capture rate ( $F_I$ ) can be calculated as:

$$F_I = 2CU_{I_{ci}}I_s \quad (7)$$

where  $I_s$  is the stopping distance of cysts (m), defined as the distance along the cyst's original trajectory over which it would stop if the fluid flow ceased instantaneously.  $I_s$  is calculated as follows (Shimeta & Jumars 1991):

$$I_s = \frac{U(\rho_{cy} - \rho_s)(2r_{cy})^2}{18\mu_s} \quad (8)$$

where  $\rho_{cy}$  is the density of fully hydrated cysts ( $1082 \text{ kg m}^{-3}$  at 23 to 25°C and 15 PSU; Clegg 1984),  $\rho_s$  and  $\mu_s$  represent temperature-dependent seawater density ( $\text{kg m}^{-3}$ ) and dynamic viscosity (Pa s) respectively, and  $r_{cy}$  is cyst radius (m).

Capture efficiency ( $N_I$ ) was calculated as follows:

$$N_I = \frac{d_{cy}^2 U (\rho_{cy} - \rho_s)}{18\mu d_{ci}} \quad (9)$$

where  $r_{ci}$  and  $d_{ci}$  are cirral radius and diameter respectively (m).

#### Gravitational deposition

Under slow water velocities, negatively buoyant cysts sink and come into contact with the cirrus. Cap-

ture rate ( $F_G$ ) and capture efficiency ( $N_G$ ) can be calculated as:

$$F_G = \frac{4Cg(\rho_{cy} - \rho_s)(r_{cy} + r_{ci})r_{cy}^2 l_{ci}}{9\mu} \quad (10)$$

$$N_G = \frac{d_{cy}^2 g (\rho_{cy} - \rho_s)}{18\mu U} \quad (11)$$

where  $g$  is gravitational acceleration ( $\text{m s}^{-2}$ ).

#### Diffusional deposition

Under very slow water velocities, cysts randomly deviate from streamlines due to Brownian motion, and can become entrained on the cirrus. Capture rate due to diffusional deposition ( $F_D$ ) and capture efficiency ( $N_D$ ) can be calculated as:

$$F_D = \pi CD^{2/3} l_{ci} \left( \frac{r_{ci} U}{2 - \ln[Re_{ci}]} \right)^{1/3} \quad (12)$$

$$N_D = \frac{KT}{3\pi\mu d_{cy} U_0 d_{ci}} \quad (13)$$

where  $Re_{ci}$  is the Reynolds number using cirral diameter as the characteristic length, cyst diffusivity ( $D$ ) =  $KT / 6\pi\mu r_{cy} = 7.4 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$  (Patterson 1991),  $K$  is the Boltzmann constant ( $1.38 \times 10^{-23} \text{ J K}^{-1}$ ), and  $T$  is temperature (K).

The influence of behavior on capture rate indices was also calculated by multiplying each by the proportion of time spent engaged in extended beating behaviors (i.e. feeding; see description below) standardized to the total time of each trial.

## RESULTS

$Re$  calculated for flow around barnacle cirri during the feeding experiments ranged from 0.1 to 14.7, increasing with faster water velocities and warmer water temperatures (Fig. 2).

#### Measured rates

Projected area of the cirral net, cyst encounter rate and capture rate were highest at  $20 \text{ cm s}^{-1}$  and  $15^\circ\text{C}$ , and decreased at extreme velocities and temperatures (Fig. 3A–C). Cyst capture rates were significantly influenced by both temperature (Kruskal-Wallis  $H = 44.87$ ,  $p < 0.001$ ) and velocity ( $H = 39.31$ ,  $p < 0.001$ ). Capture rates at  $7.5 \text{ cm s}^{-1}$  were significantly

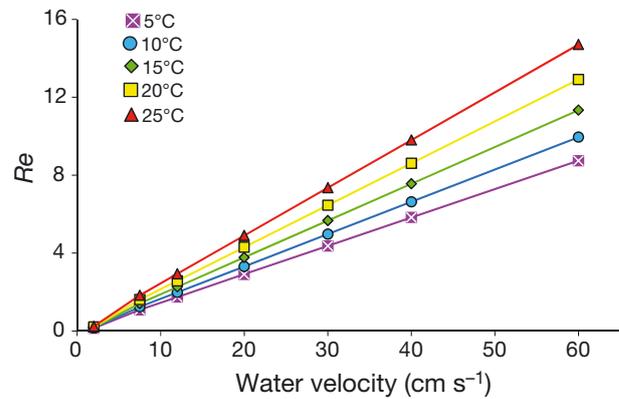


Fig. 2 Reynolds number ( $Re$ ) calculated for barnacles over a range of water velocities and temperatures. Calculations are based on the diameter of a single cirrus as the characteristic length ( $2.56 \pm 0.50 \times 10^{-4} \text{ m}$ ) and water velocities around cirri were assumed to be 9% of free stream velocities (Carrington et al. 2008)

higher than those at 1, 30, 40 and  $60 \text{ cm s}^{-1}$  (Tukey's HSD,  $p < 0.05$ ) but not different than those at 12 and  $20 \text{ cm s}^{-1}$ . Capture rates at  $15^\circ\text{C}$  were significantly higher than those at 5, 20 and  $25^\circ\text{C}$  (Tukey's HSD,  $p < 0.05$ ), but not significantly different than that at  $10^\circ\text{C}$  (Fig. 3C). The downstream position of each barnacle did not affect capture rate (Table A1).

Capture efficiencies were influenced significantly by both temperature (Kruskal-Wallis  $H = 39.26$ ,  $p < 0.001$ ) and velocity ( $H = 31.51$ ,  $p < 0.001$ ), and peaked at  $1 \text{ cm s}^{-1}$  and  $15^\circ\text{C}$  (Fig. 3D). Capture efficiencies at  $15^\circ\text{C}$  were significantly higher than those at 5, 20 and  $25^\circ\text{C}$  (Tukey's HSD,  $p < 0.05$ ), but not significantly different than that at  $10^\circ\text{C}$  (Fig. 3D). Capture efficiencies at  $1 \text{ cm s}^{-1}$  were significantly higher than those at all higher velocities (7.5, 12, 20, 30, 40 and  $60 \text{ cm s}^{-1}$ ; Fig. 3D).

#### Cirral beating behavior

Barnacles displayed high levels of cirral beating activity (Fig. 4), spending an average of  $74 \pm 3\%$  of observed time actively beating their cirri (abbreviated and extended forms combined). The total number of barnacles actively beating was significantly influenced by water velocity (Kruskal-Wallis  $H = 13.80$ ,  $p = 0.032$ ), and there was a near significant relationship with temperature ( $H = 8.97$ ,  $p = 0.06$ ). Extended beating was influenced significantly by both temperature and velocity ( $H = 12.54$ ,  $p = 0.014$  and  $H = 107.86$ ,  $p < 0.01$  respectively). Extended beating at 30, 40, and  $60 \text{ cm s}^{-1}$  was significantly lower than that at slower flows (Fig. 4; Tukey's HSD,

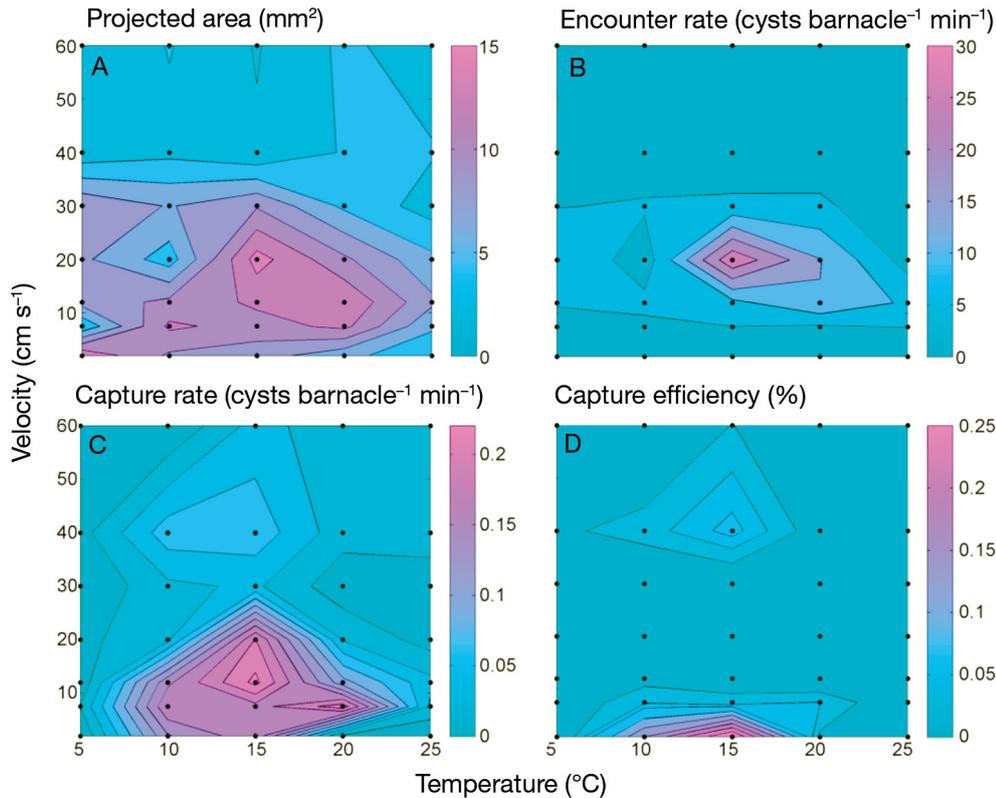


Fig. 3. Measured *Artemia* cyst capture by *Balanus glandula* over a range of water velocities and temperatures. Contour plots with linear interpolation represent: (A) projected area of the cirral net, (B) encounter rate of cysts past the cirral net, (C) capture rate and (D) capture efficiency. For capture rates, each node represents a mean of 20 barnacles

$p < 0.05$ ). The exception was between 20 and 30  $\text{cm s}^{-1}$ , where no significant differences were detected. Extended beating at 15°C was significantly higher than that at 25°C (Tukey's HSD,  $p < 0.05$ ).

Abbreviated beating was significantly influenced by both temperature (Kruskal-Wallis  $H = 21.04$ ,  $p < 0.001$ ) and velocity ( $H = 98.75$ ,  $p < 0.001$ ). Abbreviated beating at 30, 40 and 60  $\text{cm s}^{-1}$  was significantly lower than that at the slower flows (Fig. 4; Tukey's HSD,  $p < 0.05$ ). Abbreviated beating at 25°C was significantly higher than that at 5, 10, 15 and 20°C (Tukey's HSD,  $p < 0.05$ ).

Cirral beating rate increased with both water velocity and temperature (Fig. 5). Beating rates ranged from 0.05 to 1.00  $\text{beats s}^{-1}$  and were significantly influenced by both water temperature and velocity (Kruskal-Wallis  $H = 29.96$ ,  $p < 0.001$  and  $H = 43.03$ ,  $p < 0.001$  respectively). Beating rates were significantly higher at 40 to 60  $\text{cm s}^{-1}$  compared to those at all lower velocities tested and significantly lower at 5°C compared with those at all other temperatures (Tukey's HSD,  $p < 0.05$ ).

#### Cirral activity versus feeding rate

The percentage of barnacles beating their cirri during the experiment was significantly higher ( $68 \pm 3\%$ ;

Fig. 6A) than the percentage of barnacles with cysts in their gut ( $22 \pm 3\%$ ; Z-test,  $p < 0.05$ ). Whereas beating rate did not display a consistent pattern in regards to either temperature or flow, the percentage of fed barnacles peaked at moderate water velocities (7.5 to 12  $\text{cm s}^{-1}$ ) and temperatures (15 to 20°C; Fig. 6B). When the percentage of barnacles fed were plotted against the percentage beating, all measured data fell below unity (Fig. 6C). At most temperatures, there were no clear trends between cirral beating and feeding activity; only at 15°C was there a positive relationship (linear regression,  $p < 0.05$ ).

#### Model predictions

Of the 4 particle capture mechanisms, only results for direct interception ( $F_R$ ) are presented as they were on the order of 100× larger than the next largest capture mechanism for all velocity–temperature conditions. Results based on physical parameters alone (no behavior), showed capture rates increasing monotonically with increasing flow (Fig. 7A). The model also predicted that temperature had little effect on capture rates from 5 to 25°C. When behavior was incorporated into the model, however, capture rates peaked at 20  $\text{cm s}^{-1}$  and 15°C (Fig. 7B), similar to observed values.

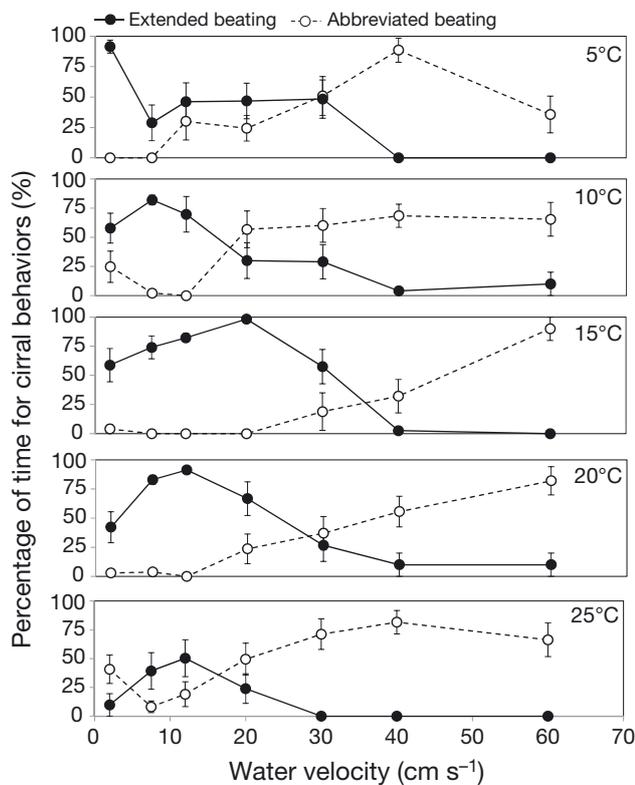


Fig. 4. Type of beating behavior displayed by barnacles under a range of water temperatures and velocities. ●: proportion of time barnacles displayed extended beating; ○: abbreviated beating. Error bars = SE. N = 10 barnacles

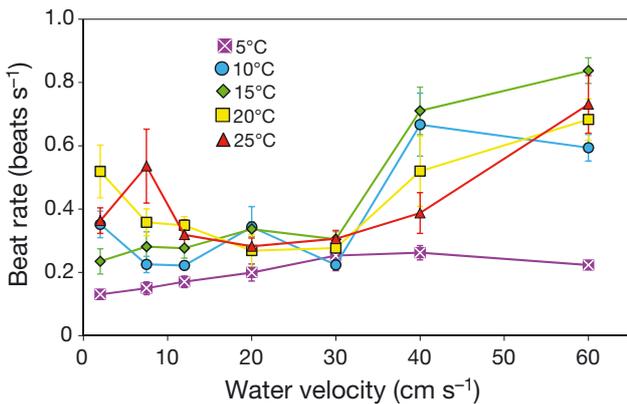


Fig. 5. Cirral beating rate in barnacles relative to water velocity and temperature. Error bars = SE. N = 10 barnacles

The capture efficiency model also suggests that direct interception ( $N_R$ ) is between  $10^1$  to  $10^8$  times larger than capture by inertial impaction, gravitational deposition or diffusional deposition. The model predicted uniform efficiencies across all treatments (~75%; data not shown).

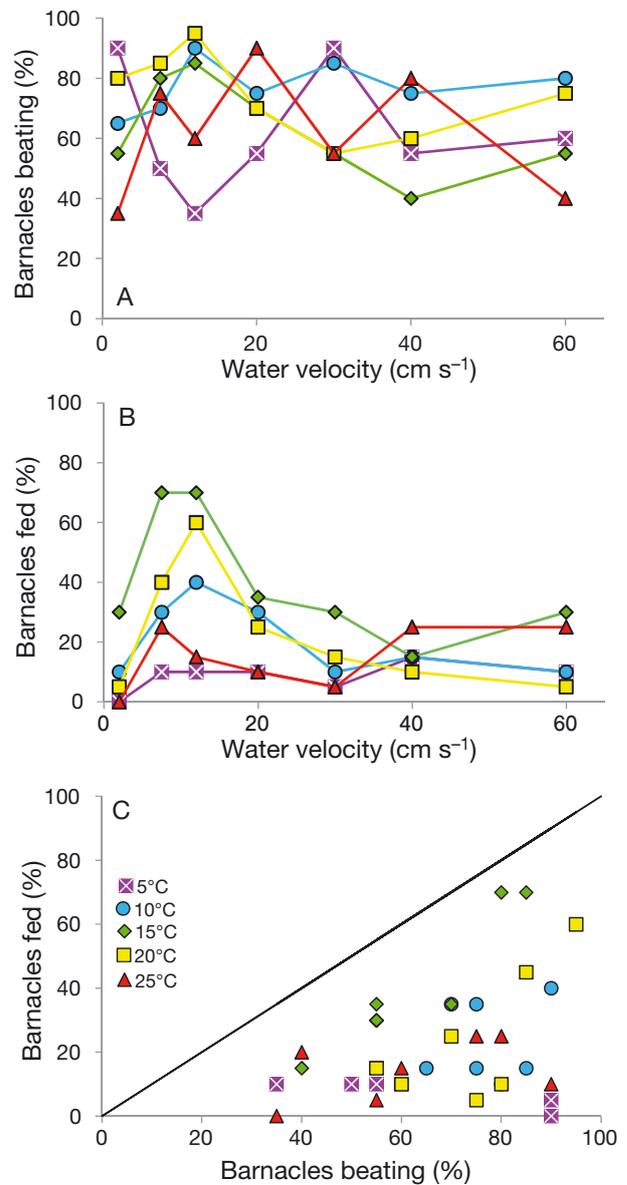


Fig. 6. Feeding activity of *Balanus glandula* in relation to water temperature and velocity showing (A) percentage of barnacles beating cirri and (B) percentage of barnacles with cysts in their gut. Each point represents a percentage estimated from 20 barnacles. (C) Relationship between the percentage of barnacles beating and the percentage of barnacles fed

**DISCUSSION**

Changes in water temperature and velocity elicited behavioral changes in cirral beating that affected projected area of the cirral net, cyst encounter rate, capture rate and capture efficiency (Fig. 3). In our experiments, cyst capture rate peaked at 7.5 to 20  $\text{cm s}^{-1}$  (Fig. 3C). At low water velocities, capture rates

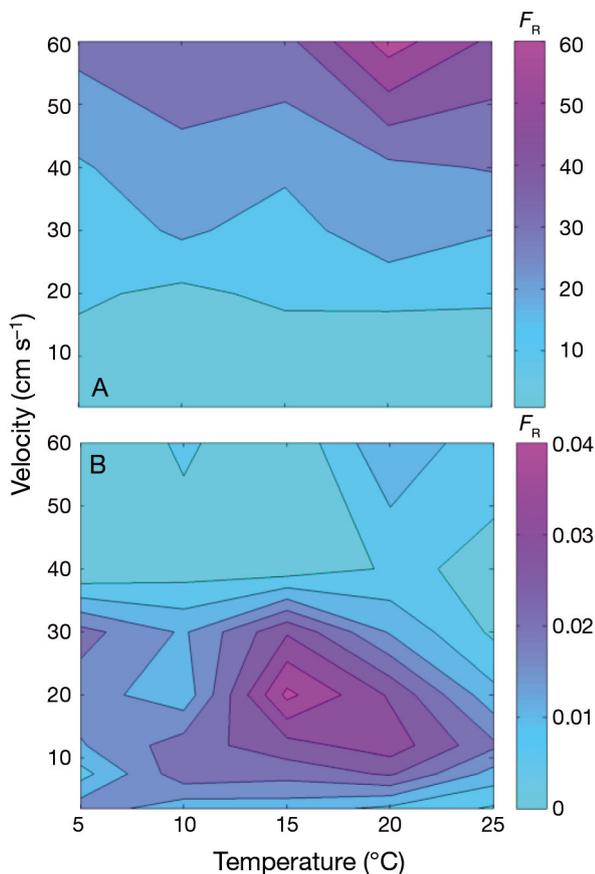


Fig. 7. Predicted patterns of *Artemia* cyst capture by *Balanus glandula* over a range of water velocities and temperatures showing (A) maximum capture rate for cylindrical cirrus without behavior, and (B) maximum capture rate of cirrus incorporating beating behavior. This model for direct interception ( $F_R$ ) was adapted from Rubenstein & Koehl (1977), Shimeta & Jumars (1991) and Humphries (2009) (see 'Results: Model predictions' for details)

were likely limited by the flux of cysts to the barnacle. At high flows, however, abbreviated beating became more common, suggesting that feeding may be constrained by mechanical limitation (Fig. 4). There was, however, a secondary peak at  $40 \text{ cm s}^{-1}$ . Shimeta & Jumars (1991) suggest that a sharp increase in capture rate may exist at  $\text{Re} \approx 10$  due to vortex formation. This is consistent with our results, as cirri at  $40 \text{ cm s}^{-1}$  were subject to  $\text{Re} = 6$  to  $10$  (Fig. 2). The effect of further increases in flow are less predictable because pressure fluctuations due to vortex shedding can reduce retention efficiency (Shimeta & Jumars 1991).

Capture rates also displayed a thermal optimum at  $15^\circ\text{C}$  (Fig. 3C). At high temperatures, barnacles employ abbreviated beating, presumably for ventilation, which may contribute to reduced capture rates

(Nishizaki & Carrington 2014). At low temperatures, capture rate may instead be limited by significantly lower beating rates (Fig. 5). Temperature had a moderate effect on the hydrodynamics of the system (i.e. water viscosity, density). Changes in feeding rates are more likely due to changes in barnacle physiology and/or behavior with temperature (Fig. 3C).

These capture rates do not account for prey escape behavior (Trager et al. 1994) and consequently may be an overestimate if applied to swimming prey. Similarly, capture rates are significantly affected by particle size (Labarbera 1978, Sutherland et al. 2010), which should be considered before extending these conclusions to smaller prey items (Crisp & Southward 1961).

The experimental results contrasted with those of the capture rate model, which is based on morphological characteristics and hydrodynamic conditions. The model using a single, continuously exposed cirrus predicted a monotonic increase in particle capture with increasing flow and no change with temperature. Only when behavior was incorporated did the model more closely predict peak capture rates at intermediate water velocities. Both capture rate and capture efficiency models suggest that direct interception is the dominant capture mechanism under a wide range of thermal and flow conditions (Fig. 7A,B). Although further refinements to the physical model are possible, any reasonable modifications are unlikely to produce accurate predictions about barnacle capture rates without accounting for flow- and temperature-dependent behavior.

Capture efficiencies were highest at low velocities and decreased rapidly as velocity increased (Fig. 3D). Lower efficiencies at high flows may be a consequence of the cirral net deforming (Marchinko 2007). As water velocities increase, the cirral net may also become increasingly 'leaky' (Geierman & Emler 2009), which would decrease capture efficiencies.

Although capture efficiency dropped with increasing water velocity, higher encounter rates likely compensated to yield the highest capture rates occurring at  $7.5$  to  $12 \text{ cm s}^{-1}$  (Fig. 3C). At very high velocities, low capture efficiencies may prevent barnacles from capitalizing on high cyst flux. Moreover, capture rates rather than efficiencies are of greater interest from an energetic perspective as energy intake will be greatest where cyst capture is highest, not efficiency.

The low capture efficiencies measured here (Fig. 3D) beg the question of why barnacles do not spend long periods of time with their cirri extended (Trager et al. 1990). One possible explanation is that cirral beating behavior balances the costs and bene-

fits of exposing cirri to predators in the water column. In *Balanus glandula*, periods of cirral withdrawal increase in response to the presence of predators (Palmer et al. 1982). Barnacles also spend less time hiding from predators when starved (Dill & Gillett 1991).

Measured capture efficiencies were in sharp contrast to the model predictions (run without 'behavior') of uniform capture rates across all velocities and temperatures. Although direct interception was predicted to be the most important capture mechanism for all conditions, it is clear that biophysical analysis alone is not sufficient to explain patterns of capture efficiency.

Cirral activity rates measured here were higher compared to barnacles tested in the absence of food ( $68 \pm 3$  vs.  $37 \pm 2\%$  respectively; Nishizaki & Carrington 2014). At low velocities, barnacles typically displayed extended beating behavior, whereas at higher velocities, barnacles showed abbreviated beating (Fig. 4). The switch from extended to abbreviated behaviors in our study typically occurred at lower velocities (20 to 30 cm s<sup>-1</sup>) compared to barnacles tested without food (40 cm s<sup>-1</sup>; Nishizaki & Carrington 2014). The difference between these 2 observations may be related to the increased risks of mechanical damage in the feeding experiment from a fast moving fluid filled with cysts. Indeed, fed barnacles may simply be less motivated to expose their cirri to potential danger in high flows (Dill & Gillett 1991).

Slow beating rates were observed at 5°C, but were similar across all other temperatures from 10 to 25°C. This is in contrast to other barnacle species such as *Semibalanus balanoides* and *Balanus amphitrite*, which display clear thermal optima in beating rate at 15 to 20°C and 30 to 37°C respectively (Southward 1955a, Anderson & Southward 1987, Sanford et al. 1994).

Barnacles in our experiments rarely displayed the passive feeding behavior that has been reported for related species such as *S. balanoides* (Trager et al. 1990). This is consistent with results that show passive beating behavior is observed in small barnacles, but largely absent in larger individuals (Geierman & Emler 2009).

This study also confirms a disparity between the percentage of barnacles actively beating their cirri ( $68 \pm 3\%$ ) and the percentage with food in their gut ( $22 \pm 3\%$ ), suggesting that beating activity is not a reliable predictor of feeding activity (Fig. 6C). Indeed, barnacles in our experiments often appeared to sweep cysts towards and then away from their mouths (M. T. Nishizaki pers. obs.), which likely contributed to the low capture efficiencies measured

(Fig. 3D). These patterns are similar to those measured in sea anemones and suspension feeding fish, where high particle encounter rates are accompanied by relatively low capture and retention rates (Robinson et al. 2013). In barnacles, estimates of feeding rates generally lack direct measures of ingestion and instead rely on behavioral proxies (Pullen & Labarbera 1991, Sanford et al. 1994, Bertness et al. 1998). Our results strongly advocate for the use of direct measures of feeding over indirect methods.

Overall, these results demonstrate that barnacle feeding is influenced by the 2 environmental factors temperature and flow. Changes in water flow mediate the physical delivery of cysts from the water column to the barnacle. In contrast, temperature has little effect on the physical encounter rate with cysts, and its influence on feeding is presumably a consequence of physiological and behavioral changes. These results suggest that environmental uncertainty in water temperature and velocity may have consequences for barnacle feeding ecology and energy acquisition.

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#### LITERATURE CITED

- Ackerman JD (1997) Submarine pollination in the marine angiosperm *Zostera marina* (Zosteraceae). I. The influence of floral morphology on fluid flow. *Am J Bot* 84: 1099–1109
- Ackerman JD, Nishizaki MT (2004) The effect of velocity on the suspension feeding and growth of the marine mussels *Mytilus trossulus* and *M. californianus*: implications for niche separation. *J Mar Syst* 49:195–207
- Anderson D, Southward AJ (1987) Cirral activity of barnacles. In: Southward AJ (ed) *Barnacle biology*. A.A. Balkema, Rotterdam, p 135–174
- Arsenault DJ, Marchinko KB, Palmer AR (2001) Precise tuning of barnacle leg length to coastal wave action. *Proc R Soc Lond B Biol Sci* 268:2149–2154
- Barnes H (1959) Stomach contents and microfeeding of some common cirripedes. *Can J Zool* 37:231–236
- Barnes M (2000) The use of intertidal barnacle shells. *Oceanogr Mar Biol Annu Rev* 38:157–187
- Bertness MD, Gaines SD, Yeh SM (1998) Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. *Ecology* 79:1382–1394
- Braimah SA (1987) Mechanisms of filter feeding in immature *Simulium bivittatum* Malloch (Diptera: Simuliidae)

- and *Isonychia campestris* McDunnough (Ephemeroptera: Oligoneuriidae). *Can J Zool* 65:504–513
- Carrington E, Moeser GM, Thompson SB, Coutts LC, Craig CA (2008) Mussel attachment on rocky shores: the effect of flow on byssus production. *Integr Comp Biol* 48: 801–807
- Clegg JS (1984) Interrelationships between water and cellular metabolism in *Artemia* cysts. *Cell Biophys* 6:153–169
- Connell JH (1961) Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecol Monogr* 31:61–104
- Crisp DJ, Southward AJ (1961) Different types of cirral activity of barnacles. *Philos Trans R Soc Biol Sci* 243:271–307
- Dill LM, Gillett JF (1991) The economic logic of barnacle *Balanus glandula* (Darwin) hiding behavior. *J Exp Mar Biol Ecol* 153:115–127
- Geierman C, Emlet R (2009) Feeding behavior, cirral fan anatomy, Reynolds numbers, and leakiness of *Balanus glandula*, from post-metamorphic juvenile to the adult. *J Exp Mar Biol Ecol* 379:68–76
- Harley CDG (2006) Effects of physical ecosystem engineering and herbivory on intertidal community structure. *Mar Ecol Prog Ser* 317:29–39
- Helmuth B, Sebens K (1993) The influence of colony morphology and orientation to flow on particle capture by the scleractinian coral *Agaricia agaricites* (Linnaeus). *J Exp Mar Biol Ecol* 165:251–278
- Hueckel C, Stayton R (1982) Fish foraging on an artificial reef in Puget Sound, Washington. *Mar Fish Rev* 44:38–44
- Humphries S (2009) Filter feeders and plankton increase particle encounter rates through flow regime control. *Proc Natl Acad Sci USA* 106:7882–7887
- Jørgensen CB (1966) *Biology of suspension feeding*. Pergamon Press, Oxford
- Jørgensen CB (1983) Fluid mechanical aspects of suspension feeding. *Mar Ecol Prog Ser* 11:89–103
- Kearney M, Porter WP (2004) Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* 85:3119–3131
- Koehl MAR (1977) Water flow and the morphology of zoanthid colonies. *Proc 3rd Int Coral Reef Symp*, Miami, FL, 1:437–444
- Labarbera M (1978) Particle capture by a Pacific brittle star: experimental test of the aerosol suspension feeding model. *Science* 201:1147–1149
- Labarbera M (1984) Feeding currents and particle capture mechanisms in suspension feeding animals. *Am Zool* 24: 71–84
- Loudon C, Alstad DN (1990) Theoretical mechanics of particle capture: predictions for hydropsychid caddisfly distributional ecology. *Am Nat* 135:360–381
- Marchinko KB (2007) Feeding behavior reveals the adaptive nature of plasticity in barnacle feeding limbs. *Biol Bull* 213:12–15
- Marchinko KB, Palmer AR (2003) Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species. *Zoology* 106:127–141
- Miller L (2007) Feeding in extreme flows: behavior compensates for mechanical constraints in barnacle cirri. *Mar Ecol Prog Ser* 349:227–234
- Navarrete SA, Menge BA, Daley BA (2000) Species interactions in intertidal food webs: prey or predation regulation of invertebrate predators? *Ecology* 81:2264–2277
- Németh Z, Bonier F, MacDougall-Shackleton SA (2013) Coping with uncertainty: integrating physiology, behavior, and evolutionary ecology in a changing world. *Integr Comp Biol* 53:960–964
- Nishizaki MT, Carrington E (2014) The effect of water temperature and flow on respiration in barnacles: patterns of mass transfer versus kinetic limitation. *J Exp Biol* 217: 2101–2109
- Okamura B (1984) The effects of ambient flow velocity, colony size, and upstream colonies on the feeding success of bryozoa. I. *Bugula stolonifera* Ryland, an arborescent species. *J Exp Mar Biol Ecol* 83:179–193
- Palmer AR, Szymanska J, Thomas L (1982) Prolonged withdrawal: a possible predator evasion behavior in *Balanus glandula* (Crustacea: Cirripedia). *Mar Biol* 67:51–55
- Patterson MR (1991) The effects of flow on polyp-level prey capture in an Octocoral, *Alcyonium siderium*. *Biol Bull* 180:93–102
- Podolsky RD (1994) Temperature and water viscosity: physiological versus mechanical effects on suspension feeding. *Science* 265:100–103
- Pullen J, Labarbera M (1991) Modes of feeding in aggregations of barnacles and the shape of aggregations. *Biol Bull* 181:442–452
- Riisgård HU, Larsen PS (2010) Particle capture mechanisms in suspension-feeding invertebrates. *Mar Ecol Prog Ser* 418:255–293
- Robinson HE, Finelli CM, Koehl MAR (2013) Interactions between benthic predators and zooplanktonic prey are affected by turbulent waves. *Integr Comp Biol* 53:810–820
- Ross DH, Craig DA (1980) Mechanisms of fine particle capture by larval black flies (Diptera: Simuliidae). *Can J Zool* 58:1186–1192
- Rubenstein DI, Koehl MAR (1977) The mechanisms of filter feeding: some theoretical considerations. *Am Nat* 111: 981–994
- Sanford E, Bermudez D, Bertness M, Gaines S (1994) Flow, food supply and acorn barnacle population dynamics. *Mar Ecol Prog Ser* 104:49–62
- Schrijver JH, Vreeken C, Wesselingh J (1981) Deposition of particles on a cylindrical collector. *J Colloid Interface Sci* 81:249–256
- Sebens KP, Koehl MAR (1984) Predation on zooplankton by the benthic anthozoans *Alcyonium siderium* (Alcyonacea) and *Metridium senile* (Actiniaria) in the New England subtidal. *Mar Biol* 81:255–271
- Sebens KP, Johnson AS (1991) Effects of water movement on prey capture and distribution of reef corals. *Hydrobiologia* 226:91–101
- Sebens K, Witting J, Helmuth B (1997) Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). *J Exp Mar Biol Ecol* 211:1–28
- Shimeta J, Jumars PA (1991) Physical mechanisms and rates of particle capture by suspension-feeders. *Oceanogr Mar Biol Annu Rev* 29:191–257
- Southward AJ (1955a) On the behaviour of barnacles: I. The relation of cirral and other activities to temperature. *J Mar Biol Assoc UK* 34:403–422
- Southward AJ (1955b) Feeding of barnacles. *Nature* 175: 1124–1125
- Southward AJ (1957) On the behaviour of barnacles III. Further observations on the influence of temperature and age on cirral activity. *J Mar Biol Assoc UK* 36:323–334
- Strathmann RR (2005) Ciliary sieving and active ciliary response in capture of particles by suspension-feeding brachiopod larvae. *Acta Zool* 86:41–54

- Sueiro MC, Bortolus A, Schwindt E (2011) Habitat complexity and community composition: relationships between different ecosystem engineers and the associated macro-invertebrate assemblages. *Helgol Mar Res* 65:467–477
- Sutherland KR, Madin LP, Stocker R (2010) Filtration of sub-micrometer particles by pelagic tunicates. *Proc Natl Acad Sci USA* 107:15129–15134
- Todgham AE, Stillman JH (2013) Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integr Comp Biol* 53:539–544
- Trager GC, Hwang JS, Strickler JR (1990) Barnacle suspension-feeding in variable flow. *Mar Biol* 105:117–127
- Trager G, Achituv Y, Genin A (1994) Effects of prey escape ability, flow speed, and predator feeding mode on zoo-

- plankton capture by barnacles. *Mar Biol* 120:251–259
- Vermeer K (1982) Comparison of the diet of the glaucous-winged gull on the east and west coasts of Vancouver Island. *Murrelet* 63:80–85
- Wang W, Qiu J, Qian P (1999) The trophic transfer of Cd, Cr, and Se in the barnacle *Balanus amphitrite* from planktonic food. *Mar Ecol Prog Ser* 187:191–204
- Wildish D, Kristmanson D (2005) Benthic suspension feeders and flow. Cambridge University Press, Cambridge
- Wright RT, Coffin RB, Ersing CP, Pearson D (1982) Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. *Limnol Oceanogr* 27:91–98
- Zar J (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ

#### Appendix. Additional data on cyst capture rate in barnacles and on experimental design

Table A1. Results of linear regression analysis on the effect of downstream position on cyst capture rate in barnacles

Temperature (°C)	Velocity (cm s <sup>-1</sup> )	N	R <sup>2</sup>	p
5	1	20	0.0820	0.221
5	7.5	20	0.1511	0.090
5	12	20	0.0030	0.819
5	20	20	0.0100	0.676
5	30	20	0.0144	0.615
5	40	20	0.0777	0.234
5	60	20	0.0163	0.591
10	1	20	0.0400	0.400
10	7.5	20	0.0211	0.542
10	12	20	0.0029	0.818
10	20	20	0.0883	0.203
10	30	20	0.0013	0.882
10	40	20	0.0065	0.736
10	60	20	0.0820	0.221
15	1	20	0.0008	0.908
15	7.5	20	0.0935	0.190
15	12	20	0.0626	0.288
15	20	20	0.0113	0.656
15	30	20	0.0117	0.649
15	40	20	0.0719	0.253
15	60	20	0.0662	0.273
20	1	20	0.0143	0.615
20	7.5	20	0.0397	0.400
20	12	20	0.0017	0.863
20	20	20	0.0010	0.894
20	30	20	0.0839	0.215
20	40	20	0.0563	0.314
20	60	20	0.0016	0.867
25	1	20	0.0820	0.221
25	7.5	20	0.0560	0.315
25	12	20	0.0792	0.229
25	20	20	0.0075	0.716
25	30	20	0.0400	0.399
25	40	20	0.0466	0.361
25	60	20	0.1443	0.099

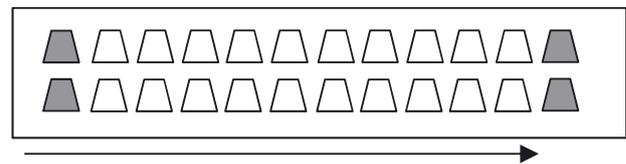


Fig. A1. Orientation of barnacles on plates used in feeding experiments. White cones: 20 barnacles used to measure capture rate; gray cones: additional barnacles placed to minimize the effects of leading and trailing edges (not included in analysis). Arrow: primary direction of water flow