Physiological and behavioral responses to temperature and flow in the barnacle *Balanus glandula* Darwin (1854).

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Abstract

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Given the scale and pace of anthropogenic change in marine environments, it is important to understand the manner in which organisms respond to environmental uncertainty. For many aquatic species, fundamental processes such respiration and feeding are potentially limited by the exchange of materials to and from their fluid environment. As a consequence, environmental factors such as water temperature and flow can profoundly impact the ecology and physiology of marine organisms. This dissertation evaluates the role of water temperature and velocity on respiration, feeding and ultimately growth in the barnacle, *Balanus glandula*. By conducting respiration experiments over a wide range of thermal (5 to 25°C) and fluid conditions (1 to 150 cm s^{-1}), I demonstrate the importance of approaches that evaluate the combined effects of multiple environmental factors when examining physiological and behavioral performance. Model analysis of the data suggests that respiration is limited by the delivery of oxygen at low velocity (< 7.5 cm s^{-1}) and high temperature (20 to 25°C). In contrast, respiration is limited by the capacity of barnacles to absorb oxygen at high flows (40 to 150 cm s^{-1}) and low temperatures

(5 to 15°C). Moreover, there are many intermediate flow-temperature conditions where both mass transfer and kinetic limitation are important. When oxygen delivery was limited (in low flow-high temperature treatments), barnacles displayed distinct "pumping" behaviors of their cirral appendages, a strategy that may serve to increase ventilation. Cirral beating behavior was also important in predicting patterns of feeding. The delivery of food particles (brine shrimp cysts) to the cirral net peaked at intermediate water velocities (7.5 to 20 cm s^{-1}) and temperatures (15°C) likely due to short, abbreviated beating strokes at high velocities and high temperature, low cyst delivery rates at low velocities and slow beating rates at low temperatures. Capture efficiency, or the ratio of cysts captured to cysts encountered, was highest under the slowest flow (1 cm s^{-1}). Model analysis of these observations demonstrated that detailed characterization of cirral beating behavior (i.e., whether they are employ fully extended versus abbreviated beating behavior) are required to accurately predict patterns of cyst capture. Model predictions of barnacle growth were generated using these respiration and feeding data. Peak growth rates are predicted at moderate water temperatures (15°C) and velocities (20 to 30 cm s^{-1}). Barnacles at slow velocities should experience lower growth, due to lower encounter rates with suspended food particles, whereas at high velocities, barnacles experience lower feeding efficiencies, which also reduces their potential for growth. At low temperatures, cirral beating behavior slows, reducing feeding capacity and in turn growth, whereas at high temperatures, high metabolic can impose limits on growth. These predictions were consistent with growth rates measured in two experiments - one that manipulated water temperature and velocity and a second that measured growth under field conditions at different temperatures and velocities. Moreover, these results underscore the importance of considering the interaction between multiple environmental factors and provide evidence that together, they shape responses in physiology, behavior and growth.

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Chapter I

THE EFFECT OF WATER TEMPERATURE AND FLOW ON RESPIRATION IN BARNACLES: PATTERNS OF MASS TRANSFER VERSUS KINETIC LIMITATION.

1.1. Abstract

In aquatic systems, physiological processes such as respiration, photosynthesis, and calcification are potentially limited by the exchange of dissolved materials between organisms and their environment. The nature and extent of physiological limitation is, therefore, likely to be dependent on environmental conditions. Here, we assess the metabolic sensitivity of barnacles under a range of water temperatures and velocities, two factors that influence their distribution. Respiration rates increased in response to changes in temperature and flow, with an interaction where flow had less influence on respiration at low temperatures, and a much larger effect at high temperatures. Model analysis suggested that respiration is mass transfer limited under conditions of low velocity (< 7.5 cm s^{-1}) and high temperature (20 to 25°C). In contrast, kinetic limitation, where respiration is limited by the biotic capacity of barnacles to absorb oxygen, prevailed at high flows (40 to 150 cm s^{-1}) and low temperatures (5 to 15°C). Moreover, there are many intermediate flow-temperature conditions where both mass transfer and kinetic limitation are important. Behavioral monitoring revealed that barnacles fully extend their cirral appendages at low flows and display abbreviated "testing" behaviors at high flows, suggesting some form of mechanical limitation. In low flow-high temperature treatments, however, barnacles displayed distinct "pumping" behaviors that may serve to increase ventilation. Our results suggest that in slow moving waters, respiration may become mass transfer limited as temperatures rise, whereas faster flows may serve to ameliorate the effects of elevated temperatures. Moreover, these results underscore the necessity for approaches that evaluate the

combined effects of multiple environmental factors when examining physiological and behavioral performance.

1.2. Introduction

Given the fluctuating nature of intertidal zones, marine biologists have had long-standing interests in the degree to which environmental variation influences the distribution and abundance of species (Barry et al., 1995; Southward et al., 1995; Underwood et al., 1983). Predicting the outcomes of species-environment interactions, however, can be limited by a poor understanding of physiological sensitivity (Denny and Helmuth, 2009; Seebacher and Franklin, 2012; Shelford, 1911). Indeed, recent attempts to forecast species distributions under changing climatic conditions now focus on key physiological mechanisms (Denny and Gaylord, 2010; Gaston, 2009; Kearney et al., 2009). One such mechanism is metabolism, which reflects an organisms' ability to convert energy into materials that support important life functions such as movement, growth and reproduction (Hochachka and Somero, 2002). Metabolic activity, when used to construct physiological performance curves, can provide a means of describing an organism's sensitivity to changing environmental conditions (Huey and Kingsolver, 1989).

Although temperature is among the most important factors influencing metabolic rate in marine invertebrates, a range of patterns have been documented including linear increase, decrease or the existence of thermal optima (see Table 1). Similarly, the effect of flow on respiration is unclear as some organisms show a positive relationship (Patterson and Sebens, 1989; Thomas and Atkinson, 1997), whereas others display little to no sensitivity (Edmunds, 2005). These discrepancies are likely rooted in: 1) the narrow range of conditions tested compared to those experienced by organisms in their natural environments and/or; 2) the interactive effects of temperature and flow on physiological rates (Edmunds, 2005). Here, we combine experiments and models to assess the metabolic sensitivity of barnacles to a wide range

of water temperatures and velocities, two environmental factors that correlate with their distribution (Leonard et al., 1998; Wethey, 1983a).

Many biological functions such as respiration, photosynthesis and calcification depend on the uptake of dissolved nutrients and/or gases (Burris et al., 1983; Cornelisen and Thomas, 2004; Sebens et al., 1997). These uptake rates, in turn, are potentially limited by: 1) the transport rates of dissolved material from the water column to the surface of an organism (known as mass transfer limitation) or; 2) reaction kinetics at the boundary that limit the ability of an organism to assimilate the dissolved material across the body wall (reaction kinetic limitation) (Gerard, 1982; Patterson and Sebens, 1989; Stevens and Hurd, 1997). Knowing whether uptake rates are governed by mass transfer versus kinetic limitation is important in understanding whether physiological processes are regulated by factors internal versus external to the organism. For instance, if an organism is mass transfer limited, adaptations that increase the physical delivery of oxygen to the organism will be favored, such as increased ventilation rates in *Mytilus edulis* under reduced oxygen tension (Bayne, 1971) or the use of respiratory proteins with high oxygen affinities by crustaceans in low-oxygen environments (Childress and Seibel, 1998). In contrast, during periods of kinetic limitation, physiological control of uptake is relatively more important (Seibel and Childress, 2013), resulting in a different set of responses. For instance, when marine snails experience aerial exposure, oxygen delivery is rarely limiting, yet metabolic responses to thermal change do occur (Marshall and McQuaid, 2011; Mcmahon and Russell-Hunter, 1977). These thermal responses are likely limited by physiological processes that are based on enzyme reaction rates (Somero, 1969). Indeed, determining whether uptake rates are mass transfer versus kinetically limited is a pervasive theme in biology that spans across the majority of the

world's taxa (e.g., plants and animal) and environments (e.g., air or water) (see Denny, 1993; Dubinsky and Stambler, 2011; Koch et al., 2006 for review).

Mass transfer limitation occurs as a consequence of dissolved materials needing to be physically delivered from the water column to the surface of the organism. To do so, solutes must penetrate through the boundary layer that surrounds various surfaces of an organism and the factors that limit this delivery are largely physical in nature. Increasing velocities reduce the thickness of diffusional boundary layers (Schlichting et al. 2000) and Fick's law predicts that the flux of a solute to or from a surface is inversely related to boundary layer thickness (Falter et al., 2004; Mass et al., 2010; Patterson et al., 1991). Thus, mass transfer between an organism and the water column should increase with faster flow (Reidenbach et al., 2006; Thomas and Atkinson, 1997). In contrast, the effects of water temperature on mass transfer rates are far less clear. Cooler waters contain more dissolved oxygen, which should increase oxygen delivery rates. However, lower temperatures also increase viscosity, and thus boundary layer thickness, which may decrease mass transfer rates. In considering these two opposing processes, it is unclear what the net effect temperature will have on oxygen mass transfer.

A second process, kinetic limitation, can occur when there is sufficient solute delivered to the organism and mass transfer limitation eases. Under these conditions, uptake rates may be limited by reaction kinetics, related to the ability of an organism to assimilate oxygen across the body surface. For instance, uptake of nutrients in freshwater plants has been shown to occur more slowly than does transport across their diffusional boundary layers (Nishihara and Ackerman, 2006). Reaction kinetics, in this case, may be more limiting than any rate of mass transfer.

Performance curves link physiological responses to environmental factors and are important in identifying whether an organism experiences mass-transfer versus kinetic limitation. In aquatic systems, mass transfer limitation has been documented at low water velocities (> 5 to 30 cm s^{-1}) in algae (Hurd et al., 1996), corals (Patterson et al., 1991; Thomas and Atkinson, 1997; Mass et al., 2010) and seagrasses (Mass et al., 2010). In contrast, kinetic limitation has been documented in freshwater plants (Nishihara and Ackerman, 2009) and algae (Gerard, 1982; Hurd et al., 1996). The degree of mass transfer limitation also varies with solute concentration and type. For instance, seagrasses maintained under replicate flow conditions were mass transfer limited for ammonia, but not nitrate (Cornelisen and Thomas, 2004). Together, these results underscore the need for a detailed examination of how environmental conditions may or may not influence uptake rates.

Many organisms engage in behavioral strategies that have significant effects on their physiology (Huey and Stevenson, 1979). In barnacles, the activity of modified appendages called cirri may represent an important coupling of physiological and behavioral systems. Cirri may contribute to respiratory exchange in barnacles (Anderson and Southward, 1987; Newell and Northcroft, 1965), in addition to serving as feeding appendages. Cirral activity is known to vary with both temperature (Anderson and Southward, 1987; Newell and Northcroft, 1965; Ritz and Foster, 1968) and flow (Marchinko, 2007a; Miller, 2007). Simultaneous monitoring of respiration and beating rate under a range of temperatures and flows will provide a more comprehensive comparison of barnacle physiology and behavior.

In this study, we use the barnacle *Balanus glandula* Darwin (1854) to investigate physiological and behavioral responses to varying water temperature and velocity. *B. glandula* is a well-known, cosmopolitan species that can be found on temperate rocky shores in both the

northern and southern hemispheres (Barnes and Barnes, 1956; Geller et al., 2008). As an intertidal organism, *B. glandula* is subject to a wide range of both water temperatures (Berger, 2009) and velocities (Marchinko, 2007a; Miller, 2007; Neufeld and Palmer, 2008). Although much interest exists in the effect of multiple factors on respiratory physiology, few studies have sufficient resolution to produce appropriate performance curves in a fully crossed design (Moran and Woods, 2010). In this study, we: 1) construct a series of performance curves to explore the influence of water temperature and velocity on respiration rate; 2) assess the relative importance of mass transfer versus kinetic limitation of respiration rates and; 3) measure cirral activity under different water temperatures and velocities to explore the interactions between environment, physiology and behavior.

1.3. Materials and methods

1.3.1. Organism collection

Adult barnacles (*Balanus glandula*) attached to mussel shells (*Mytilus trossulus*) were collected from Argyle Creek (N 48° 31.728' W 123° 00.802') on San Juan Island, WA, USA between August and September 2010. Flow in this saltwater creek is largely unidirectional as the shallow corridor (~10 m across) connects a lagoon to a bay that fills and drains during tidal exchanges. Water depth at the site varied between 10 cm and 50 cm and maximum creek width was approximately 10 m. Water velocities in Argyle Creek ranged from 0.01 to 1.37 m s^{-1} over a 12 hour tidal cycle as measured with an Acoustic Doppler Velocimeter (Sontek/YSI Inc., San Diego, CA, USA) at two locations spanning ~30 m in the streamwise direction. Water velocities at each site were sampled at 25 Hz for 180 second every hour from a sampling volume that was

maintained more than 1 cm above the substratum (where barnacles were found) to avoid boundary layer effects.

Temperatures at Argyle Creek were measured every 15 minutes from June 2011 to August 2012 with a submersible temperature probe (HOBO U22 Water Temp Pro v2; Onset Computer Corporation, Bourne, MA, USA) to estimate the range of thermal conditions that barnacles experience in the field throughout a typical year.

All barnacles were maintained in unfiltered, flowing seawater at the Friday Harbor Labs where water temperatures ranged from 11 to 14°C and salinity remained relatively constant at 30 psu. Barnacles were maintained under laboratory conditions for less than 2 weeks before use in experiments. Individual barnacles, with their calcareous basal plate intact, were gently removed from mussel shells with a razor blade and attached to an acrylic plate (10 cm x 3 cm) using ZSpar (A-788 Splash Zone Epoxy, Kop-Coat Inc., Pittsburgh, PA, USA). Each plate contained between 69-73 barnacles and a total of three replicate plates were used in each experimental treatment.

1.3.2. Measuring respiration rate

Experiments were conducted in a closed, recirculating flow chamber of 600 mL volume (Fig. 1.2). A clear acrylic test chamber (3 cm x 3 cm x 15 cm, H × W × L) was connected to a submersible pump (Models 25D/27D, Rule Industries, Gloucester, MA, USA) via low-gas-permeability Tygon tubing (19 mm ID). Water velocities along the centerline of the testing chamber were estimated by tracking the displacement of glass microbeads at each flow setting (mean particle diameter = 9 μm, density = 2.0 g cm^{-3}, Potters Industries, Malvern, PA, USA).

The entire flow chamber was submersed in a water bath that was temperature-regulated by a re-circulating water chiller (± 0.1°C; Ecoline RE 106, Lauda, Germany).

Oxygen concentrations were measured using a fiber-optic oxygen sensor equipped with a needle-like probe tip of 1.5 mm diameter (FOXY-R, Ocean Optics, Dunedin, FL, USA). The probe contained a complex constructed from a hydrophobic material called ruthenium. When blue light (475 nm) is directed through the probe, the ruthenium complex excites and fluoresces at a wavelength of 600 nm. In the presence of oxygen, the intensity of this emission decays predictably and the rate of this emission quenching is used to estimate oxygen concentration. It should be noted that the rate of Ru^{2+}-O_2 association is five times faster than disassociation, thus attempts to measure changes in O_2 concentrations greater than 166 nM O_2 s^{-1} do not allow for the establishment of proper binding equilibrium (Glazer et al., 2004). In our experiments, the rate of change in O_2 concentration was lower than this threshold and the results are, therefore, considered accurate. The probe was calibrated at each temperature with a two-point calibration at 0% (oxygen reduced with sodium dithionite) and 100% (oxygen saturated) seawater filtered to one micron. The probe was extremely sensitive to temperature fluctuations, necessitating tight control of temperature in the flow chamber (± 0.1°C). Samples were recorded at a rate of 0.5 Hz and drift of the probe was negligible (< 0.3% over 30 minutes at 20°C and 7.5 cm s^{-1}).

Barnacles were first acclimated in fully oxygenated water at testing temperature for 60 minutes before being acclimated for five minutes in the testing chamber. Barnacles were exposed fifteen times to the same order of nine randomized water velocities (12, 20, 2, 40, 0.7, 7.5, 30, 60 and 150 cm s^{-1}) with the first velocity (12 cm s^{-1}) being repeated at the end of the trials to ensure that the barnacles physiology had not changed over the course of the experiment ($t_{(14)}$ = 2.14, p = 0.33). Barnacles were also tested three times under a specific order of

temperature treatments (20, 10, 5, 15 and 25°C) with no two temperatures tested on the same day. No differences in respiration rates were found at the beginning and end of the experimental trials ($t_{(2)} = 4.30$, $p = 0.30$). A total of 45 trials were run until approximately 25% of the oxygen in the flow chamber was consumed (typically 30 minutes to 2 hours) and a stable rate of decline could be identified. Oxygen concentrations were standardized by dry barnacle body mass (g), where barnacle body (prosoma + cirri) was removed from the test with watchmaker forceps and dried at 60°C for 72 hours.

1.3.3. Analysis

Temperature coefficients (Q_{10}) describing the magnitude of change in respiration with increasing temperature were calculated,

$$Q_{10} = \left(\frac{R_2}{1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (1),$$

where R_1, R_2 are respiration rates (μmol O_2 g^{-1} h^{-1}) and T_1, T_2 are corresponding temperatures (°C).

Respiration rates were analyzed using a two-way repeated-measures ANOVA, with water temperature and velocity as repeated factors. When the assumption of sphericity, that covariances between each level of a repeated measures factor are equal, was not satisfied, a Huynh- Feldt correction was employed (Zar, 1999). Paired comparisons were made using the Holm–Sidak method. Analysis was conducted using MATLAB R2011a (Mathworks, Natick, MA, USA).

1.3.4. Sherwood –Reynolds number analysis

Two non-dimensional indices were calculated to examine how advection affects the mass flux of oxygen from the water column to barnacles. The first, called the Reynolds number (Re), is a ratio of inertial to viscous forces of a fluid:

$$Re = \frac{U\rho l}{\nu} \quad (2),$$

where U is water velocity (m s^{-1}), ρ is water density (kg m^{-3}), l is the characteristic dimension of the organism (barnacle diameter, m), and ν is the kinematic viscosity (m^2 s^{-1}).

The Sherwood number (Sh) represents the ratio of advective mass (oxygen) flux to diffusive mass flux (Campbell, 1977):

$$Sh = \frac{h_m l}{D} \quad (3),$$

where D is the diffusion coefficient for oxygen (m^2 s^{-1}) and h_m is the mass transfer coefficient (m s^{-1}), which was determined empirically from the ratio of the average mass flux of oxygen assisted by convection to the oxygen concentration difference between the chamber and the site of aerobic respiration and photosynthesis.

If barnacles are under kinetic limitation, mass transfer coefficients calculated from total oxygen consumption in the chamber under conditions may underestimate the potential for

advective mass transfer. For comparison, h_m can also be calculated as (Nishihara and Ackerman, 2006),

$$h_m = 0.1\, u_* \, Sc^{-0.67} \quad (4),$$

where Sc is the Schmidt number, a ratio that compares the kinematic viscosity of seawater ($m^2\ s^{-1}$) to the diffusivity of O_2 in seawater ($m^2\ s^{-1}$) and u_* ($m\ s^{-1}$) is shear velocity. Shear velocity was determined by multiplying the von Karman's constant ($\kappa = 0.4$) by the slope obtained from the logarithmic region of the boundary layer estimated from water velocities measured through particle tracking (Nishizaki and Ackerman, 2005). For each water velocity, u_* was calculated at 15°C and used for all temperatures in the model.

Plots of Sh (ordinate) versus Re (abscissa) were used to describe how water motion affects mass transfer (Patterson and Sebens, 1989),

$$Sh = aRe^b \quad (5),$$

where *a* is an empirical coefficient that depends on barnacle shape and *b* is the flow-dependent exponent (Patterson and Sebens, 1989). Slopes from least squared regression analysis of the Sh-Re relationship were used to estimate the flow exponent of equation 5.

1.3.5. Determining mass transfer vs. kinetic limitation.

A comparison of the relative importance of mass transfer versus reaction kinetics in limiting respiration rates was conducted using the non-dimensional approach described by Sanford and Crawford (2000). A short description of the method is provided below.

To begin, mass transfer flux (F) of oxygen can be expressed as,

$$F = \beta(C_\infty - C_0) \quad (5),$$

where β is the mass transfer velocity (m s^{-1}), C_∞ is the bulk fluid concentration (nmol O_2 ml^{-1}) at a distance from the boundary and C_0 is the concentration at the boundary (nmol O_2). C_∞ was measured from the free stream portion of the flow chamber. β can be estimated as,

$$\beta = 0.1\, Sc^{-2/3}\, u_* \quad (6),$$

Reaction kinetics, or the uptake rate at a solid-liquid boundary can be described by Monod kinetics, with uptake rates saturating at high concentrations:

$$R = \frac{V_m C_0}{K_m + C_0} \quad (7),$$

where R is the reaction rate (μmol O_2 m^{-2} h^{-1}), V_m is the maximum uptake rate (nmol O_2 m^{-2} h^{-1}) and K_m is oxygen concentration at which the uptake rate is one half of its maximum (nmol O_2).

To estimate the kinetic parameters V_m and K_m, oxygen uptake rates were fitted to the Michaelis-Menten model using nonlinear regression analysis (Kemmer and Keller, 2010).

Solving for steady-state uptake rate can then be achieved by equating equations 5 and 7,

$$R = \frac{V_m C_0}{K_m + C_0} = \beta (C_\infty - C_0) \quad (8),$$

A non-dimensional solution (see Sanford and Crawford 2000 for further details) is derived by dividing equation 8 by $V_m C_\infty / K_m$,

$$\frac{R K_m}{V_m C_\infty} = \left[\frac{C_\infty}{K_m} + \frac{1}{2} \left(\gamma + \sqrt{\gamma^2 + 4 \frac{C_\infty}{K_m}} \right) \right]^{-1} \quad (9),$$

where

$$\gamma = 1 + \left(\frac{\beta K_m}{V_m} \right)^{-1} - \frac{C_\infty}{K_m}$$

From this relationship, one can calculate the non-dimensional uptake rate ($RK_m/V_m C_\infty$), the non-dimensional mass transfer coefficient ($\beta K_m/V_m$) and the non-dimensional oxygen concentration coefficient (C_∞/K_m). A non-dimensional plot of mass transfer rate versus the oxygen concentration is presented with thresholds delineating whether barnacles in the different treatments are under mass transfer versus kinetic limitation (see results). Following the methods

of Sanford and Crawford (2000), thresholds are defined as 25% deviations from the full solution for uptake rates. For instance, conditions under mass transfer limitation were defined as,

$$\frac{\beta K_m}{V_m} < \frac{0.25}{1+\frac{C_\infty}{K_m}} \quad (10),$$

whereas conditions where reaction kinetics were limiting were defined according to Sanford and Crawford (2000) as,

$$\frac{\beta K_m}{V_m} > \frac{4 + 0.8\frac{C_\infty}{K_m}}{(1 + \frac{C_\infty}{K_m})^2} \quad (11),$$

These thresholds for kinetic and mass transfer limitation were used to delineate mass transfer limitation, kinetic limitation and intermediate regions on plots of $\beta K_m/V_m$ versus C_∞/K_m.

1.3.6. Cirral beating b*ehavior*

During the respiration experiment, cirral beating behavior of barnacles was recorded directly to a PC using a 3-CCD digital video camera (Model PV-GS150, Panasonic of North America, Secaucus, NJ, USA). The digital video was used to assess cirral motion using an open-source processing software package (Avidemux 2.5.4). Preliminary tests indicated that a capture rate of 15Hz was sufficient to measure all forms of cirral behavior. Ten barnacles were randomly selected for each of three replicate trials at each of the temperature × velocity treatments (N = 45, based on 1350 barnacles). For each barnacle, cirral behaviors were classified and the proportion

of time barnacles spent engaged in each behavior was calculated. Behaviors were classified using the criteria described by Anderson and Southward (1987) and subsequently assigned to one of two categories: 1) extended behaviors, which included *normal beating, slow beating, fast beating and cirral extension* and; 2) abbreviated behaviors which included *testing, pumping and gaping* (Anderson and Southward, 1987). Cirral beating behaviors were assessed from ten minute video clips coinciding with respiration trials.

Two-way RMANOVAs with Holm-Sidak method for individual comparisons were used to assess differences in the frequency of beating activity under different temperatures and water velocities. Frequencies were assessed for: 1) abbreviated beating, 2) extended beating and 3) total beating (abbreviated + extended beating). When the assumption of sphericity was not satisfied, a Huynh-Feldt correction was employed (Zar, 1999). Analysis was conducted using MATLAB R2011a (Mathworks, Natick, MA, USA).

1.4. Results

1.4.1. Field conditions

A total of 38,759 water temperature measurements were recorded at Argyle Creek between June 2011 and August 2012. Temperatures were not recorded from August 9, 2011 to August 17, 2011 when probes were collected and redeployed. Temperatures varied between 2.5°C and 26.7°C (Fig. 1.1).

1.4.2. Respiration rates

Mean respiration rates ranged twenty-fold in response to changing water temperature and flow conditions (2.8 to 60.4 nmol O_2 g^{-1} s^{-1}; Fig. 1.3). Respiration rates rose more rapidly from

5 to 15°C than 15 to 25°C (Q_{10} averaged over all flows (+/- SEM) = 2.90 ± 0.30 and, 1.57 ± 0.10, respectively).

Respiration rates displayed a curvilinear response to increased water velocities, saturating at velocities above 7.5 to 12 cm s^{-1} (Fig. 1.3). Mean respiration rates were typically low at slow velocities (e.g., 8.6 nmol O_2 g^{-1} s^{-1} ± 2.9 at 0.7 cm s^{-1}), rose rapidly as flows increased to 7.5 cm s^{-1} (33.8 ± 7.1 nmol O_2 g^{-1} s^{-1}) , and remained stable through 150 cm s^{-1} (38.0 ± 7.2 nmol O_2 g^{-1} s^{-1}).

There was a significant interaction between the two main effects, water temperature and water velocity ($F_{(32,64)}$ = 3.756, $p < 0.05$). At low temperatures (5 to 10°C), flow had little influence on respiration rate, whereas at high temperatures (20 to 25°C), flow had a much larger effect (Fig. 1.3).

1.4.3. Sherwood number - Reynolds number analysis.

For Sherwood numbers derived from total oxygen consumption rates, Sh-Re plots indicate that oxygen uptake was flow-dependent at slow water velocities (< 7.5 cm s^{-1}; Fig. 1.4) as evidenced by high Re exponents (0.73 ± 0.13). In contrast, lower Re exponents at higher velocities (0.18 ± 0.05 for > 7.5 cm s^{-1}), indicated that uptake was flow-independent. Alternatively, Sh derived from fluid transport processes displayed higher Re exponents at both high and low flows (1.01 ± 0.04 for velocities < 7.5 cm s^{-1} and 0.98 ± 0.03 for velocities > 7.5 cm s^{-1}). For both methods of calculation, Sh had similar Re exponents across the five temperatures (0.49 ± 0.01 from oxygen consumption and 0.99 ± 0.01 from fluid motion).

1.4.4. Mass transfer vs. kinetic limitation

The non-dimensional plot based on Sanford and Crawford (2000) demonstrates that barnacle respiration rate can be mass transfer and/or, kinetically limited, depending on the temperature-flow conditions they experience (Fig. 1.5). Barnacles in low flow conditions (0.7 cm s^{-1}) were under mass-transfer limitation at all temperatures above 5°C. Barnacles at slightly higher flows, between 1 and 7.5 cm s^{-1}, were generally under mass transfer limitation at warmer temperatures but entered kinetic limitation under cooler temperatures. Barnacles at 12 to 40 cm s^{-1} were in the intermediate region, where both mass transfer and kinetic limitation occurs, at warm temperatures and kinetic limitation at high temperatures. Barnacles at or above 60 cm s^{-1} were generally limited by reaction kinetics.

1.4.5. Cirral beating behavior

At low temperature (5°C), the frequency of both abbreviated and extended beating behaviors were limited (< 23% and 26% respectively; Fig. 1.6). At intermediate temperatures (10 to 20°C), barnacles generally displayed extended beating at low flows and abbreviated beating at high flows. These abbreviated beats primarily consisted of *gaping* or *testing beats*. At the highest temperature (25°C), barnacles displayed elevated levels of abbreviated beating when water velocities were low (< 2 cm s^{-1}). These abbreviated beats consisted mainly of *pumping* behavior.

The results of the two-way repeated measures ANOVA indicated that temperature was a significant predictor of total beating activity ($F_{(4,8)} = 4.886$, $p < 0.05$), whereas water velocity was not ($F_{(8,16)} = 1.295$ $p > 0.05$). Water velocity was a significant factor influencing both extended ($F_{(8,16)} = 6.018$ $p < 0.05$) and abbreviated beating ($F_{(8,16)} = 2.616$ $p < 0.05$). In contrast,

water temperature had little effect on either extended ($F_{(4,8)}$ = 0.818, p > 0.05) or abbreviated ($F_{(4,8)}$ = 3.057, p > 0.05) beating.

1.5. Discussion

1.5.1. Metabolic response to temperature and flow

Barnacle respiration rates varied between 3 and 60 μmol g^{-1} h^{-1} and showed a positive relationship with temperature regardless of flow condition. Respiration increased from low to moderate temperatures (5 to 20°C), and saturated at high temperatures (20 to 25°C). Although respiration rates generally increased with increased temperature, this temperature dependency was stronger at low temperatures (Q_{10} = 2.90 ± 0.30 for 5 to 15°C) than at high temperatures (Q_{10} = 1.57 ± 0.10 for 15 to 25°C), suggesting a peak at or above 20 to 25°C.

The rates reported here are consistent with previous measures of barnacle respiration (14 μmol g^{-1} h^{-1}, (Barnes and Barnes, 1969); 4 μmol g^{-1} h^{-1}; 67 μmol g^{-1} h^{-1}, Wu and Levings 1978). Similarly, our temperature coefficients approximate those measured in mussels (Q_{10} = 1.4 to 2.1; Widdows 1973), urchins (Q_{10} = 1.7 to 3.0; Siikavuopio et al. 2008), hermit crabs (Q_{10} = 1.4 to 1.6; Burggren and McMahon 1981), and shore crabs (Q_{10} = 2.2 to 2.4; Greenaway et al. 1996).

Our respiratory response curves also displayed a pattern of hyperbolic saturation as small increases in water velocity at the lowest flows resulted in large increases in respiration rate, whereas further increases at high velocities had little effect on respiration (Fig. 1.3). Our flow-mediated response was similar to the respiratory response of corals (Finelli et al., 2005; Patterson and Sebens, 1989; Patterson et al., 1991), nutrient uptake in seagrasses (Cornelisen and Thomas, 2004) and photosynthetic response in aquatic plants (Nishihara and Ackerman, 2006; Stewart and Carpenter, 2003).

Our non-dimensional analyses indicate that respiration rates are generally mass transfer limited under low velocity-high temperature conditions and kinetically limited at high velocity-low temperature conditions (Fig. 1.5). Lower water temperatures may lead to kinetic limitation due to higher levels of dissolved O_2. One of the few previous reports of kinetic limitation comes from measurements of photosynthesis in the freshwater plant, *Vallisneria americana* (Nishihara and Ackerman, 2009). The thermal maximum for respiration in *V. americana* from the Great Lakes region is 32.6°C (Titus and Adams, 1979), which is higher than the temperature (24°C) tested by Nishihara and Ackerman (2009). This is consistent with our conclusion that kinetic limitation is likely to be limited to the lower temperature range of a species. A second example of kinetic limitation has been reported for coral respiration (Edmunds, 2005). Again, respiration rates were independent of flow (kinetic limitation) at low temperatures, but were dependent on flow (mass transfer limited) at high temperatures (Edmunds, 2005).

Given the dependence of respiration rates on temperature and flow, it is reasonable to ask how frequently barnacles experience mass transfer vs. kinetic limitation in the field. At wave-sheltered sites, water velocities are typically in the mass transfer limited region (0.96 ± 0.1 cm s^{-1}; measured 5 cm above the substrate; Marchinko 2003), whereas at more exposed barnacle sites (mean velocity = 98 cm s^{-1}; Miller 2007), respiration rates are most likely to be limited by reaction kinetics. However, these velocities may themselves be an overestimate of those experienced by barnacles, as water motion near boundaries where barnacles are found may be greatly reduced from free-stream velocities. For instance, velocities within a mussel aggregation may be as little as 0.1 to 9% of free stream (Carrington et al., 2008; O'Donnell, 2008). Similarly, water temperatures monitored at the Argyle Creek collection site suggest that barnacles potentially experience mass transfer limited conditions (e.g., water temperatures above

20°C), for 5.2% of the year (Fig. 1.1). Furthermore, if one considers a 1.8 to 4.0°C rise in temperature as is predicted over the next century (Solomon, 2007), the time that barnacles experience mass transfer limited conditions rises to between 9.6 and 21.4%. Clearly, conditions of mass transfer limitation exist for many barnacles under field conditions.

1.5.2. Cirral behavior

Temperature influenced the proportion of barnacles displaying extended beating (i.e., thermal optima at 20°C; Fig. 1.6), consistent with many related species of barnacle (see Anderson and Southward 1987 for review). Under low flows (< 20 cm s^{-1}), barnacles typically displayed extended beating behaviors. Marchinko (2007) observed extended cirral activity from wave exposed barnacles up to the maximum tested velocity (49 cm s^{-1}), whereas wave-sheltered barnacles ceased feeding when water velocities reached between 7.5 and 21.4 cm s^{-1}. We had similar results for our wave-sheltered barnacles; animals in high water velocities (> 40 cm s^{-1}) switched to "testing" behavior. This is likely due to the mechanical deformation of cirri experience under high velocities (≥ 21.4 cm s^{-1}) and a subsequent switch to abbreviated lower-drag behaviors (≥ 33 cm s^{-1} reported in Marchinko 2007). Increased "pumping" behavior under low flow-high temperature conditions is similar to observations made by Anderson and Southward (1987) who describe a "respiratory pumping beat". In corals, a similar, but slower behavior of tentacle extension has been interpreted as a strategy to increase the diffusive surface available for O_2 exchange (Kuhl et al., 1995; Shashar et al., 1993). For barnacles, rapid cirral beating may increase oxygen uptake through both passive (i.e., increasing surface area) and active (i.e., disturbing boundary layers) means.

Our results underscore the need to consider multiple environmental factors when assessing physiological performance. The degree to which barnacle respiration is under mass transfer versus kinetic limitation depends on both water temperature and velocity. For example, studies conducted under low flows might only observe mass transfer limitation, whereas experiments run only at cool temperatures might only see kinetic limitation. As our results demonstrate, only a comprehensive survey of the temperature-flow landscape may reveal patterns of mass transfer and kinetic limitation.

The advantages of employing factorial experiments become even more pronounced when one considers the impacts of rising ocean temperatures (Levitus et al., 2000). Our results suggest that we might expect different physiological responses to elevated temperatures on wave sheltered versus wave exposed shores. For instance, in areas with slow moving waters, barnacle physiology may become increasingly mass transfer limited as water temperatures rise. In contrast, at wave exposed sites, faster water velocities may ameliorate the effects of rising temperatures on mass transfer limitation. Our results are consistent with the hypothesis that oxygen limitation may restrict the ecological distribution of marine organisms by lowering thermal tolerance (Pörtner and Knust, 2007). Moreover, our results demonstrate the limitation of inferences drawn from single-factor designs, and strongly advocate for approaches that consider interactions among multiple factors.

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Table 1.1. Respiration rates of benthic marine invertebrates.

Organism	Temperature (°C)	Velocity (cm s^{-1})	Source
Sponges	18	---	Hoffmann et al. 2008
Sponges	22.5 ± 1	---	Hadas et al. 2008
Corals	15 – 17	7 – 16	Patterson & Sebens 1989
Corals	---	2 – 7	Patterson et al. 1991
Corals	28.5 ± 1.5	2 – 10	Sebens et al. 2003
Corals	---	0.1 – 15	Finelli et al. 2005
Corals	29.5	3 – 8	Bruno & Edmunds 1998
Coral recruits	27 – 30	0.6	Edmunds 2005
Sea anemone	5 – 25	---	Navarro et al. 1981
Mussels	10 – 27	---	Jansen et al. 2009
Mussels	8 – 10	---	Thomsen & Melzner 2010
Mussels	4 – 30	---	Bruce 1926
Mussels	18 – 28	---	Anestis et al. 2010
Oysters	5 – 32	---	Bougrier et al. 1995
Oysters	10 – 18	---	Lejart et al. 2012
Snails	1 – 38	---	Newell 1973
Littorines	5 – 40	---	Mcmahon & Russell-Hunter 1977
Limpets	10 – 30	---	Bannister 1974
Limpets	10 – 17	---	Martin et al. 2006
Nudibranch egg masses	(-1) – 12	---	Moran and Woods, 2010
Sea urchins	6 – 24	---	Ulbricht & Pritchard 1972
Brittle stars	19 – 25	---	Christensen et al. 2011
Barnacles	15	---	Prasada Rao & Ganapati 1968
Barnacles	5 - 20	---	Barnes & Barnes 1969
Barnacles	5 - 20	---	Wu & Levings 1978
Barnacles	5 - 25	0.7 – 150	This study

Figure legends

Figure 1.1. Seasonal water temperatures from Argyle Creek, Washington, USA.

Figure 1.2. Schematic diagram of recirculating flow chamber used to measure respiration rate. Flow rates were controlled by a submersible pump (Rule Model 25D/27D) and temperatures were set using a water chiller (± 0.1°C; Ecoline RE 106, Lauda, Germany). Dark arrows indicate direction of water flow through the system. Oxygen concentrations were measured via an optical probe inserted into the chamber and recorded to a laptop (PC1). Drift in control runs without barnacles was less than 0.6% h^{-1}. Barnacle behavior was recorded directly to a computer (PC2) in digital video format. Dimensions are not to scale.

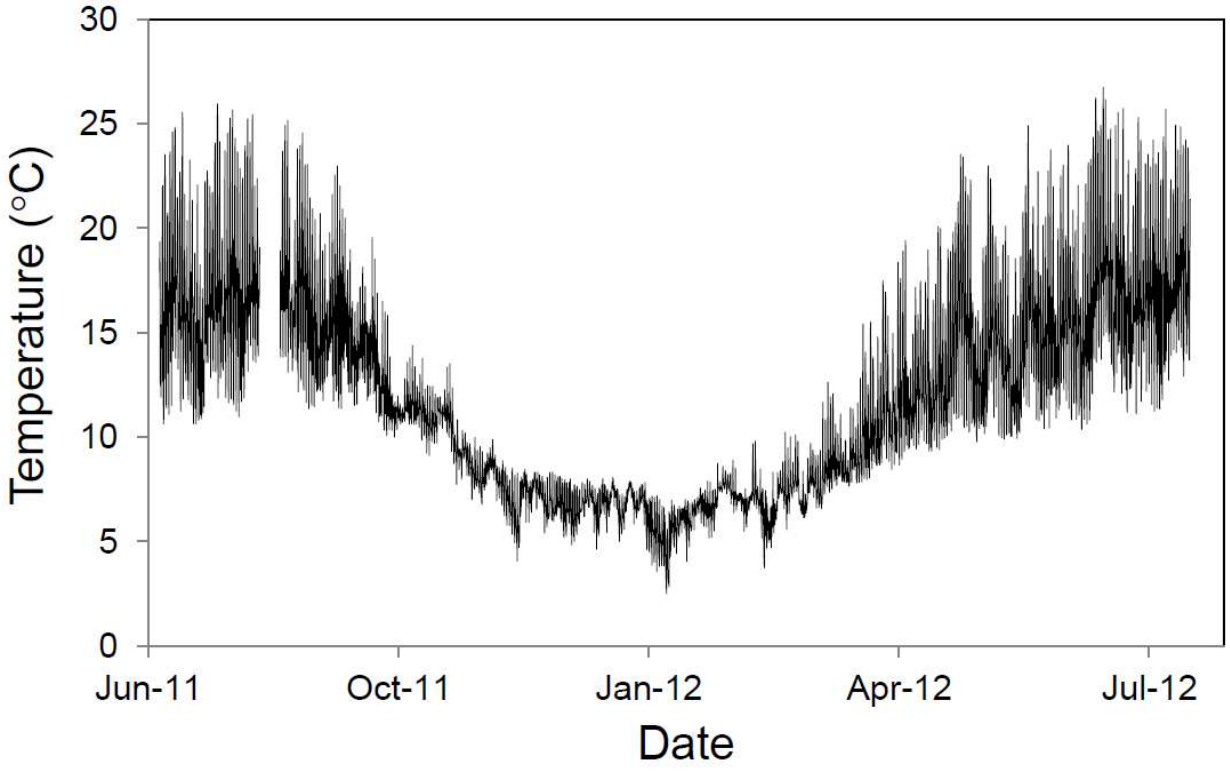
Figure 1.3. Response surface of respiration rate as a function of temperature and water velocity for *Balanus glandula*. Error bars represent one standard error, N = 3 plates.

Figure 1.4. Non-dimensional plot of Sherwood number as a function of Reynolds number. Values based on total oxygen consumption rates are represented by diamonds and are fitted by the dotted line in the form $Sh = 33.82Re^{0.26}$. Circles represent Sherwood numbers based on advective/diffusive transport and are fitted by the solid line, $Sh = 0.93Re^{0.99}$.

Figure 1.5. Non-dimensional mass transfer coefficient ($\beta K_m/V_m$) plotted against non-dimensional oxygen saturation (C_b/K_m). Each circle is calculated from averages of 3 trials of the respiration experiment. Colored circles indicate different temperature treatments and each line represents trials conducted under the same water velocity, as indicated on the graph. Solid line represents the upper limit for conditions of mass transfer limitation (pink region) and the hatched line represents the lower limit for conditions of kinetic limitation (green region). The white region represents intermediate conditions where both mass transfer limitation and kinetic limitation are important and the black region is undefined.

Figure 1.6. Cirral behavior of barnacles under varying water temperatures and velocities. Behaviors, as described by Anderson and Southward (1987) include "*extended beating*" with cirri fully extended (i.e., *normal beating, slow beating, fast beating,* and *cirral extension*) and a category of abbreviated beating (*testing*, *pumping* and *gaping*). The pink region represents conditions of mass transfer limitation, the green region represents kinetic limitation and the white

region indicates intermediate conditions, as described in Fig 5. Error bars represent one standard error. N=3 plates.


30
25
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Temperature (°C)
Jun-11
Oct-11
Jan-12
Apr-12
Jul-12
Date

Figure 1.1

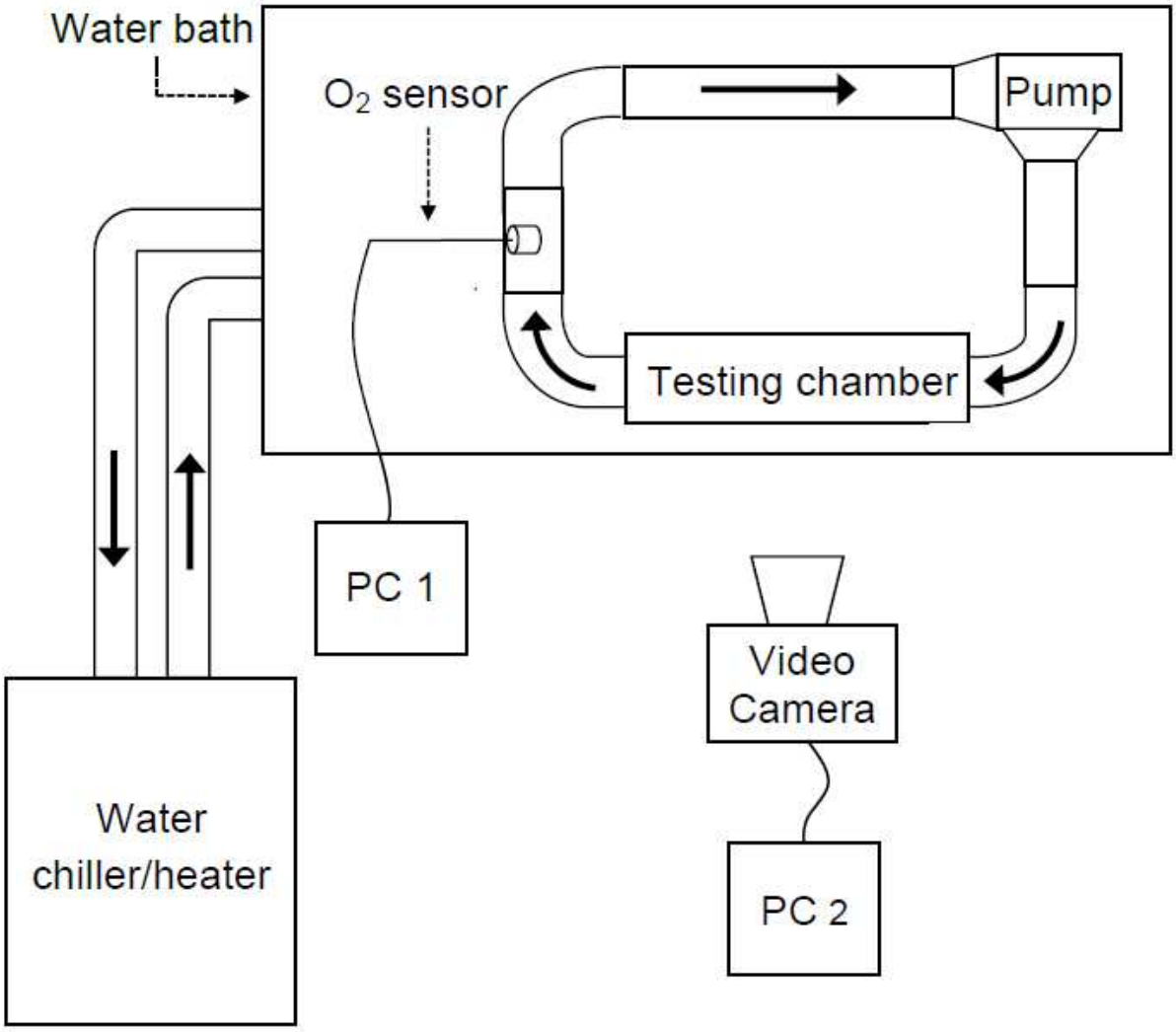
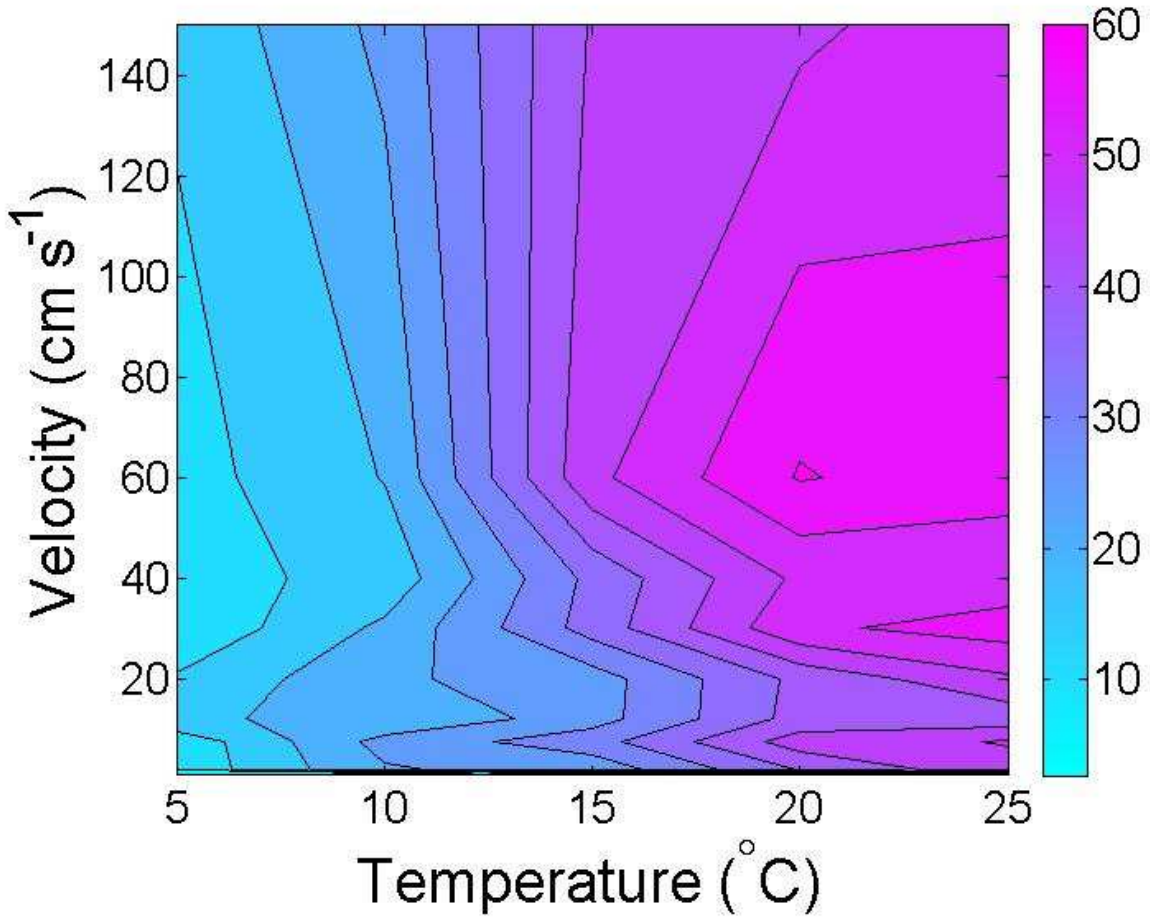

Water bath
O_2 sensor
Pump
Testing chamber
PC 1
Video Camera
PC 2
Water chiller/heater

Figure 1.2


Respiration rate (μmol g-1 h-1)
Velocity (cm s-1)
140
120
100
80
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40
20
5
10
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20
25
Temperature (°C)
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40
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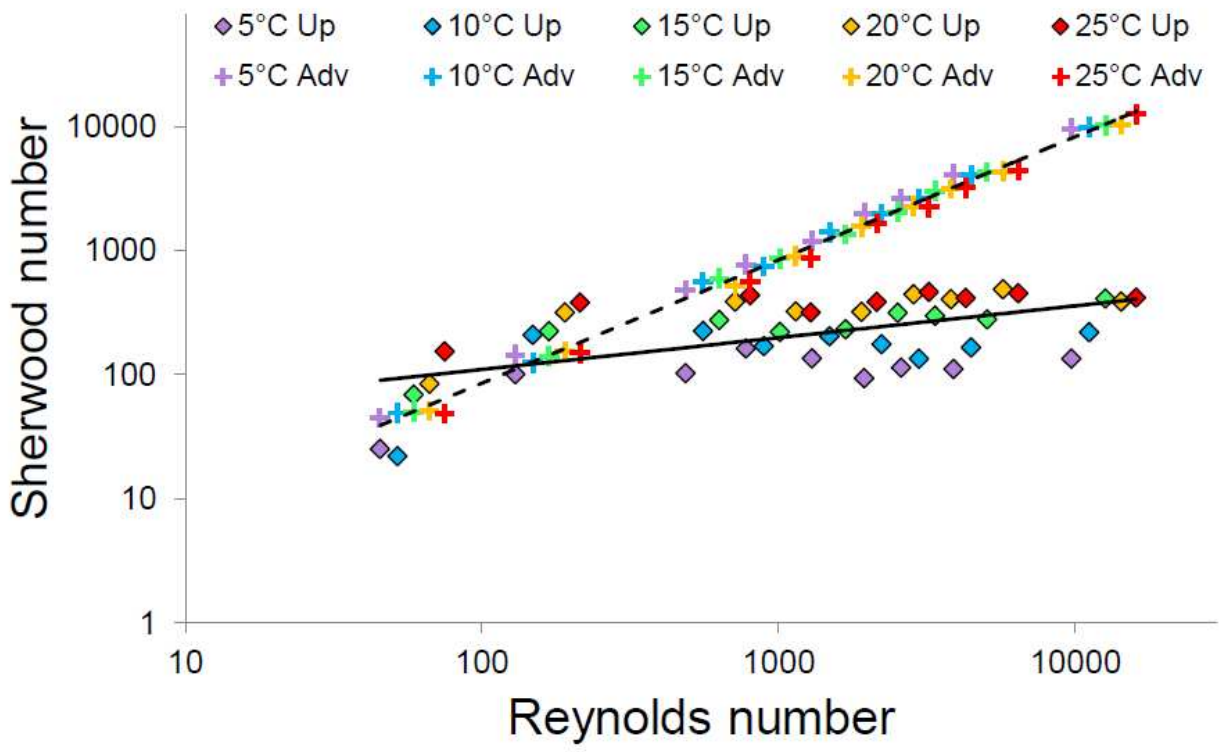
20
10

Figure 1.3


5°C Up
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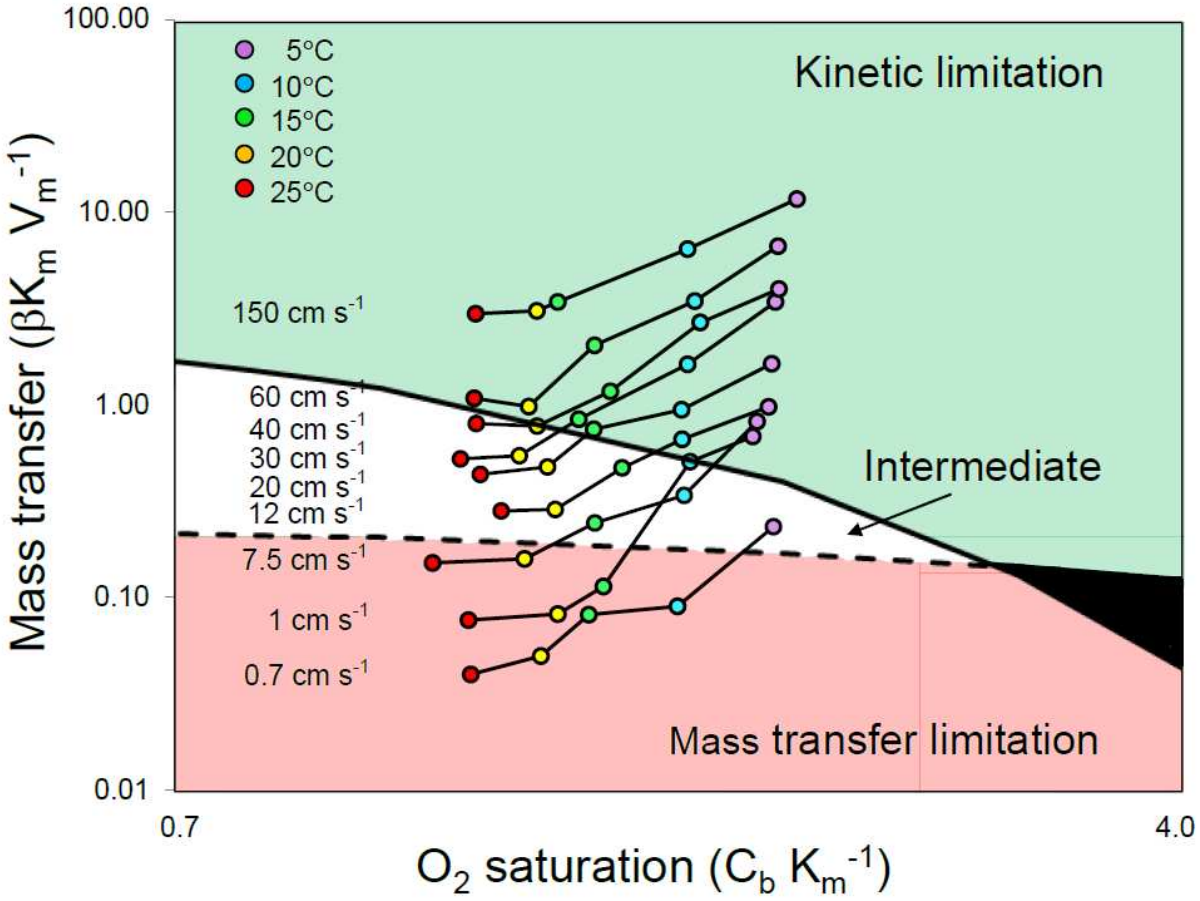
Sherwood number
10000
1000
100
10
1
10
100
1000
10000
Reynolds number

Figure 1.4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4.0
Mass transfer (βK$_m$ V$_m$$^{-1}$)
O$_2$ saturation (C$_b$ K$_m$$^{-1}$)
5°C
10°C
15°C
20°C
25°C
Kinetic limitation
Intermediate
Mass transfer limitation
150 cm s^{-1}
60 cm s^{-1}
40 cm s^{-1}
30 cm s^{-1}
20 cm s^{-1}
12 cm s^{-1}
7.5 cm s^{-1}
1 cm s^{-1}
0.7 cm s^{-1}

Figure 1.5

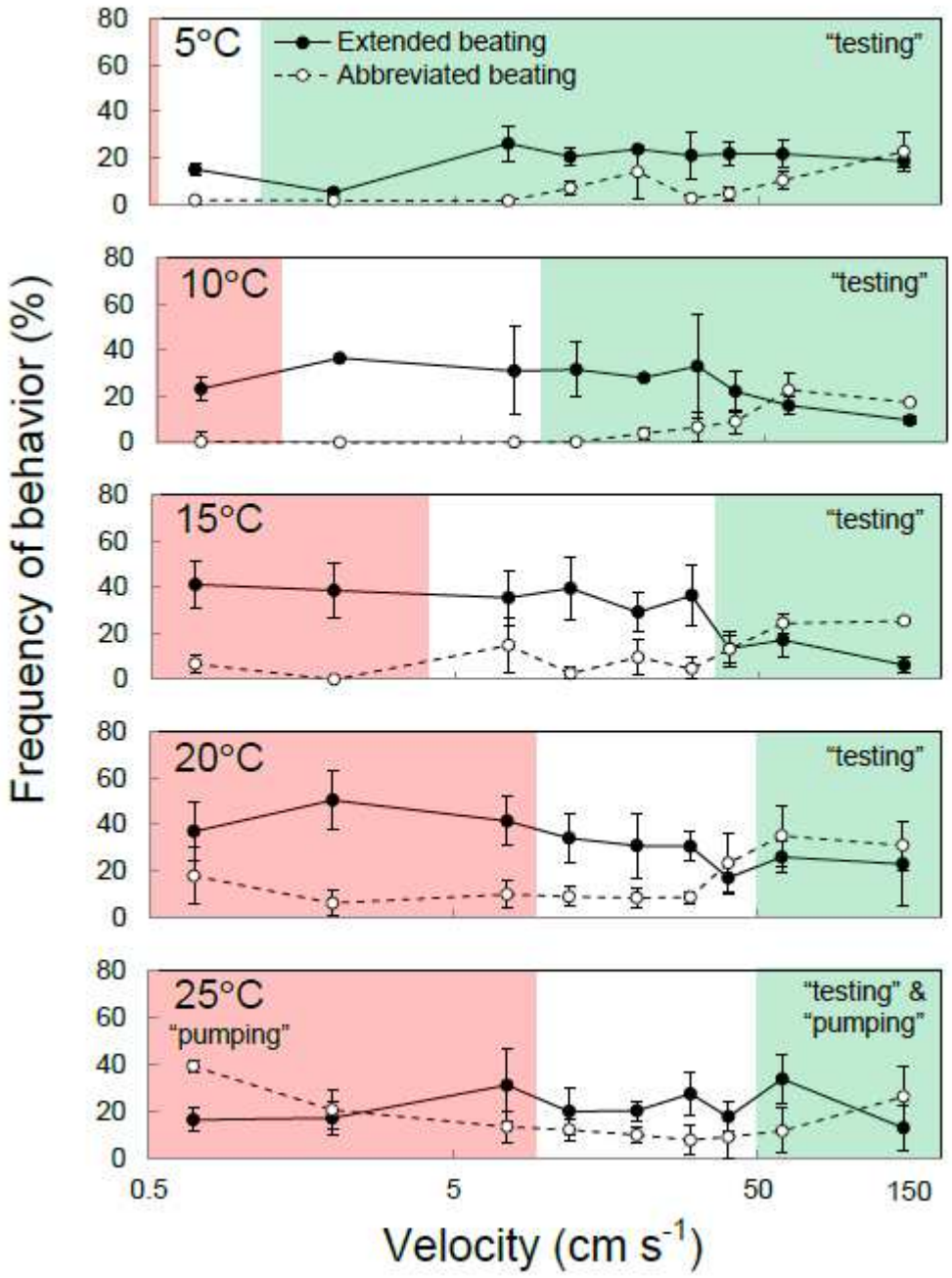
5°C
Extended beating
Abbreviated beating
"testing"
10°C
"testing"
15°C
"testing"
20°C
"testing"
25°C
"pumping"
"testing" &
"pumping"
Frequency of behavior (%)
0
20
40
60
80
0.5
5
50
150
Velocity (cm s-1)

Figure 1.6

Chapter II

THE EFFECTS OF FLOW AND TEMPERATURE ON BARNACLE SUSPENSION FEEDING

2.1. Abstract

Suspension feeding, which entails the capture of food particles from the water column, is a common energy acquisition strategy for many marine organisms. Acquisition rates depend on both the flux of particles past an organism and the efficiency with which organisms retain those particles. As such, the success of many suspension feeders is inherently influenced by conditions in the fluid environment. We investigate barnacle feeding under a range of water velocities and temperatures using gut dissections to directly quantify capture rates of food particles.

Overall, the proportion of barnacles observed beating was typically high (68 ± 3%), yet gut dissections confirmed that a far lower proportion had actually ingested food particles (hydrated *Artemia* cysts; 22 ± 3%). This discrepancy suggests that cirral activity may serve other functions and simple behavioral descriptions provide a poor proxy for barnacle feeding rate.

Both the delivery of cysts to the cirral net and cyst capture rates peaked at intermediate water velocities (7.5 to 20 cm s^{-1}). This is likely due to behavioral changes in cirral beating as barnacles employed short, abbreviated beating strokes at higher velocities. Capture efficiency, or the ratio of cysts captured to cysts encountered, was highest under the slowest flow (1 cm s^{-1}). Model analysis of these observations demonstrated that detailed characterization of cirral beating behavior are required to accurately predict these patterns of flow-dependent cyst capture.

Barnacles also displayed a clear thermal optimum for capture rate (15°C). At high temperatures, barnacles employed abbreviated, ventilating beating behaviors that likely reduce both capture rates and efficiencies, whereas at low temperatures, slow beating rates may reduce

particle capture rate and efficiency. Again, only when beating behavior was incorporated into model analyses were patterns of temperature-dependent cyst capture accurately predicted. This suggests that the nature of thermal limitation is not biophysical, but likely physiological or behavioral in nature.

2.2. 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 and Stayton, 1982) and birds (Vermeer, 1982). These ecosystem engineers alter environmental conditions on shore 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 communities through their procurement of small food particles, called seston, from surrounding waters. 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 and Kristmanson, 2005) and *capture efficiency*, the ratio of particles captured to those encountered by feeding structures (Rubenstein and Koehl, 1977). Both measures of feeding are likely influenced by two potentially important environmental factors, water motion and temperature (Jørgensen, 1983; Labarbera, 1984; Wildish and Kristmanson, 2005).

Water motion can enhance capture rate by increasing the flux of particles past feeding structures (Ackerman and Nishizaki, 2004; Helmuth and Sebens, 1993; Sanford et al., 1994). However, the proportion of water that passes through a feeding structure, or "leakiness" also increases with increasing flow (Geierman and Emlet, 2009; Loudon and Alstad, 1990), 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, 2007b; Miller, 2007).

Water temperature can affect feeding rate through changes in physiology and through changes in fluid dynamic conditions. Barnacles deploy their feeding appendages, called 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 Anderson and Southward 1987, review). From a physical perspective, temperature may also affect suspension feeding as a consequence of changing water density and viscosity (Podolsky, 1994).

Sieving has been assumed to be the primary particle capture mechanism for suspension feeders, with particles larger than the space between filter elements unable to pass (Riisgrd and Larsen 2010, review). However, Jørgensen (1966) notes that limited pressure differentials across these filters suggests sieving may not be the dominant capture mechanism. An alternate approach based on aerosol-filtration theory (Rubenstein and Koehl 1977) provides four non-dimensional indices that describe capture efficiencies related to each of four 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 and Jumars 1991, but see Labarbera 1978). Together, the four models allow for predictions about the effect of water motion particle capture. Shimeta and Jumars (1991) extended this approach by introducing four similar indices describing particle encounter rate, rather than efficiency, for each of the four 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 (Sanford et al., 1994; Southward, 1955b).

Although beating rate has been related to both temperature (Southward, 1957) and flow (Marchinko, 2007b; Miller, 2007), surprisingly little data exists measuring actual capture rates of suspended food particles (but see Trager et al. 1994; Bertness et al., 1998). Here, we present results from experiments directly measuring feeding rates of *B. glandula* under a range of water temperatures and velocities.

In this study, we: 1) conduct feeding experiments to explore the influence of water temperature and velocity on particle capture rate and particle capture efficiency; 2) make detailed measurements of cirral activity under different water temperatures and velocities to explore the interactions between environment, feeding and behavior and; 3) employ models to predict patterns of particle capture rate and particle capture efficiency under a range of water velocities and temperatures.

2.3. Materials & methods

2.3.1. Organism collection

Barnacles (*Balanus glandula*) attached to mussel shells (*Mytilus trossulus*) were collected from Argyle Creek (N 48° 31.728' W 123° 00.802') 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 in a recirculating seawater system for one week before use in experiments (8°C). Shell height and basal diameter of each barnacle were measured with Mitutoyo 500-196-20 digital calipers (Mitutoyo America Corporation, Aurora, IL; ± 0.01 mm). Cirri were dissected, photographed under a dissecting microscope with a CoolPix 995 digital camera (Nikon Inc., Tokyo, Japan) 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, Bethesda, MD; ± 0.001 mm).

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

$$Re = \frac{\rho l U}{\mu} \quad (1),$$

where ρ is the density of seawater (kg m^{-3}), l is the diameter of the sixth cirrus (m), U is water velocity (m s^{-1}), and μ is the dynamic viscosity of seawater (kg m^{-1} s^{-1}).

Before each trial, barnacles were attached to an acrylic plate (10 cm × 3 cm) using modeling clay. Ten barnacles were placed on each side of the centerline of the plate, forming two columns that extended along the primary axis of water flow (twenty barnacles total). An additional row of barnacles 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).

2.3.2. Measuring feeding rates

Feeding trials were conducted in a closed, recirculating flow chamber of 600 mL volume as described by Nishizaki and Carrington (*in review*). Barnacles were fed hydrated *Artemia* cysts (hydrated diameter = 228.1 ± 4.1 μm, 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 μm filtered seawater for 30 min and then fractionated to isolate near neutrally buoyant (slight negative buoyancy) cysts. Cysts were used as we were unable to

quantify plankton prey and found no published identification of *Balanus 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 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 account for 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 for 45 min to experimental conditions. This test duration was based on preliminary observations to provide sufficient time for barnacles to commence feeding, while avoiding feeding saturation (data not shown). Each 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 cm s^{-1}). Water temperatures varied by less than 0.1°C during the course of each trial. After removal from the flow chamber, barnacles 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 minute, per barnacle. Capture efficiency (%) was calculated as cyst capture rate (cysts s^{-1}) divided by the encounter rate of cysts to the cirral net (cysts s^{-1}).

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

Encounter rate was calculated as the delivery of cysts passing through the area projected by the cirral net,

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

where C_{cy} is the concentration of cysts in the water column (cysts m^{-3}), U is free-stream water velocity (m s^{-1}) and $A_{cirral\ net}$ is the projected area of the cirral net (m^2). The projected area of the cirral net was calculated assuming a triangular shape ($^1/_2$bh), where height (h) is measured as cirral length (m) and base (b) is test diameter (m). Video analysis was used to measure cirral height during different cirral beating behaviors (N = 5 barnacles per behavior), which varied with flow and temperature. Cirral behaviors were then categorized (see descriptions in *Cirral behavior* section below) every two seconds for ten minutes (N = 10 barnacles per trial) 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). 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.

2.3.3. Cirral behavior

Cirral beating behavior was recorded during each feeding trial using a PowerShot SX20 IS digital camera (Canon Inc., Tokyo, Japan). Ten minutes of cirral behavior was assessed for each trial using an open-source processing software package (Avidemux 2.6.0). Cirral behaviors were classified according to Nishizaki and Carrington (in review) 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 (i.e., pumping, testing, gaping) beating. 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. Ten minutes of feeding experiment video was analyzed to determine the proportion of barnacles actively beating their cirri. These numbers were compared to the number of barnacles with cysts found in their gut by dissection.

2.3.4. Analysis

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

Differences in the proportion of barnacles beating and proportion with cysts in their gut were tested for using 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 Inc, Natik, MA) and SPSS v.19 (IBM Corporation, Chicago, IL).

2.3.5. Predicted cyst capture

For each of the four particle capture mechanisms (Fig. 2.1), two 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 fiber in a moving fluid.

The indices were calculated across a range of velocities (1, 7.5, 12, 20, 30, 40 and 60 cm s^{-1}) and temperatures (5, 10, 15, 20 and 25°C), as described below.

Direct interception. As cysts in seawater move along streamlines, a proportion approach the distance within one cyst radius from a single cirrus (r_{cy} = 1.28 × 10^{-4} m). Capture rate (F_R) and capture efficiency (N_R) due to direct interception can be calculated as,

$$F_R = 2CUr_{cy}l_{ci} \quad (4),$$

$$N_R = \frac{d_{cy}}{d_{ci}} \quad (5),$$

where C is cyst concentration (cells ml^{-1}) U is water velocity (m s^{-1}), r_{cy} represents cyst radius (m), l_{ci} is exposed cirrus length (m), 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. When the momentum of a cyst causes it to deviate from the path of a streamline and is intercepted by the cirrus. Capture rate (F_I) and capture efficiency (N_I) can be calculated as,

$$F_I = 2CUr_{ci}l_{ci} \quad (6),$$

$$N_I = \frac{{d_{cy}}^2 U(\rho_{cy}-\rho_s)}{18\mu d_{ci}} \quad (7),$$

where l_{ci} is cirral length (m), ρ_{cy} is the density of fully hydrated cysts (1082 kg m^3 at 23-25°C and 15 PSU; Clegg 1984), ρ_s and μ represent temperature-dependent seawater density (kg m^3) and dynamic viscosity (Pa s) respectively and d_{ci} is cirral diameter (m).

Gravitational deposition. Negatively buoyant cysts sink and come into contact with the cirrus. Capture rate (F_G) and capture efficiency (N_G) can be calculated as,

$$F_G = \frac{4Cg(\rho_{cy}-\rho_s)(r_{cy}+r_{ci}){r_{cy}}^2 l_{ci}}{9\mu} \quad (8),$$

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

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

Diffusional deposition. When a cyst randomly deviates from streamlines due to Brownian motion, it becomes entrained on the cirrus. Capture rate due to diffusional deposition (F_D) and capture efficiency (N_D) can be calculated as,

$$F_D = \pi C D^{2/3} \left(\frac{r_{ci}U}{2-\ln Re_{ci}}\right)^{1/3} l_{ci} \quad (10),$$

$$N_D = \frac{KT}{3\pi\mu d_{cy} U_0 d_{ci}} \quad (11),$$

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

Capture rate indices were also calculated with a term that incorporated cirral beating behavior. The proportion of time that barnacles in each treatment spent engaged in extended beating behaviors (i.e., feeding; see description below) was standardized to the total time of each trial.

2.4. Results

Reynolds number during the feeding experiments ranged from 0.1 to 15.4 increasing with faster water velocities and warmer water temperatures (Fig. 2.2).

2.4.1.Measured rates

Both the projected area of the cirral net and the cyst encounter rate were highest at 20 cm s^{-1} and 15°C, and decreased at extreme velocities and temperatures (Fig. 2.3A, B). Similarly, capture rates were highest at 7.5 cm s^{-1} and 15°C (Fig. 2.3C). Cyst capture rates were significantly influenced by both temperature (Kruskal-Wallis test $H = 45.38$, $p < 0.05$) and velocity (Kruskal-Wallis test $H = 42.65$, $p < 0.05$). Pairwise comparison tests indicated that capture rates at 7.5 cm s^{-1} were significantly higher than 1, 30, 40 and 60 cm s^{-1}, while 12 and 20 cm s^{-1} were not significantly different. Pairwise comparison tests also indicated that capture rates at 15°C were significantly higher than 5, 20 and 25°C (Fig. 3C).

Capture efficiencies were significantly influenced by both temperature (Kruskal-Wallis test $H = 41.35$, $p < 0.05$) and velocity (Kruskal-Wallis test $H = 39.20$, $p < 0.05$), and peaked at 1 cm

s^{-1} and 15°C (Fig. 2.3D). Pairwise comparison tests indicate that capture efficiencies at 15°C were significantly higher than 5, 20 and 25°C. Capture efficiencies at 1 cm s^{-1} were significantly higher than all higher velocities (7.5, 12, 20, 30, 40 and 60 cm s^{-1}; Fig. 2.3D).

2.4.2. Cirral beating behavior

Barnacles displayed high levels of cirral beating activity, spending an average of 74 ± 3% of observed time actively beating their cirri (abbreviated and extended forms combined, Fig. 2.4). The total number of barnacles actively beating was significantly influenced by water velocity (Kruskal-Wallis test $H = 13.80$, $p < 0.05$) and there was a near significant relationship with temperature (Kruskal-Wallis test $H = 8.97$, $p = 0.06$). Extended beating was significantly influenced by both temperature and velocity (Kruskal-Wallis tests, $H = 12.54$, $p < 0.05$ and $H = 107.86$, $p < 0.05$ respectively). Pairwise comparison tests indicate that extended beating at 30, 40 and 60 cm s^{-1} was significantly lower than at slower flows (Fig. 2.4). The exception was between 20 and 30 cm s^{-1}, where no significant differences were detected. Pairwise comparison tests also indicate that extended beating at 15°C was significantly higher than at 25°C.

Abbreviated beating was significantly influenced by both temperature (Kruskal-Wallis test $H = 21.04$, $p < 0.05$) and velocity (Kruskal-Wallis test $H = 98.75$, $p < 0.05$). Pairwise comparison tests indicate that abbreviated beating at 30, 40 and 60 cm s^{-1} was significantly lower than the slower flows (Fig. 2.4). Pairwise comparison tests also indicate that abbreviated beating at 25°C was significantly higher than at 5, 10, 15 or 20°C.

Cirral beating rate increased with both water velocity and temperature (Fig. 2.5). Beating rates ranged from 0.05 to 1.00 beats s^{-1} and was significantly influenced by both water temperature and velocity (Kruskal-Wallis tests, $H = 29.96$, $p < 0.05$ and $H = 43.03$, $p < 0.05$

respectively). Post-hoc comparisons revealed beating rates were significantly higher at 40 to 60 cm s^{-1} compared to all lower velocities tested and significantly lower at 5°C compared with all other temperatures.

2.4.3. Cirral activity versus feeding rate

The proportion of barnacles beating their cirri during the experiment was significantly higher (68 ± 3%; Fig. 2.6A) than the proportion of barnacles with cysts in their gut (22 ± 3%; Z-test, $p < 0.05$). Whereas beating rates did not display a consistent pattern in regards to either temperature or flow, the proportion of fed barnacles peaked at moderate water velocities (7.5 to 12 cm s^{-1}) and temperatures (15 °C; Fig. 2.6B). When the proportion of barnacles fed were plotted against the proportion beating, all measured data fell below unity (Fig. 2.6C).

2.4.4. Model predictions

Of the four particle capture mechanisms, results for direct interception (F_R) are presented alone 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. 2.7A). 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 cm s^{-1} and 15°C (Fig. 2.7B), similar to observed values.

The capture efficiency model also suggests that direct interception (N_R) is between 10 to 1,000,000× larger than capture by inertial impaction, gravitational deposition or diffusional deposition. The model predicted uniform efficiencies across all treatments (Fig. 2.7C).

2.5. Discussion

Changes in water temperature and velocity elicited behavioral changes in cirral beating that affected cyst encounter rate, capture rate and capture efficiency (Figs. 2.3A-D).

2.5.1. Capture rates

In our experiments, water velocity influenced particle capture rates, with peak capture at 7.5 cm s^{-1} (Fig. 2.3C). At high flows, abbreviated beating became more common and capture rate decreased, suggesting that feeding may be constrained by mechanical limitation (Fig.2.4).

Capture rates also displayed a thermal optimum at 15°C (Fig. 2.3C). At high temperatures, barnacles employ abbreviated beating, presumably for ventilation that may contribute to reduced capture rates (Nishizaki and Carrington, in review). At low temperatures, capture rate may instead be limited by significantly lower beating rates (Fig. 2.5). Temperature had a moderate effect on the hydrodynamics of the system (i.e., water viscosity, density), and so changes in feeding rate are more likely due to changes in barnacle physiology and/or behavior with temperature (Fig. 2.3C).

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 and 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 versions of the model suggest that direct interception is the dominant capture mechanism under a wide range of thermal and flow conditions (Figs. 2.7A,B).

2.5.2. Capture efficiencies

Capture efficiencies were highest at low velocities and decreased rapidly as velocity increased (Fig. 2.3D). Lower efficiencies at high flows may be a consequence of the cirral net deforming (Marchinko, 2007b). As water velocities increase, it is also possible that the cirral net may become increasingly "leaky" (Geierman and Emlet, 2009), which would decrease capture efficiencies.

Although capture efficiency dropped with increasing water velocity, higher encounter rates likely compensate to yield the highest capture rates occurring at 7.5 cm s^{-1} (Fig. 2.3C). 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 predicted capture efficiencies calculated here are based on the assumption that cirri are continually extended, and ignore periods of withdrawal. The low capture efficiencies measured here (Fig. 2.3D) beg the question of why barnacles do not spend long periods of time with their cirri extended (Trager et al., 1990). On possible explanation is that cirral beating behavior balances the costs and benefits 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 and Gillett, 1991).

Measured capture efficiencies were in sharp contrast to the model predictions (no behavior) of uniform capture rates across all velocities and temperatures (Fig. 2.7C). 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.

2.5.3. Cirral activity

This study reports higher overall cirral activity rates compared to barnacles tested in the absence of food (67 ± 4 to 37 ± 2% respectively; Nishizaki and Carrington, In review). At low velocities, barnacles typically displayed extended beating behavior, whereas at higher velocities, barnacles showed abbreviated beating (Fig. 2.4). The switch between from extended to abbreviated behaviors typically occurred at lower velocities (20 to 30 cm s^{-1}) compared to barnacles tested without food (40 cm s^{-1}; Nishizaki and Carrington *In prep*). The difference between these two 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 and Gillett, 1991).

Slow beating rates were observed at 5°C, but were similar across all other temperatures from 10°C 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 (Anderson and Southward, 1987; Sanford et al., 1994; Southward, 1955a).

This study also confirms a disparity between the proportion of barnacles actively beating their cirri (67 ± 3%), and the proportion with food in their gut (21 ± 3%), suggesting that beating activity is not a reliable predictor of feeding activity (Fig. 2.6C). Indeed, barnacles in our experiments often appeared to sweep cysts towards and then away from their mouths (personal obs.), which likely contributed to the low capture efficiencies measured (Fig. 2.3D).

Overall, these results demonstrate that barnacle feeding is influenced by two 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.

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Table 2.1. Reported mechanisms of particle capture for various aquatic organisms.

Capture Mechanism	**Organism**	**Velocity (cm s^{-1})**	**Particle diameter (μm)**	**Source**
Sieving	Brachiopods	---	~4.5	Strathmann 2005
	Mussels	---	0.2-1	Wright et al. 1982
Direct interception	Corals		200	Sebens et al. 1997
	Corals	3-20	200	Patterson 1991
	Brittlestars	20-360	20-340	Labarbera 1978
	Seagrasses	20	7.5-2700	Ackerman 1997
	Blackfly larvae	---	0.091-30	Ross & Craig 1980
	Tunicates	2-4	0.5-3	Sutherland et al. 2010
Inertial impaction	Brittlestars	---	20-340	Labarbera 1984
	Octocorals	---	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
Diffusive deposition	Blackfly larvae	---	56	Braimah 1987
	Glass Fibers	0.1-1	5	Schrijver et al. 1981
Electrostatic attraction	Brittlestars	---	20-340	Labarbera 1978

Figure Legends

Figure 2.1. Mechanisms of aerosol particle capture. ***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.

Figure 2.2 Reynolds number 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 (2.56×10^{-4} m) and water velocities around cirri were assumed to be 9% of free stream velocities (Carrington et al., 2008).

Figure 2.3. *Artemia* cyst capture by *Balanus glandula* over a range of water velocities and temperatures. A) projected area of the cirral net, B) encounter rate past the cirral net, C) capture rate and, D) capture efficiency. For capture rates, each node represents a mean of 20 barnacles and error bars represent standard error.

Figure 2.4. Type of beating behavior displayed under a range of water temperatures and velocities. Filled circles represent proportion of time barnacles displayed extended beating and open circles represent abbreviated beating. Error bars represent standard error. N = 10 barnacles.

Figure 2.5. Cirral beating rate in barnacles. Beating rates relative to water velocity and temperature. Rates averaged from 10 barnacles.

Figure 2.6. Feeding activity of *Balanus glandula* in relation to water temperature and velocity. A) the proportion of barnacles beating cirri and B) the proportion of barnacles with cysts in their gut. Each point represents a proportion estimated from 20 barnacles. C) relationship between the proportion of barnacles beating and the proportion of barnacles fed.

Figure 2.7. Generalized patterns of *Artemia* cyst capture by *Balanus glandula* over a range of water velocities and temperatures. A) Maximum capture rate (F) adapted from Rubenstein and Koehl (1977) and Shimeta and Jumars (1991); B) Capture rate indices incorporating extended beating behavior C) predicted effects of flow and temperature on *particle capture efficiency* (N) adapted from Rubenstein and Koehl (1977) and Shimeta and Jumars (1991)

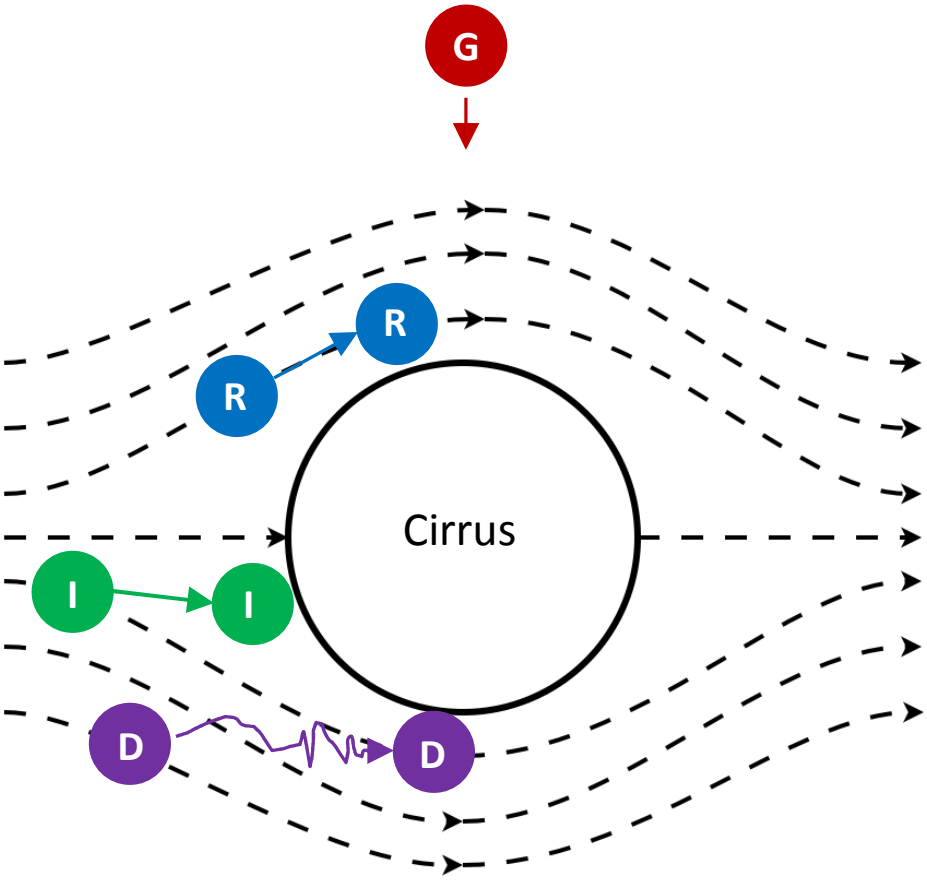

Figure 2.1

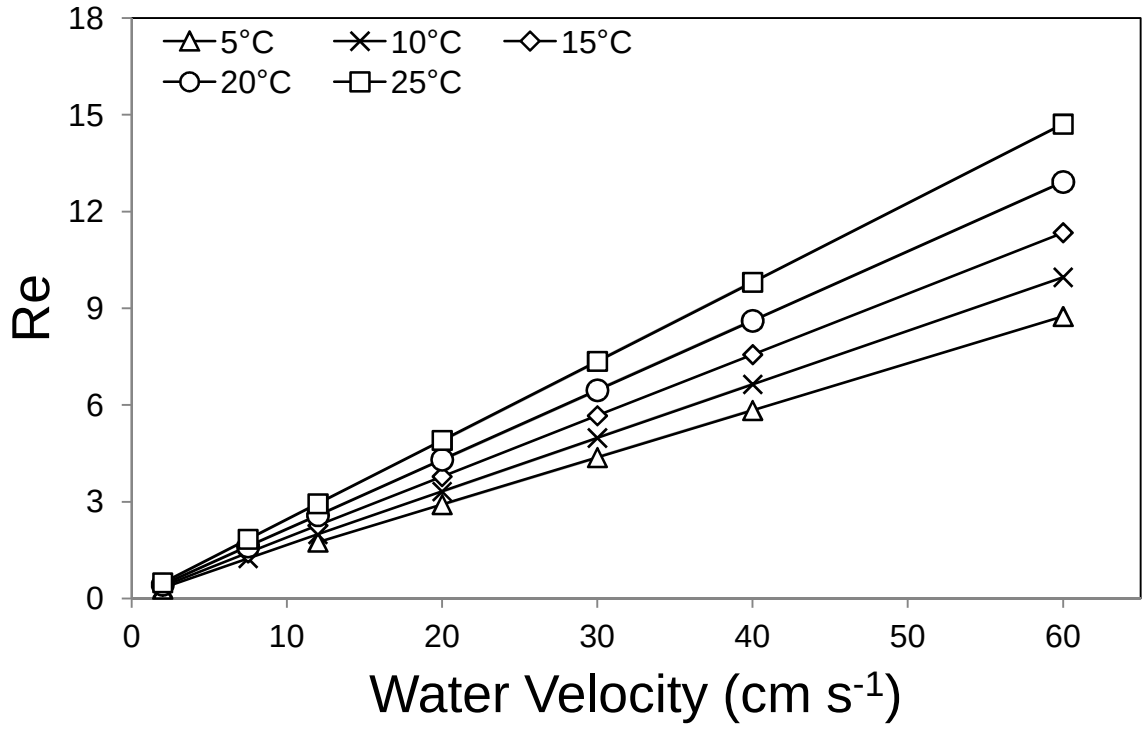
5°C
10°C
15°C
20°C
25°C
Re
Water Velocity (cm s-1)
0
3
6
9
12
15
18
0
10
20
30
40
50
60

Figure 2.2

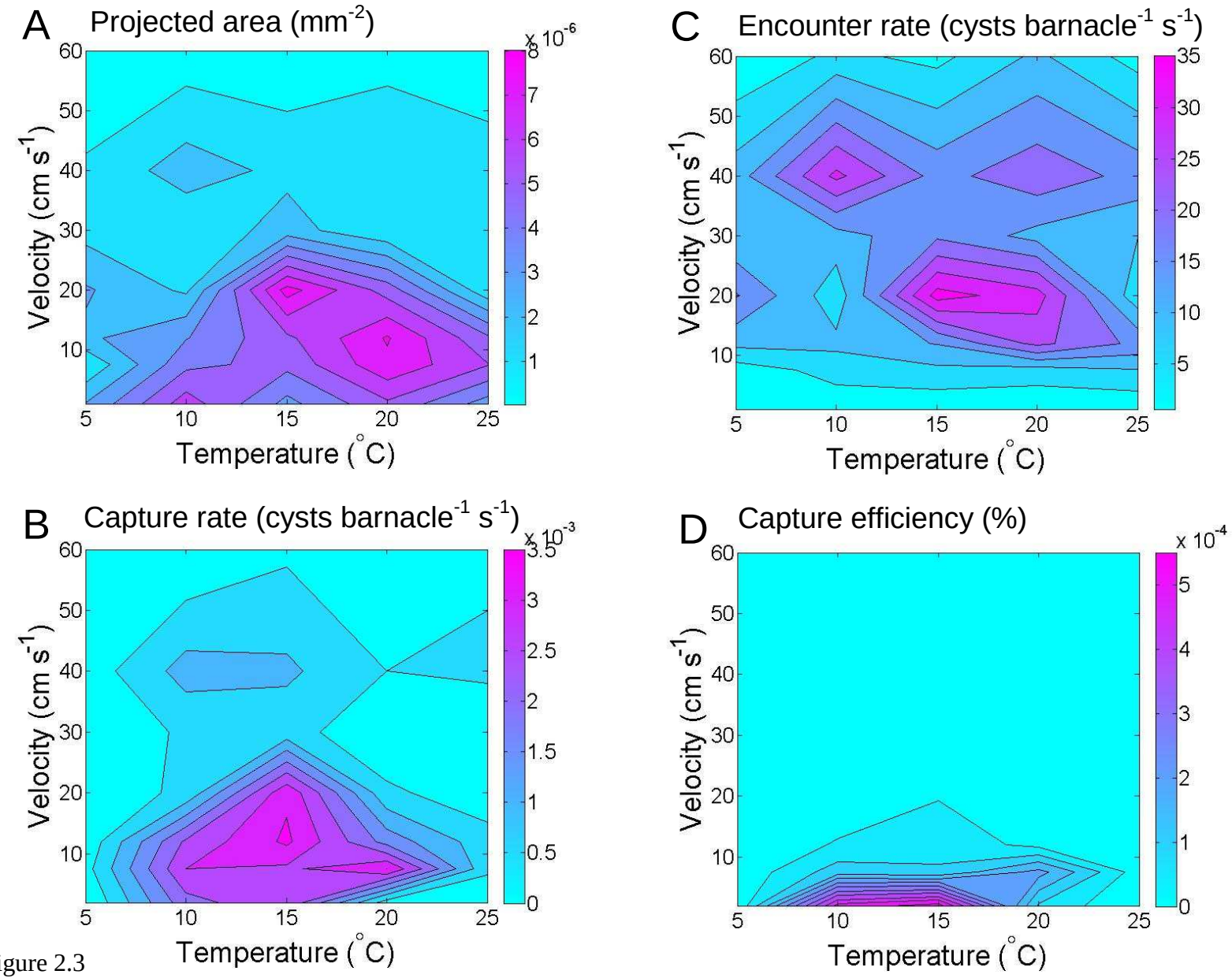
A
Projected area (mm-2)
x 10-6
8
7
6
5
4
3
2
1
Velocity (cm s-1)
60
50
40
30
20
10
5
10
15
20
25
Temperature (°C)
C
Encounter rate (cysts barnacle-1 s-1)
35
30
25
20
15
10
5
B
Capture rate (cysts barnacle-1 s-1)
x 10-3
3.5
3
2.5
2
1.5
1
0.5
0
D
Capture efficiency (%)
x 10-4
5
4
3
2
1
0

Figure 2.3

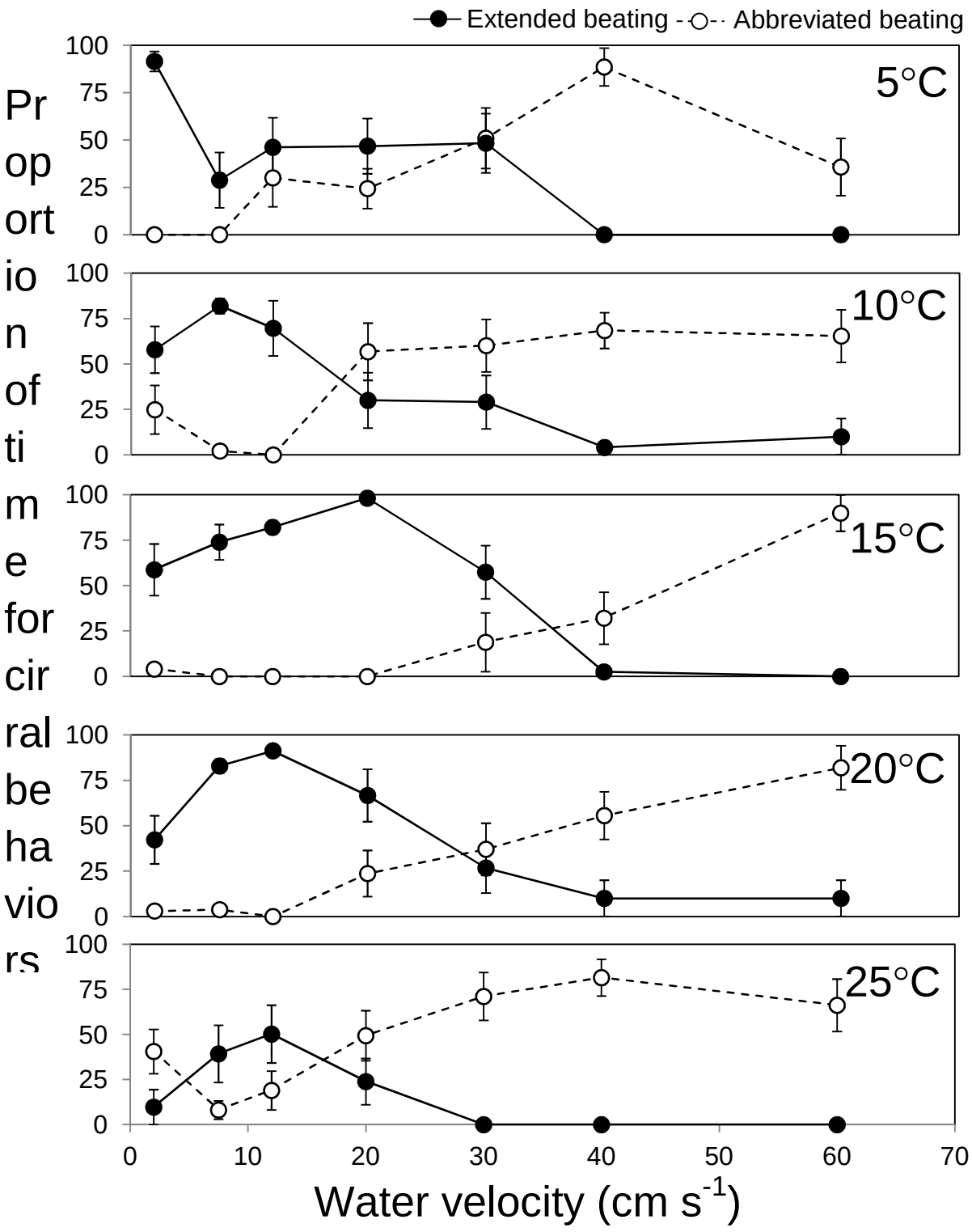
Extended beating
Abbreviated beating
Proportion of time for circral behaviors
5°C
10°C
15°C
20°C
25°C
100
75
50
25
0
0
10
20
30
40
50
60
70
Water velocity (cm s-1)

Figure 2.4

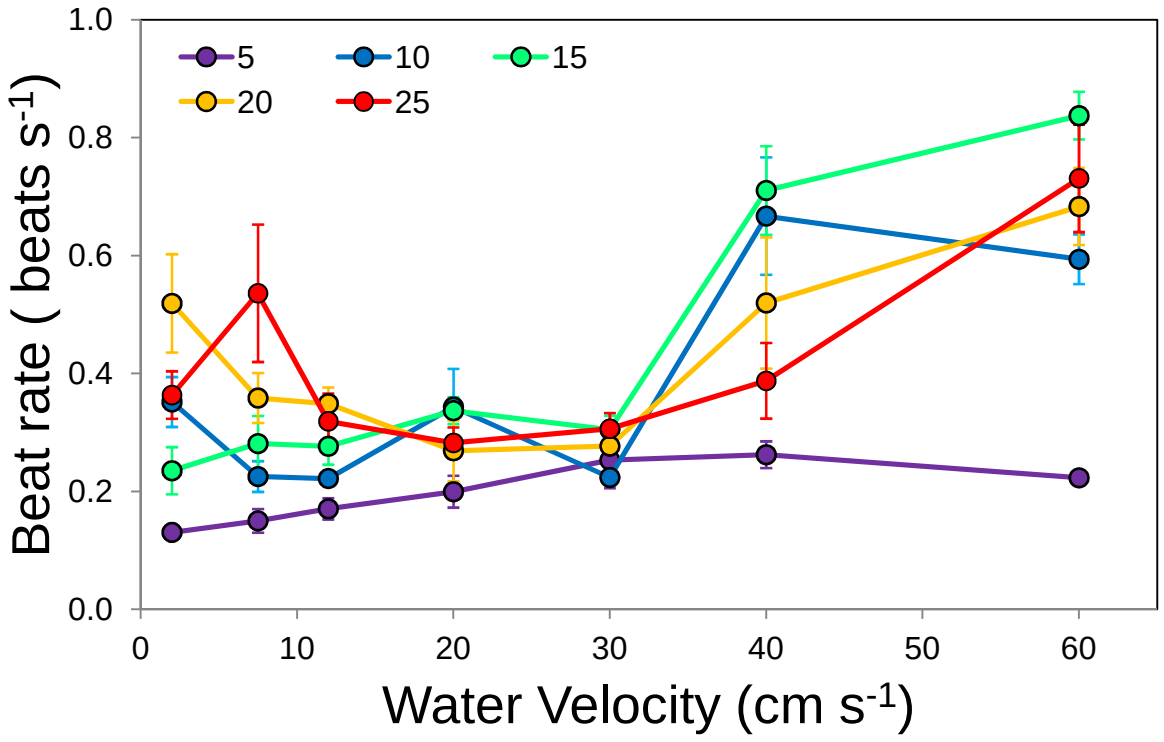
1.0
0.8
0.6
0.4
0.2
0.0
Beat rate (beats s-1)
5
10
15
20
25
0
10
20
30
40
50
60
Water Velocity (cm s-1)

Figure 2.5

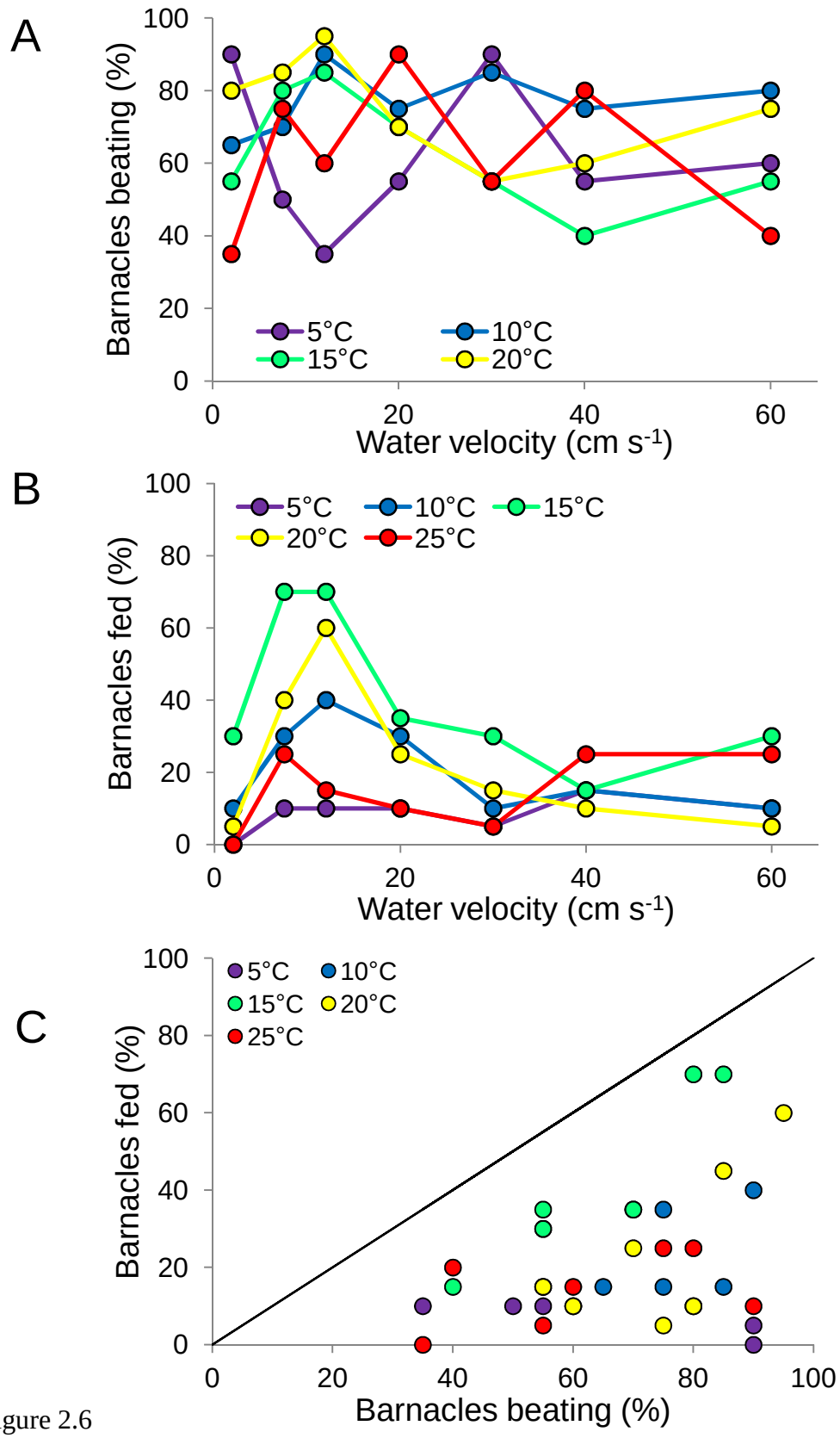
A
Barnacles beating (%)
100
80
60
40
20
0
0
20
40
60
Water velocity (cm s-1)
5°C
10°C
15°C
20°C
B
Barnacles fed (%)
100
80
60
40
20
0
0
20
40
60
Water velocity (cm s-1)
5°C
10°C
15°C
20°C
25°C
C
Barnacles fed (%)
100
80
60
40
20
0
0
20
40
60
80
100
Barnacles beating (%)
5°C
10°C
15°C
20°C
25°C

Figure 2.6

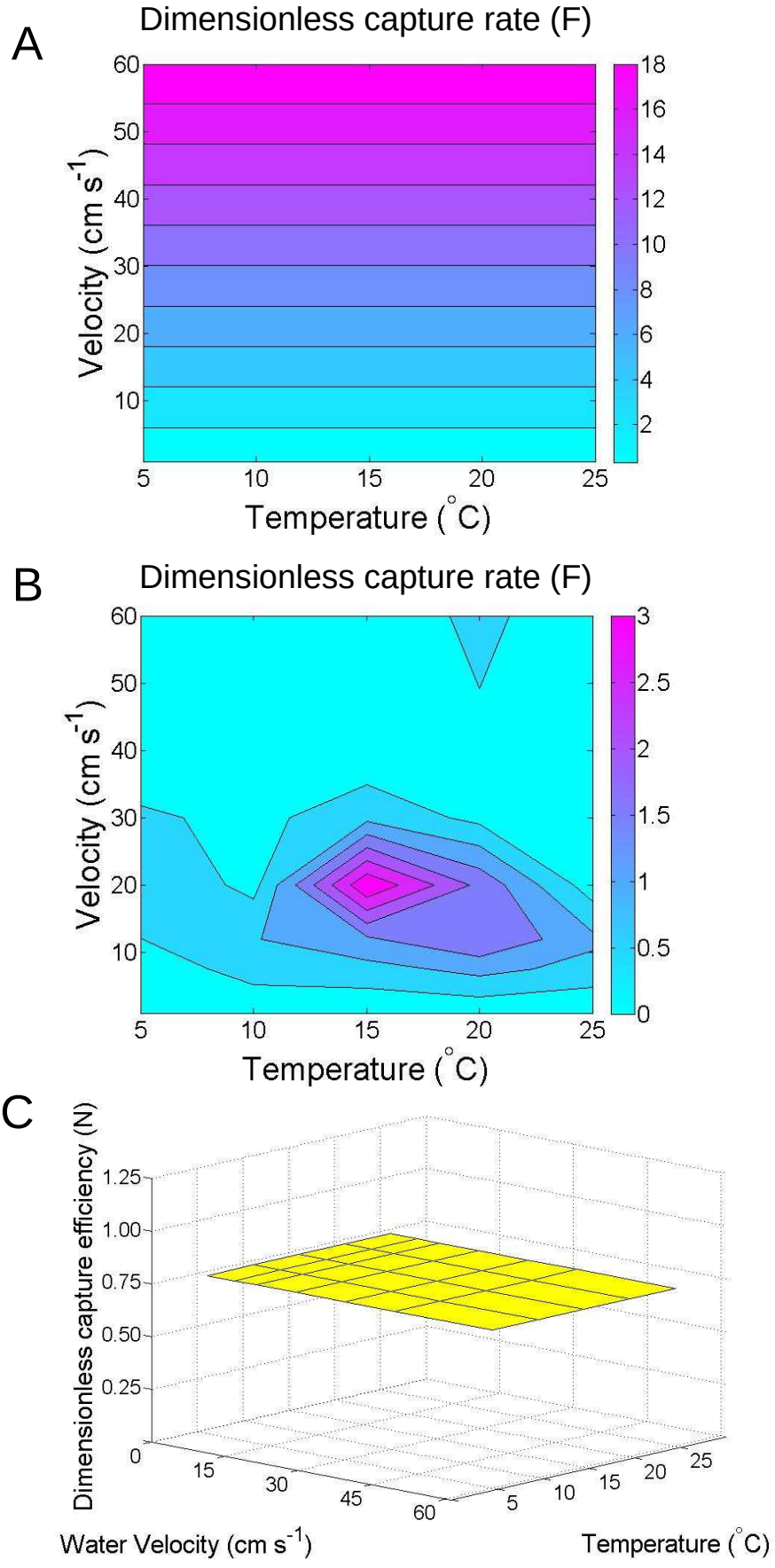
A
Dimensionless capture rate (F)
Velocity (cm s-1)
Temperature (°C)
B
Dimensionless capture rate (F)
Velocity (cm s-1)
Temperature (°C)
C
Dimensionless capture efficiency (N)
Water Velocity (cm s-1)
Temperature (°C)

Figure 2.7

Chapter III

THE EFFECT OF TEMPERATURE AND FLOW ON THE GROWTH OF THE BARNACLE *BALANUS GLANDULA*

3.1. Abstract

For benthic invertebrates, physiological functions such as respiration and feeding depend on the conditions of the physical environment. How these processes integrate into the overall growth of an organism, however, remains unclear. Growth rates of the barnacle, *Balanus glandula*, were measured relative to changes in the fluid environment, namely water velocity and temperature. Barnacles in a dockside flow-through chamber were raised in raceways under water velocities of 2 versus 20 cm s^{-1} and temperatures of 11.5 versus 14°C. Over 37 days, barnacles grew an average of 1.3 ± 0.1 mm^2 day^{-1} and growth rates increased with both water velocity and temperature. At 20 cm s^{-1} and 14°C, where growth rates were the highest (1.5 ± 0.1 mm^2 day^{-1}), barnacle shell growth slowed relative to increases in body mass and gonad.

A separate experiment in the field confirmed these patterns of temperature- and flow-dependent growth over 41 days. Outplanted barnacles exposed to the faster water velocities (up to 1.90 m s^{-1}) and warmest temperatures (16.8°C) experienced higher growth compared to individuals at lower velocities (<0.1 m s^{-1}) and temperatures (13.7°C). Growth rates in the field were lower than those measured in the mesocosm (0.6 to 0.8 mm^2 day^{-1}).

In both experiments, barnacles in slower and warmer waters consistently grew longer cirri than individuals in faster, cooler waters. This plastic response was in the opposite direction of many other morphological traits, and thus longer feeding appendage lengths are unlikely to account for differences in body growth.

Estimates of energy available for growth based on previously measured feeding and respiration rates predict peak growth at moderate temperatures (15°C) and velocities (20 to 30 cm s^{-1}). Barnacles growth is predicted to be lower at both low and high velocities due to lower encounter rates with suspended food particles and lower capture efficiencies respectively. At high temperatures, barnacles growth is limited by high metabolic costs and at low temperatures, slow beating rates lead to low feeding and growth rates. Barnacle growth rates measured in these experiments conform to model predictions and suggest that both increases and decreases in temperature or flow will impact barnacle growth.

3.2. Introduction

For many benthic marine organisms, body size has direct implications for an organism's abundance and distribution (Cohen et al., 1993; Levitan, 1991; Sebens, 1982). Indeed, growth is enhanced by high wave energies (Leigh et al., 1987; Leonard et al., 1998) and warm water temperatures (Sanford and Menge, 2001). For suspension feeders that procure food from the water column, the relationship between flow and growth can be positive, negative or unimodal (i.e., peak rate at one optimal condition; Table 1). The effect of water temperatures on growth can also be positive (Angilletta et al., 2004) or unimodal (Schöne et al., 2002).
However, linkages between the effects of water temperature and flow remain less well studied.

Although growth rates have been measured or estimated for many barnacles species (Bertness et al., 1998; Sanford and Menge, 2001; Thiyagarajan et al., 2003), evidence linking growth to changing environmental conditions remains largely correlative. For instance, growth in *Balanus glandula* is known to follow a seasonal pattern, with highest growth in the spring and lower growth rates in the fall and winter (Barnes, 1955; Moore, 1934; Wethey, 1983b). *Semibalanus balanoides* at high flow sites had higher growth rates than individuals at low flow sites (Crisp, 1960; Sanford et al., 1994). Similarly, Sanford and Menge (2001), found that barnacle growth was highest at sites with high wave-exposure during periods of elevated water temperatures. Character displacement in cirral morphology has also been documented among different species of barnacles, with increased water motion leading to shorter cirri (Arsenault et al., 2001). Although such correlative patterns suggest a link between growth and environmental conditions, little experimental evidence exists to test these relationships.

Energy budget models, based on quantitative experiments, can generate predictions about growth under different environmental conditions. Their value lies, in part, in their ability to generate predictions about how a species will respond to novel environmental conditions that may not exist today (Kearney and Porter, 2009). Such predictions, however, require careful measurement of physiological responses like feeding and respiration under a range of environmental conditions. For barnacles, two important environmental factors are water temperature and velocity. Response curves for feeding and respiration have been constructed (Nishizaki and Carrington *in review*; Nishizaki and Carrington *in prep*) for both temperature and velocity allowing for predictions about patterns of growth in the barnacle, *Balanus glandula*. In this study, we aim to measure barnacle growth responses to different water temperatures and velocities to compare with model predictions.

We conducted a pair of growth experiments to examine the roles of water temperature, velocity and their interaction on barnacle growth. In the first experiment, barnacle growth rates were measured in a dockside experiment where water temperatures and velocities were controlled. In second experiment, growth rates were measured for juvenile barnacles outplanted in the field at three sites.

3.3. Materials & Methods

Experiments were conducted at the Friday Harbor Laboratories of the University of Washington (FHL-UW) on San Juan Island, WA, USA to determine barnacle growth rates in response to changes in water temperature and velocity. A field experiment measured growth

rates of barnacles that were outplanted in the field at sites of varying water temperatures and velocities.

3.3.1 Barnacle growth in dock mesocosm experiment

To examine the influence of water temperature and velocity on growth, juveniles settled on plates were raised under different thermal and flow conditions in a dockside experiment from July 19 to August 25, 2011. Fresh seawater was drawn off the FHL-UW floating dock, from depth of 1 m below the surface using a 2800 GPH submersible bilge pump (Rule Industries, Gloucester, MA, USA) fitted with a mesh (pore size 2 cm × 2 cm) over the inlet. Water was transported via a 2" diameter PVC pipe into one of two header tanks (180 L; 65 cm height; 60 cm diameter). The water in one of the tanks was heated with seven 15" 1000 W submersible heating elements (Biotherm 1000 watt Titanium Heating Element, Blueline Aquatics, San Antonio, TX, USA), and the other tank was left unheated. Both tanks were wrapped in 2" thick foil and fiberglass insulation (SP55, Frost King, Mahwah, NJ). Each header tank emptied via a 2" diameter bottom standpipe into a 2" PVC manifold that supplied four raceways (total of 8 raceways; Fig. 3.1). Each raceway measured 5 cm width 150 cm length 3 cm depth and flow in each raceway was controlled with a 2" diameter PVC ball valve preceding the entrance end. At the exit end of the raceway, a 50 mm tall gate was placed to maintain sufficient water depth to cover the barnacle plates. The raceways were shaded with 1/8" plywood covered in reflective mylar thermal blankets (Primacare, Dallas, TX, USA).

Temperatures were monitored in all eight raceways by a vacuum-sealed Thermocron iButton datalogger (Maxim Integrated/Dallas Semiconductor, San Jose, CA, USA), every 15 minutes for the duration of the experiment. Water velocities directly over the barnacle plates were measured

every week by timing the downstream movement of fluorescein dye. Five measurements were made over each plate every week and small adjustments with the ball valve were made to maintain water velocities. Water velocities and temperatures were tightly controlled throughout the experiment. (19.1 ± 0.7 and 2.0 ± 0.1 cm s^{-1} and 11.5 ± 0.2 and 14.1 ± 0.4 °C, respectively, means +/- SE; N = 6 weeks for velocities and N = 3550 samples every 15 minutes for temperatures).

Barnacles were settled on 10 cm × 10 cm PVC plates with SafetyWalk Tape® (Product number 7740, 3M Company, Saint Paul, MN, USA). The plates were set out under the Friday Harbor Labs dock in April, 2011 and once barnacles had naturally settled (July, 2011), each plate was cut in half, to produce a 5 cm × 10 cm plate. At the beginning of the experiment, barnacles were thinned and individuals were of similar size among plates (basal area = 1.53 ± 0.07 mm^2 with no statistical differences among plates). Plates were photographed weekly to measure the basal area (mm^2) of ten individual barnacles on each plate for five weeks.

At the end of the experiment, the ten barnacles on each plate were dissected to separate any gonadal material from the body. Gonad and barnacle body were both dried at 60°C in a drying oven for 48 hours and weighed. As cirral morphology is known to undergo plastic responses to flow (Arsenault et al., 2001), the length of the sixth cirrus was also measured at the end of the experiment with a dissection microscope equipped with a CoolPix 995 digital camera (Nikon Inc., Tokyo, Japan). All images were processed using ImageJ software (v.1.45s; NIH, Bethesda, MD, USA).

3.3.2. Barnacle growth in the field

Barnacle growth rates in the field were monitored at Argyle Creek on San Juan Island, WA, USA (a marine research preserve, 48.52° N, 123.01° W). The creek feeds into a saltwater lagoon that fills at high tide and empties during low tide. As such, the creek experiences a range of water velocities and temperatures due to its tidal interactions with the lagoon.

Juvenile barnacles were settled onto Safety-Walk plates as described above. Plates were placed uncut (10 cm × 10 cm) at two sites within Argyle Creek. The two sites, separated by 20 m, were chosen as representative of two different flow regimes (fast and slow). At each site, three concrete blocks (40 cm × 19 cm × 4 cm) were deployed amongst the bottom cobble and arranged to span the creek width. On each block, two barnacle plates were fastened for a total of 6 plates per site. Barnacles were also outplanted to a nearby (250 m) floating dock on the ocean side of the creek that experiences relatively slow water velocities. Two plates were attached to the left and right side of the dock at approximately 20 cm depth below the surface. Barnacles were continuously submerged throughout the experiment. Since block effects of plates and concrete blocks were not significant (ANOVA p-values from 0.12 to 0.45), individual barnacles were considered replicates.

Water temperatures at each site were monitored throughout the experiment using TidbiT v2 temperature probes sampling every 15 minutes (Onset Computers, Bourne, MA, USA).

Measuring water flow is a complex problem as hydrodynamic processes act over multiple spatial and temporal scales. In an attempt to characterize this variation, water velocities were measured via three methods. First, water velocities were measured at high frequency over nine

hours to quantify variation throughout the tidal cycle using an Acoustic Doppler Velocimeter (ADV; Nortek, Norway). The ADV was deployed on July 23, 2011, and made three measurements at each site about once an hour. Measurements were made 1 cm above the substratum and sampled at 25 Hz. Weekly measurements were also made at the sites using a Marsh-McBirney flowmeter (model 523, Frederick, MD). From June 29 to August 9, water velocities were measured at the three sites 5 cm above the substratum. Relative water motion at each site was also estimated indirectly from mass loss of standard Plaster of Paris spheres (45 cm diameter) over 24 hours (spheres deployed at 17:00 on June 7, 2011 and collected at 17:00 on June 8, 2011).

Food concentration was measured weekly by filtering water sampled at the site. 1L seawater samples were run through glass GF/C Whatman filters and placed in a drying oven (Model 255G, Fisher Scientific, Hampton, New Hampshire, USA) for 48 hours to measure total dry weight using an electronic analytical balance (Sartorius 1602 MP8-1, ± 0.1 mg; Göttingen, Germany). Filters were then placed in a muffle furnace (Omegalux, LMF-3550) at 500°C for 12 hours and weighed again to procure organic/inorganic ratios.

3.3.3 Analysis

The effect of water temperature and velocity on barnacle growth (e.g., shell, body and gonad) were analyzed with two-way ANOVAs. Where the assumptions of the general linear model could not be met, non-parametric Kruskal-Wallis tests were employed. Proportional data were arcsin-square root transformed before using ANOVA. For the mesocosm experiment,

Student t-tests were run to determine if there were any differences between two raceways in a given treatment. For the field experiment, ANOVA was used to confirm similarity among plates within each site. Cirral lengths were analyzed using ANCOVA, using $\log_{10}$ transformed data to meet test assumptions. Dry body weight was used as a covariate as differences in growth were expected among the treatments and cirral length is known to vary with body size (Arsenault et al., 2001; Crisp and Maclean, 1990). To remove body-size effects, the least-squares mean cirral length was calculated for a standard body mass for each treatment using ANCOVA according to the methods of Marchinko and Palmer (2003). If the slopes of each treatment were not significantly different, the least-squares means for each treatment was plotted against water temperature and velocity. For all parametric tests, where significant differences were found, pairwise comparisons were made using Bonferroni post-hoc tests. All analyses were conducted with SPSS v.19 (IBM Corporation, Chicago, IL).

Temperature coefficients (Q_{10}) describing the magnitude of change in growth with increasing temperature were calculated for the mesocosm experiment,

$$Q_{10} = \left(\frac{G_2}{G_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (1),$$

where G_1, G_2 are growth rates ($mm^2\ day^{-1}$) measured as the change in basal diameter between the beginning and end of the experiment and T_1, T_2 are corresponding temperatures (°C).

Energy available for barnacle growth was estimated from feeding and respiration data collected over different temperatures and velocities (Nishizaki and Carrington, in review; Nishizaki and Carrington, in prep). The total energy available for growth per day was calculated as (Wu and Levings, 1978):

$$E_P = E_C - E_R - E_M - E_F \quad (2),$$

where E_P is the energy available for production (i.e., growth and reproduction ; calories day^{-1}), E_C is the rate of energy gained from consumption (calories day^{-1}), E_R is the rate of energy used for respiration (calories day^{-1}), E_M is the rate of energy lost to molting and E_F is the rate of energy loss to fecal production. Values of E_F were calculated by multiplying E_C by the assimilation efficiency for *Balanus glandula* range from 92.5 to 99% (Wu and Levings, 1978). Although energies lost to molting were not measured here, they contribute 2% to the overall barnacle energy budget and are ignored (Wu and Levings, 1978). The rate of energy gained from consumption was calculated as,

$$E_C = C \times TSM \times CS \times Q \quad (3),$$

where C is the relative capture rate (% of maximum capture rate) , which is temperature and flow dependent and measured by Nishizaki and Carrington (in prep), TSM is the total suspended matter concentration measured from the Friday Harbor dock (g L^{-1}), CS is the caloric content of phytoplankton in seawater (2730 cal $g^{-1;}$; Platt and Irwin, 1973) and Q is the volume of water that moves through an area the size of the cirral net (L day^{-1}).

The rate of energy used in respiration can be calculated as,

$$E_R = \mathrm{R} \times \mathrm{OC} \quad (4),$$

where R is the temperature- and flow-dependent respiration rate (L O_2 day^{-1}) as measured by Nishizaki and Carrington (in review) and OC is the oxycaloric value of 4800 cal L O_2^{-1} (Crisp, 1971, 1971).

3.4. Results

3.4.1. Barnacle growth in dock mesocosm experiment

Growth rates were lowest in the low temperature-low flow treatment (1.0 ± 0.1 mm^2; Figs. 3.2A,B). Growth was higher for both the low temperature-high flow treatment and the high temperature-low flow treatment (1.4 ± 0.1 mm^2 day^{-1} for both). At the high temperature-high flow treatment, growth was the highest (1.5 ± 0.1 mm^2 day^{-1}). Growth rates were significantly

higher at faster velocities ($F_{(1,76)} = 4.086$, $p < 0.05$) and high temperatures ($F_{(1,76)} = 16.872$, $p < 0.05$). There was also a significant interaction between velocity and temperature ($F_{(1,76)} = 4.798$, $p < 0.05$); Q_{10} for barnacles at 2 and 20 cm s^{-1} were 2.7 and 1.2, respectively.

Dry shell masses at the end of the experiment were lowest for the low temperature-low flow treatment (0.05 ± 0.01 g; Fig. 3.3A). Shell mass was higher at both the low temperature-high flow and high temperature-low flow treatments (0.07 ± 0.01 g for both). Masses were highest in the high temperature-high flow treatment (0.09 ± 0.01 g). Shell mass was significantly influenced by both water velocity ($F_{(1,76)} = 4.074$, $p < 0.05$) and temperature ($F_{(1,76)} = 10.167$, $p < 0.05$). The interaction was not significant ($F_{(1,76)} = 0.001$, $p = 0.999$; Table 2).

At low velocities (2 cm s^{-1}), dry body masses were lower at 11.5°C compared to 14°C (2.2 ± 0.1 g to 2.6 ± 0.2 g; Fig. 3.3B). Masses were higher at 20 cm s^{-1} and again increased from 11.5°C to 14°C (2.8 ± 0.1 g to 3.7 ± 0.1 g). Dry body mass was significantly affected by flow (Mann-Whitney U, $p < 0.05$) and temperature (Mann-Whitney U, $p < 0.05$).

In our experiments, more barnacles produced eggs in the high velocity-high temperature treatment (70%) compared to all other treatments (45-50%; data not shown). At low velocities (2 cm s^{-1}) gonad mass increased from 11.5°C to 14°C ($1.9 \pm 0.9 \times 10^{-3}$ g to $2.6 \pm 1.0 \times 10^{-3}$ g). At 20 cm s^{-1}, gonad mass increased from 11.5°C to 14°C ($2.7 \pm 0.6 \times 10^{-3}$ g to $6.2 \pm 0.6 \times 10^{-3}$ g respectively; Fig 3.3C).

Cirral lengths were longer under slow water velocities and warmer water temperatures ($F_{(1,75)} = 147.04$, $p < 0.05$ and $F_{(1,75)} = 125.89$, $p < 0.05$ respectively; Fig. 3.4).

3.4.2. Barnacle growth in the field

Water temperatures at the slow flow site near the ocean (13.67 ± 0.02°C; N = 3840) were 23% lower compared to the intermediate (16.81 ± 0.05°C; N = 3840) and fast flow sites (16.87 ± 0.05°C; N = 3840 Fig. 3.5). Significant differences were found among all three sites ($F_{(2,11520)}$ = 1689, $p < 0.05$) with post-hoc tests indicating that the slow site was significantly cooler than the intermediate and fast sites.

Throughout the tidal cycle, water velocities were higher at the intermediate and fast flow sites (0.32 ± 0.01 m s^{-1} and 0.34 ± 0.01 m s^{-1} respectively) compared to the slow site (0.01 ± 0.01 m s^{-1}; Fig. 3.6A). Consistent differences among the sites were found at all times tested (for all times $p < 0.05$ from ANOVA, N = 3), with post-hoc tests indicating that each site was significantly different from the other two. Weekly measurements made over the duration of the experiment also indicated that water velocities at the slow site were slower (0.01 ± 0.01 m s^{-1} averaged over all days), than the intermediate and fast sites (0.83 ± 0.21 m s^{-1} and 1.09 ± 0.22 m s^{-1} respectively; Fig. 3.6B). Consistent differences in weekly velocities existed among the sites (Fig. 3.6B; Kruskal-Wallis test, $p < 0.05$; N = 8 sample days). Similarly, dissolution rates were significantly higher at the intermediate and high flow sites (140 to 285% respectively) compared to the low flow site (Fig. 6C; Kruskal-Wallis test, $p < 0.05$; N = 6 spheres).

Although there were fluctuations in seston concentration on a daily time scale (6.4 to 27.7 mg L^{-1}), there were no significant differences among the three sites ($F_{(2,12)}$ = 36.552, p = 0.993; Fig. 3.6A). Likewise, seston concentrations at the three sites varied from 0.6 to 27.7 mg L^{-1} over the duration of the experiment (Fig. 3.6B), but no difference in concentration was found among the three sites (Kruskal-Wallis tests, p = 0.359; N = 5). The organic fraction of seston ranged

from 25 to 48% over the duration of the experiment, but no significant differences were found among the three sites. ($F_{(2,12)} = 1.133$, $p = 0.354$; Fig. 3.6C).

Barnacle basal areas increased throughout the field deployment from 6.23 ± 0.24 mm^2 to 34.34 ± 0.76 mm^2 (450% increase; Fig. 3.8A). Although barnacle growth varied among sites (high flow site > intermediate site > slow site), these differences were not significant (Fig. 3.8B; Kruskal-Wallis tests, $p = 0.165$).

Cirral lengths at the slowest site were significantly longer (30%) than those found at the intermediate and fast sites (Fig. 3.9A; ANOVA, $p < 0.05$). In addition, lengths at the slow site were more closely related to the covariate, body mass (Fig. 9B; ANCOVA, $p < 0.05$).

3.4.3. Model predictions

Scope for growth predictions estimated from respiration and feeding rates are presented in Fig. 3.10. Barnacles at 10°C were predicted to have low levels of energy available for production at both 1 and 20 cm s-1 (0.3 to 0.7 calories per day respectively). Whereas, the available energy at 15°C and 1 cm s^{-1} was also low (0.1 cal day^{-1}), barnacles at 15°C and 20 cm s^{-1} were expected to have much more energy available for production (3.7 cal day^{-1}).

3.5. Discussion

Our model of barnacle growth, based on measured respiration and feeding rates, suggests that peak rates should occur at moderate water temperatures (15°C) and velocities (20 to 30cm s^{-1}; Fig. 3.10C). Barnacles at slow velocities should experience lower growth, due to lower encounter rates with suspended food particles, whereas at high velocities, barnacles experience lower feeding efficiencies, which also reduces their potential for growth (Nishizaki and Carrington in prep). At low temperatures, cirral beating behavior slows, reducing feeding capacity and in turn growth, whereas at high temperatures, high metabolic can impose limits on growth (Nishizaki and Carrington, in review). These predictions provide a comparison for barnacle growth data collected in the mesocosm and field experiments.

Average growth rates for barnacles in the outdoor mesocosms ranged from 0.6 to 1.5 mm^2 day^{-1} and 0.6 to 0.8 mm^2 day^{-1} in the field transplant experiments (Figs. 3.2 and 3.8). In both experiments, increased barnacle growth was associated with higher water temperatures and velocities (Figs. 3.2 and 3.8). In addition, cirral lengths in both the mesocosm and the field were reduced at higher velocities (Figs. 3.4 and 3.9).

In the mesocosm experiment, barnacles experienced greater growth under warmer temperatures (14°C versus 11.5°C) and faster velocities (20 cm s^{-1} versus 2 cm s^{-1}), consistent with model predictions (Fig. 3.10). The positive relationship between temperature and growth, with Q_{10} values of 1.2 to 2.7, is consistent with those reported for other intertidal ectotherms (2.0 to 4.1; Dame, 1972; Green and Hobson, 1970). In the field, barnacles also grew larger at sites with higher temperatures (17°C versus 14°C) as predicted by the model.

Increased growth at moderate temperatures (14°C in mesocosm and 17°C in field experiment) may reflect similar increases in feeding activity for barnacles. At low temperatures, cirral activity and ingestion increases up to 15-20°C and decreases at temperatures ≥ 25°C (Nishizaki and Carrington, in review; Anderson and Southward, 1987). Although such temperatures were only briefly seen at midday in Argyle Creek, future increases in water temperature may reduce growth at the two warm sites while increasing growth at the slow, cool site.

Growth rates in both experiments were also higher at faster water velocities, consistent with model predictions (Figs. 3.2, 3.8 and 3.10). In the mesocosm experiment, barnacles under the high temperature and high velocity treatment were predicted to have more energy available for production. Although body and shell growth was higher under these conditions, there was also a notable increase in reproductive output (Fig. 3.3). Bertness et al. (1991) suggest that larger barnacles produce proportionally more reproductive material, which appears consistent with these results. In the field experiment, barnacle growth was also influenced by water velocity as predicted, with barnacles growing fastest at the fast site (Fig. 3.8).

In contrast to our results, Eckman and Duggins (1993) found that subtidal *Balanus glandula* growth rate was relatively insensitive to changes in water velocity from 2 to 16 cm s^{-1}. These experiments ran from June until the end of November, and it is possible that slow growing barnacles caught up to faster growing barnacles by the end of the season if larger barnacles divert proportionally more energy towards reproductive production and away from growth. Lacking measures of reproductive output from the Eckman and Duggins (1993) study, however, prevents more detailed comparison of the two experiments.

At low flows (< 5 cm s^{-1}), increasing velocity will increase the rate of food delivery (Taghon et al., 1980) and will also increase respiration rates up to some limit (Nishizaki and Carrington, in review). At high velocities, water motion will impose mechanical forces that may damage cirri (Marchinko, 2007a) or make them less effective. Thus, growth rates should be optimal at intermediate water velocities (20 to 30 cm s^{-1}).

Barnacles exposed to slower water velocities had longer cirri compared with individuals grown under faster flows. This flow-dependent response is consistent with the findings for a number of barnacle species (Arsenault et al., 2001; Marchinko and Palmer, 2003), and do not explain the observed differences in barnacle growth. Whereas cirri were longest under slow velocities, both encounter rate and growth rate were lowest under slow flows. Cirral lengths were slightly longer (14%) at 14°C compared to 11.5°C, possibly reflecting the need for increased oxygen ventilation or particle capture at elevated temperatures (Nishizaki and Carrington, in review). Longer cirri may facilitate respiration as they serve both as a surface for gas exchange and as a means of increasing flow (Anderson, 1994).

These results demonstrate that water temperature and velocity affect the growth of the barnacle, *Balanus glandula*. Both factors had a positive effect on growth over the ranges tested. Growth rates from both experiments conform to model predictions calculated from temperature- and flow-dependent feeding and respiration rates. These predictions are also consistent with an increase in reproductive material produced by large individuals under high temperatures and flows. Such patterns are unlikely to be a result of plastic responses in cirral length as individuals at high flows had shorter cirri and would thus result in lower growth. Although high growth rates of barnacles at high temperatures may be a consequence of longer cirri, it remains possible

that these differences in cirral morphology are related increased oxygen ventilation requirements. Moreover, peak growth rates are predicted for intermediate water temperatures (15°C) and velocities (20 to 30cm s^{-1}). Reduced growth is predicted at extreme temperatures and velocities, each due to a different physiological or behavioral responses in respiration or feeding.

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Table 3.1. Growth responses for marine suspension feeders. "+" = higher growth with increasing temperature or flow, "-" = lower growth with increasing temperature or flow, Unimodal = a single peak at an optimal temperature or flow and None = no relation. NR = not reported. Values in brackets are peak velocities/temperatures.

Organism	Flow ($cm\ s^{-1}$)	Relation	Temperature (°C)	Relation	Source
Various corals	NR	NR	18-26	Unimodal (26°C)	(Jokiel and Coles, 1977)
Alcyonium siderium	10-20	+	NR	NR	(Sebens, 1984)
Anthopleura elegantissima	NR	NR	5-25	Unimodal (5-10°C)	(Sebens, 1980)
Argopecten irradians	0.2-12.8	Unimodal (0.76 $cm\ s^{-1}$)	14-27	+	(Kirby-Smith, 1972)
A. irradians	0-15	Unimodal (1-6.5 $cm\ s^{-1}$)	18-23	NR	(Cahalan et al., 1989)
Placopecten magellannicus	0.5-23.5	Unimodal (2-6 $cm\ s^{-1}$)	2-12	NR	(Wildish et al., 1987)
Crassostrea virginica	1-8	Unimodal (4.2 $cm\ s^{-1}$)	21±0.3	NR	(Grizzle et al., 1992)
Mercenaria mercenaria	1-8	Unimodal (2.2 $cm\ s^{-1}$)	21±0.3	NR	
Mya arenaria	0.1-6	+	1.5-16	NR	(Emerson, 1990)
Mytllus trossulus	1-40	-	16.5-17.0	NR	(Ackerman and Nishizaki, 2004)
M. californianus	1-40	-	16.5-17.0	NR	
M. californianus	NR	NR	~10-13	+	(Menge et al., 2008)
Pseudochitinopoma occidentalis	2-15	-	NR	NR	(Eckman and Duggins, 1993)
Membranipora membranacea	2-15	-	NR	NR	
Balanus glandula	2-15	None	NR	NR	
Semibalanus cariosus	2-15	None	NR	NR	
Pollicipes	2-15	None	NR	NR	

polymerus

Organism	Flow ($cm\ s^{-1}$)	Relation	Temperature (°C)	Relation	Source
Various corals	NR	NR	18-26	Unimodal (26°C)	(Jokiel and Coles, 1977)
B. cretanus	2-15	Unimodal (8 $cm\ s^{-1}$)	NR	NR	

Figure legends

Figure 3.1. Raceways used to measure barnacle growth in response to water temperature and velocity. Tiles are numbered 1 to 16 and arrows indicate direction of water flow.

Figure 3.2. Barnacle growth in response to water temperature and flow. A) changes in basal area over time for barnacles grown in different water velocities (2 and 20 cm s^{-1}) and temperatures (11 or 14.5°C) B) basal area growth for barnacles. N = 20 barnacles, error bars represent standard error.

Figure 3.3. Effect of water velocity and temperature on barnacle morphology. A) shell mass (g), B) dry body mass (g) and, C) gonad mass (g). All measurements made at the end of the dockside experiment. N = 20 barnacles, error bars represent standard error.

Figure 3.4. Barnacle cirral lengths versus water temperature and flow. A) Log-log comparison of cirral length versus dry body mass for barnacles as a function of temperature and flow. Each point represents an individual barnacle. Differences in slope were not significant (ANCOVA, $p > 0.05$). N = 20) Average cirral length for barnacles as a function of water temperature and velocity. Bars represent the mean leg length (±SE) of 20 individuals. ANCOVA confirmed that slopes among treatments were not significantly different and thus leg lengths were standardized to a dry body mass of 0.002 g.

Figure 3.5. Water temperatures at three field sites located at Argyle Creek, WA, USA. A) time series of temperatures sampled every 15 minutes, B) mean temperatures for all three sites. N = 3848, error bars represent standard error.

Figure 3.6. Water flow at Argyle Creek. A) water velocities at three sites through tidal cycle. Hatched line depicts tide height and the red line delineates the change in direction of water flow at the site (e.g., ebb versus flood tide), B) water velocities at sites measured weekly with Marsh-Mcbirney flowmeter and, C) dissolution rate of Plaster of Paris spheres. N = 6 spheres, error bars represent standard error. Inset is a photo of a plaster sphere with a US quarter for scale (24.3 mm diameter).

Figure 3.7. Seston concentrations at field sites located at Argyle Creek, WA, USA. A) Dry seston measured through tidal cycle. Hatched line depicts tide height and the red line delineates the change in direction of water flow at the site (e.g., ebb versus flood tide), B) dry mass of seston at three field sites over the duration of the growth experiment, C) organic fraction over the duration of the experiment, error bars represent standard error.

Figure 3.8. Growth of barnacles outplanted in the field. A) changes in basal area over time for barnacles grown in one of three sites of varying water flow (slow, intermediate and fast), B) basal area growth for barnacles at each site. N = 60 barnacles for fast and intermediate, N= 40 for slow, error bars represent standard error.

Figure 3.9. Cirral lengths from barnacles at the end of field deployment. A) Cirral length N = 60 barnacles for fast and intermediate, N= 40 for slow, error bars represent standard error, B) Log-log relationships of cirral length as a function of dry body mass for the slow (thin line), intermediate (dark line) and fast flow (hatched line) sites. Each point represents an individual barnacle. Differences in slope were significant (ANCOVA, $p < 0.05$), with body mass having a greater effect on cirral length on barnacles at the slow site.

Figure 3.10. Model predictions for barnacle growth. A) Energy from feeding; B) energetic cost of respiration; C) Predicted energies available for production for *Balanus glandula* as a function of water temperature and velocity and; D) Predicted energies available for production under conditions matched to the mesocosm experiment. Based on a calories gained from measured feeding rates (Nishizaki and Carrington, in review) and respiration rates (Nishizaki and Carrington, in review) under different water velocities and temperatures.

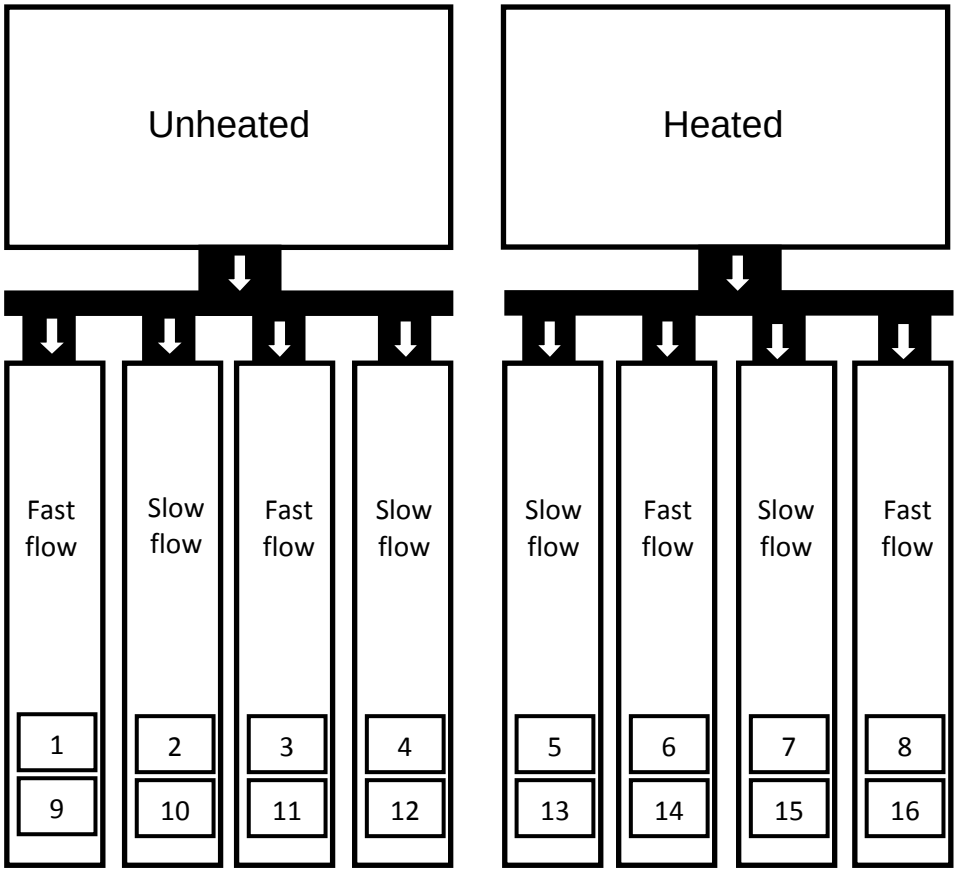
Unheated
Heated
Fast flow
Slow flow
Fast flow
Slow flow
Slow flow
Fast flow
Slow flow
Fast flow
1
9
2
10
3
11
4
12
5
13
6
14
7
15
8
16

Figure 3.1

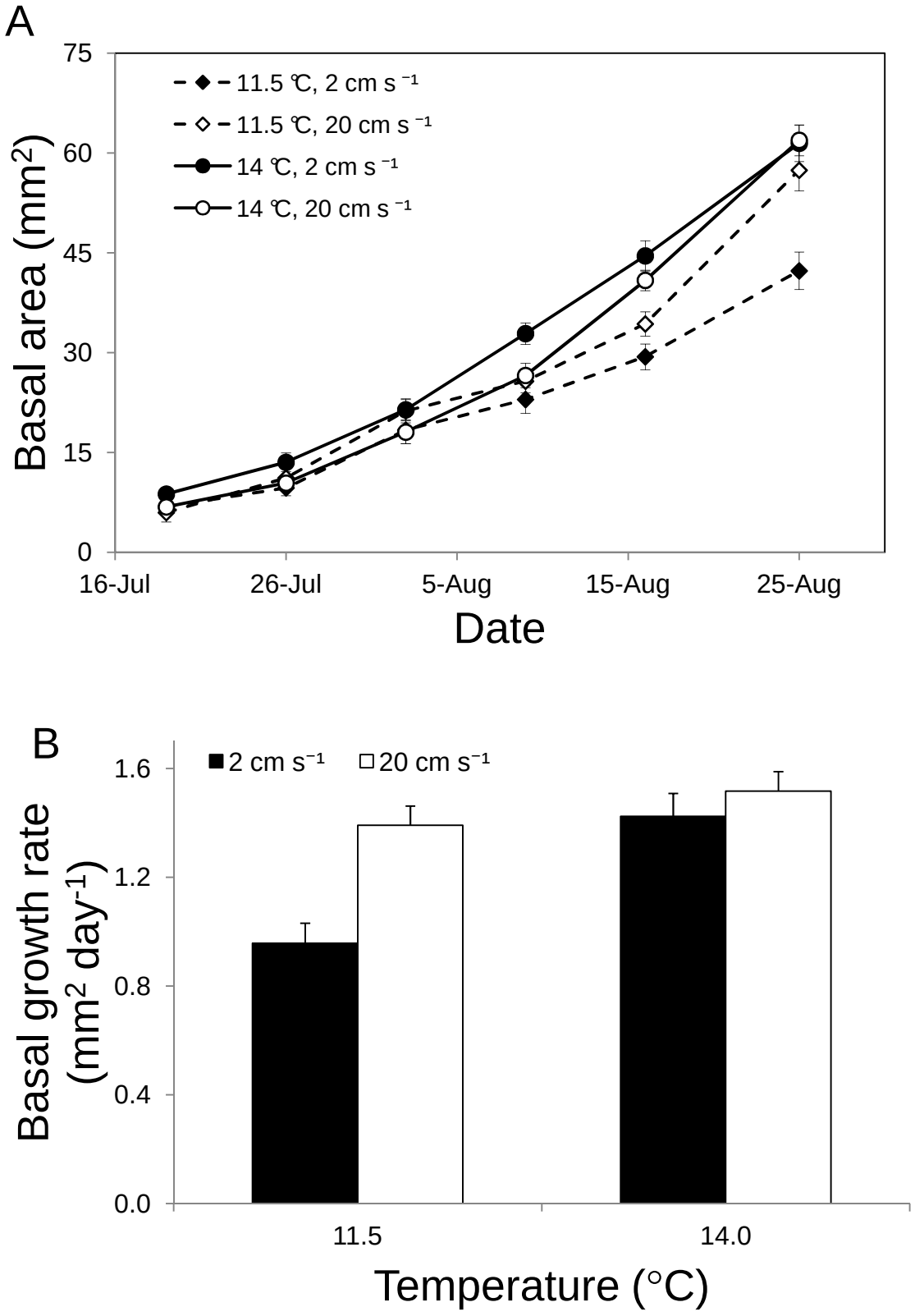
A
75
60
45
30
15
0
Basal area (mm2)
11.5 ℃, 2 cm s −1
11.5 ℃, 20 cm s −1
14 ℃, 2 cm s −1
14 ℃, 20 cm s −1
16-Jul
26-Jul
5-Aug
15-Aug
25-Aug
Date
B
2 cm s−1
20 cm s−1
1.6
1.2
0.8
0.4
0.0
Basal growth rate (mm2 day-1)
11.5
14.0
Temperature (°C)

Figure 3.2

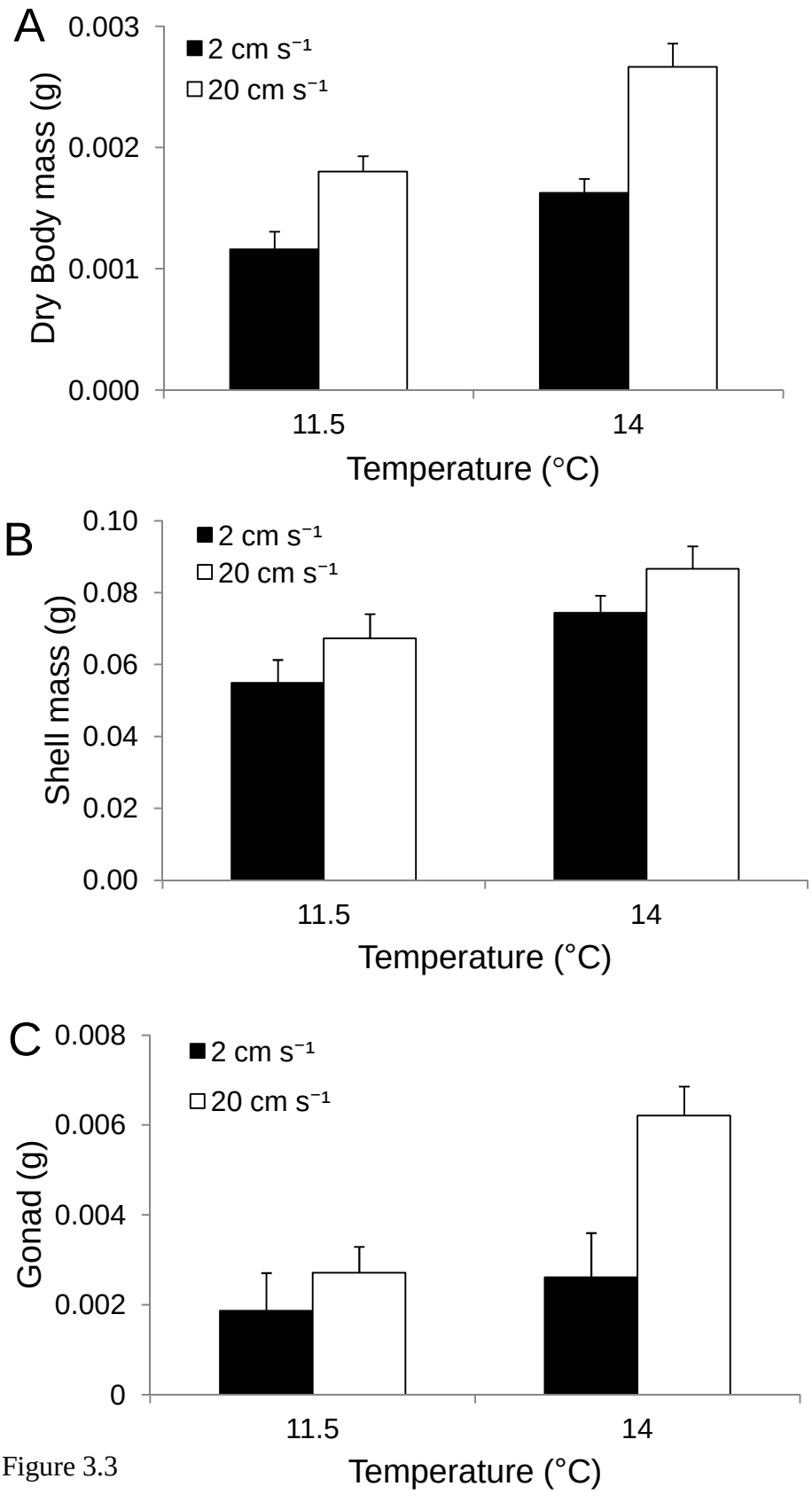
A
0.003
0.002
0.001
0.000
Dry Body mass (g)
2 cm s⁻¹
20 cm s⁻¹
11.5
14
Temperature (°C)
B
0.10
0.08
0.06
0.04
0.02
0.00
Shell mass (g)
2 cm s⁻¹
20 cm s⁻¹
11.5
14
Temperature (°C)
C
0.008
0.006
0.004
0.002
0
Gonad (g)
2 cm s⁻¹
20 cm s⁻¹
11.5
14
Temperature (°C)

Figure 3.3

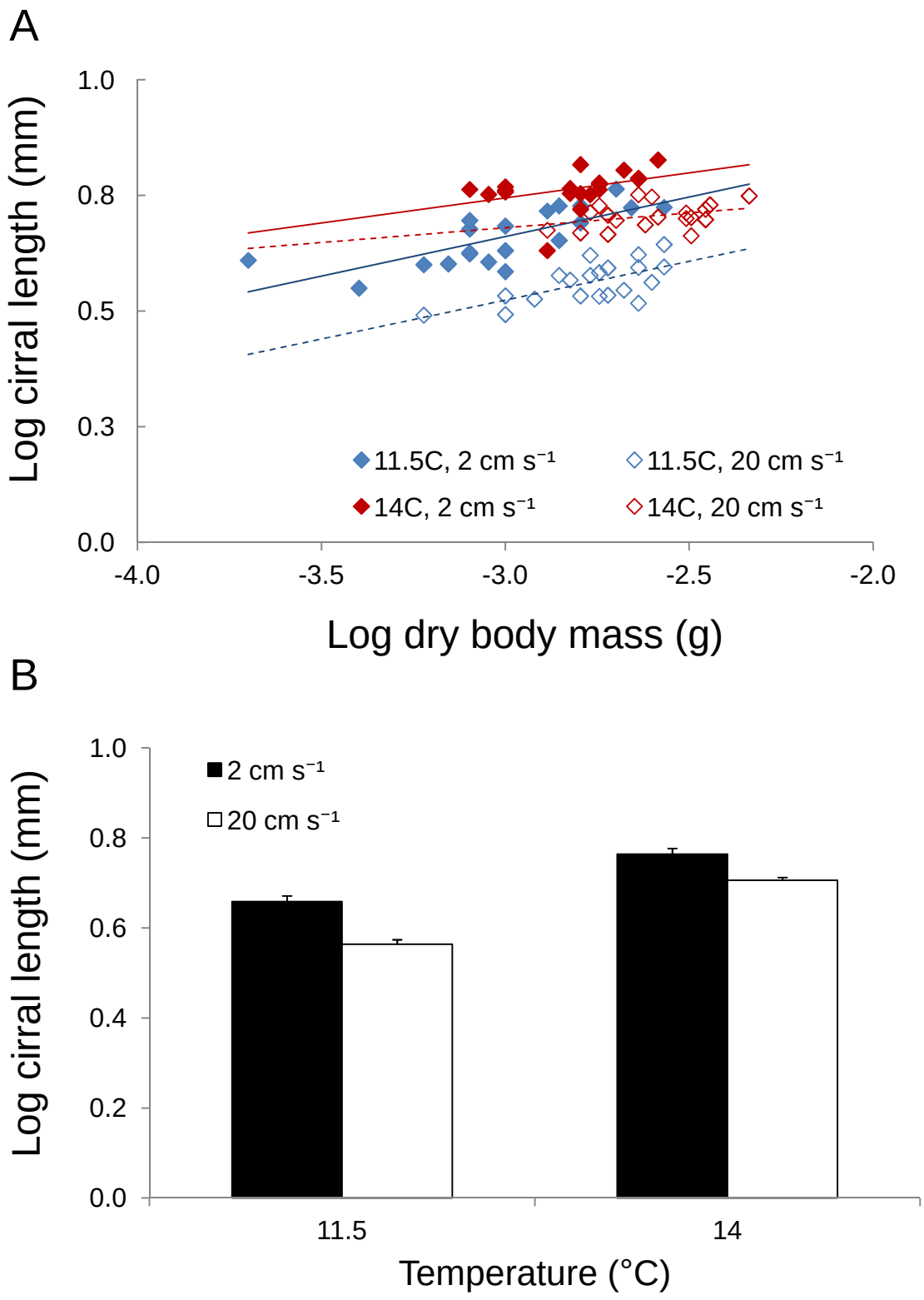
A
Log cirral length (mm)
1.0
0.8
0.5
0.3
0.0
-4.0
-3.5
-3.0
-2.5
-2.0
Log dry body mass (g)
11.5C, 2 cm s⁻¹
11.5C, 20 cm s⁻¹
14C, 2 cm s⁻¹
14C, 20 cm s⁻¹
B
Log cirral length (mm)
1.0
0.8
0.6
0.4
0.2
0.0
2 cm s⁻¹
20 cm s⁻¹
11.5
14
Temperature (°C)

Figure 3.4

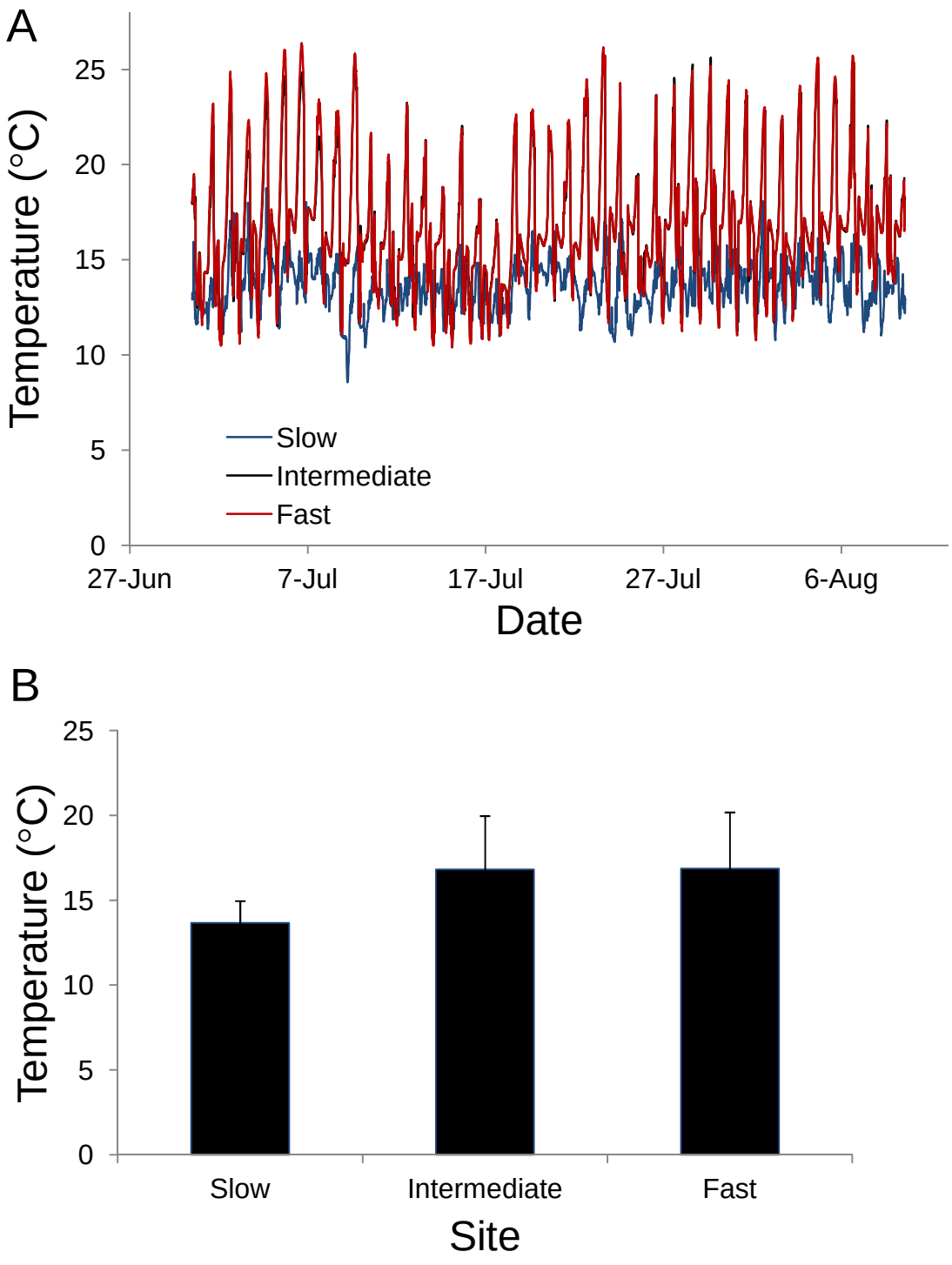
A
Temperature (°C)
25
20
15
10
5
0
Slow
Intermediate
Fast
27-Jun
7-Jul
17-Jul
27-Jul
6-Aug
Date
B
Temperature (°C)
25
20
15
10
5
0
Slow
Intermediate
Fast
Site

Figure 3.5

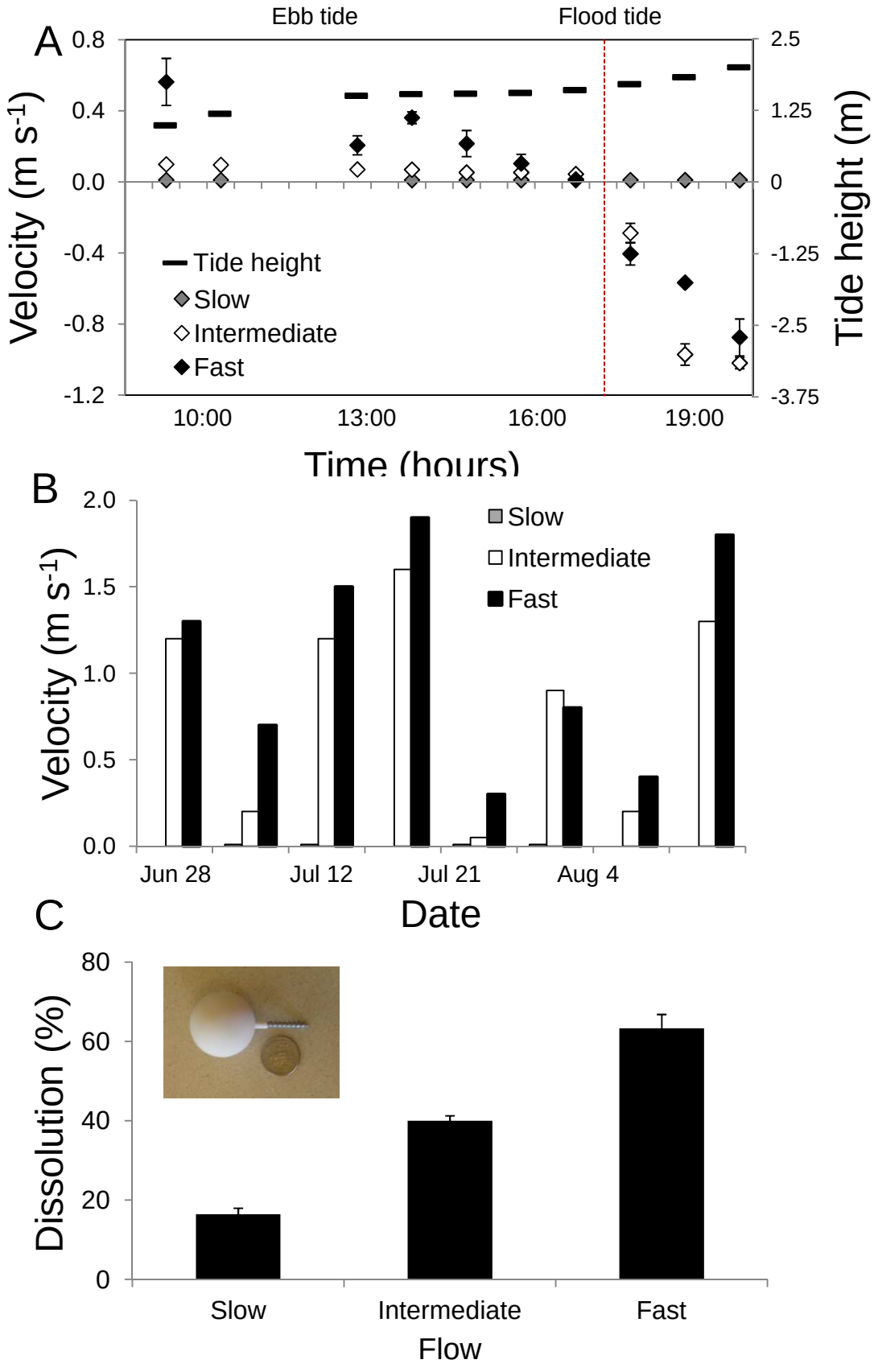
A
Ebb tide
Flood tide
Velocity (m s-1)
Tide height (m)
0.8
0.4
0.0
-0.4
-0.8
-1.2
2.5
1.25
0
-1.25
-2.5
-3.75
Tide height
Slow
Intermediate
Fast
10:00
13:00
16:00
19:00
Time (hours)
B
Velocity (m s-1)
2.0
1.5
1.0
0.5
0.0
Slow
Intermediate
Fast
Jun 28
Jul 12
Jul 21
Aug 4
Date
C
Dissolution (%)
80
60
40
20
0
Slow
Intermediate
Fast
Flow

Figure 3.6

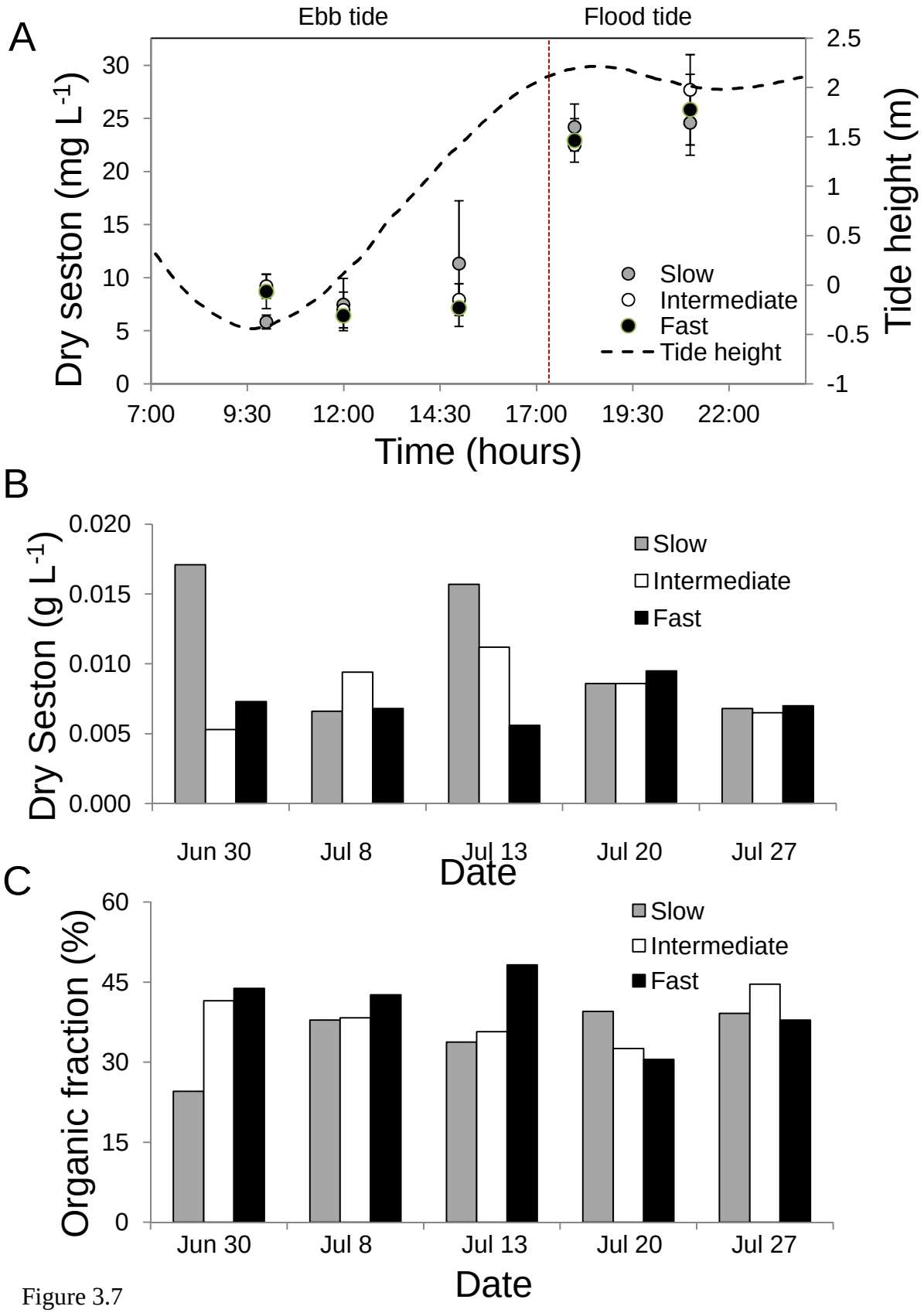
A
Ebb tide
Flood tide
Dry seston (mg L-1)
Tide height (m)
Slow
Intermediate
Fast
Tide height
7:00
9:30
12:00
14:30
17:00
19:30
22:00
Time (hours)
B
Dry Seston (g L-1)
Slow
Intermediate
Fast
Jun 30
Jul 8
Jul 13
Jul 20
Jul 27
Date
C
Organic fraction (%)
Slow
Intermediate
Fast
Jun 30
Jul 8
Jul 13
Jul 20
Jul 27
Date

Figure 3.7

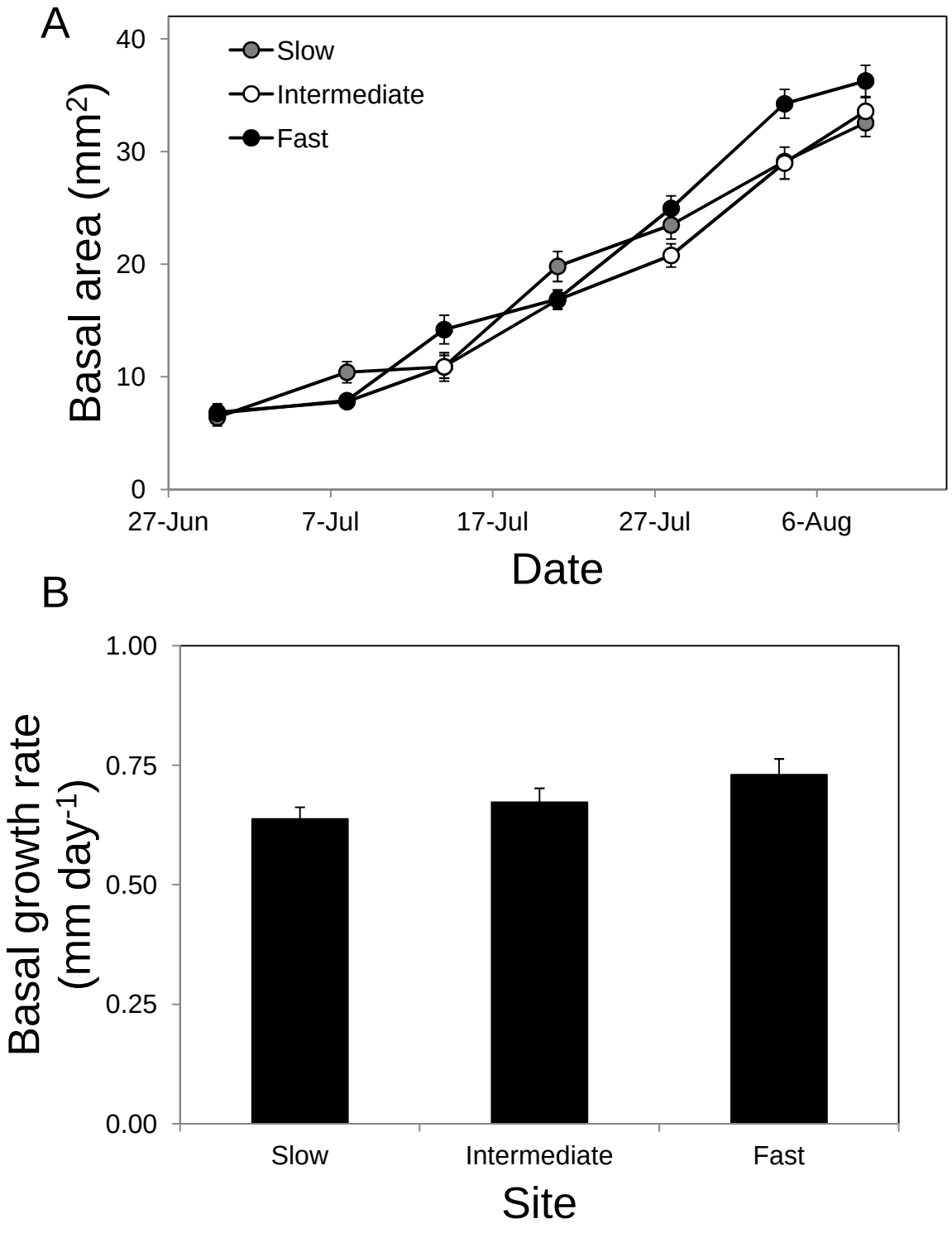
A
Basal area (mm2)
40
30
20
10
0
Slow
Intermediate
Fast
27-Jun
7-Jul
17-Jul
27-Jul
6-Aug
Date
B
Basal growth rate (mm day-1)
1.00
0.75
0.50
0.25
0.00
Slow
Intermediate
Fast
Site

Figure 3.8

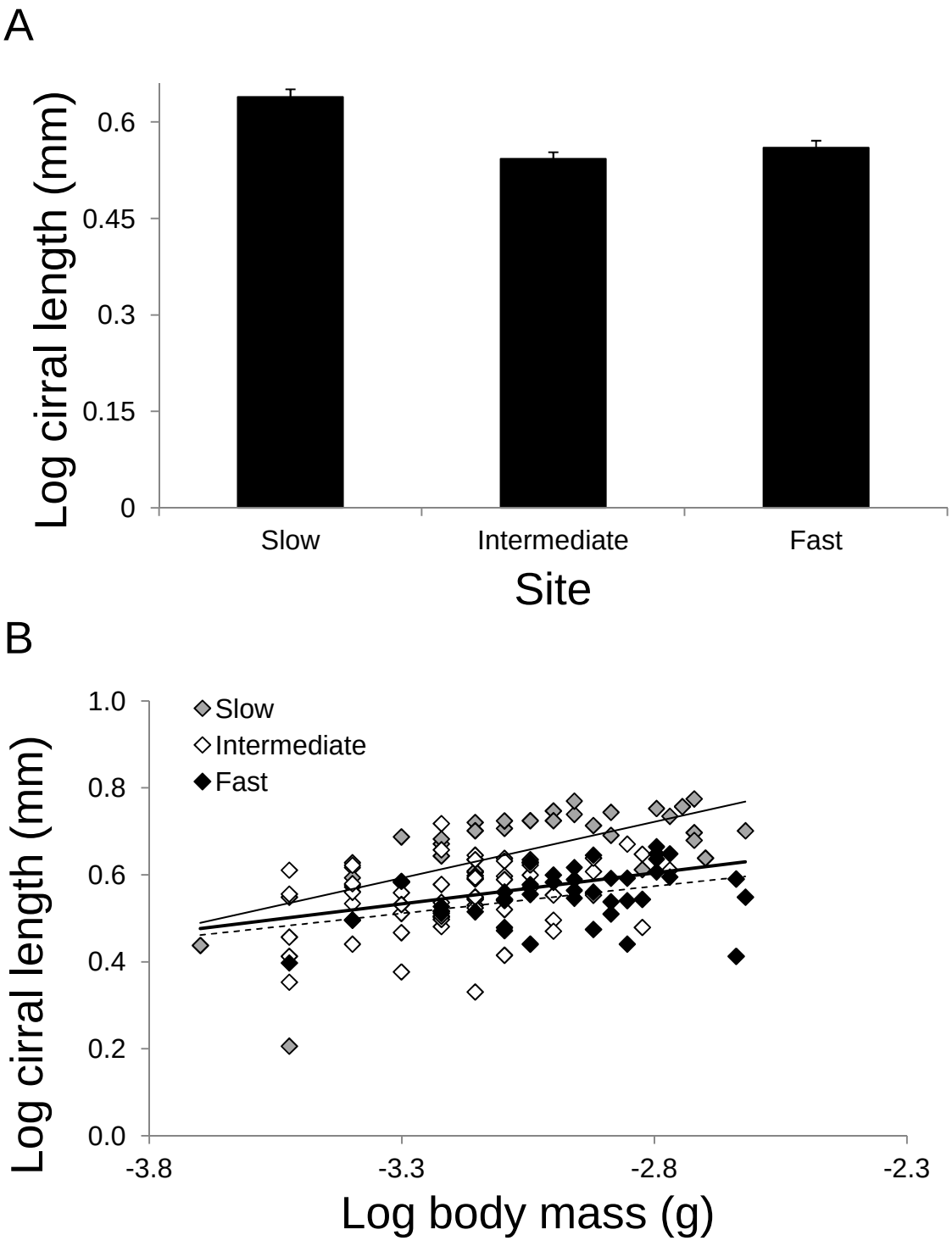
A
Log cirral length (mm)
0.6
0.45
0.3
0.15
0
Slow
Intermediate
Fast
Site
B
Log cirral length (mm)
1.0
0.8
0.6
0.4
0.2
0.0
Slow
Intermediate
Fast
-3.8
-3.3
-2.8
-2.3
Log body mass (g)

Figure 3.9

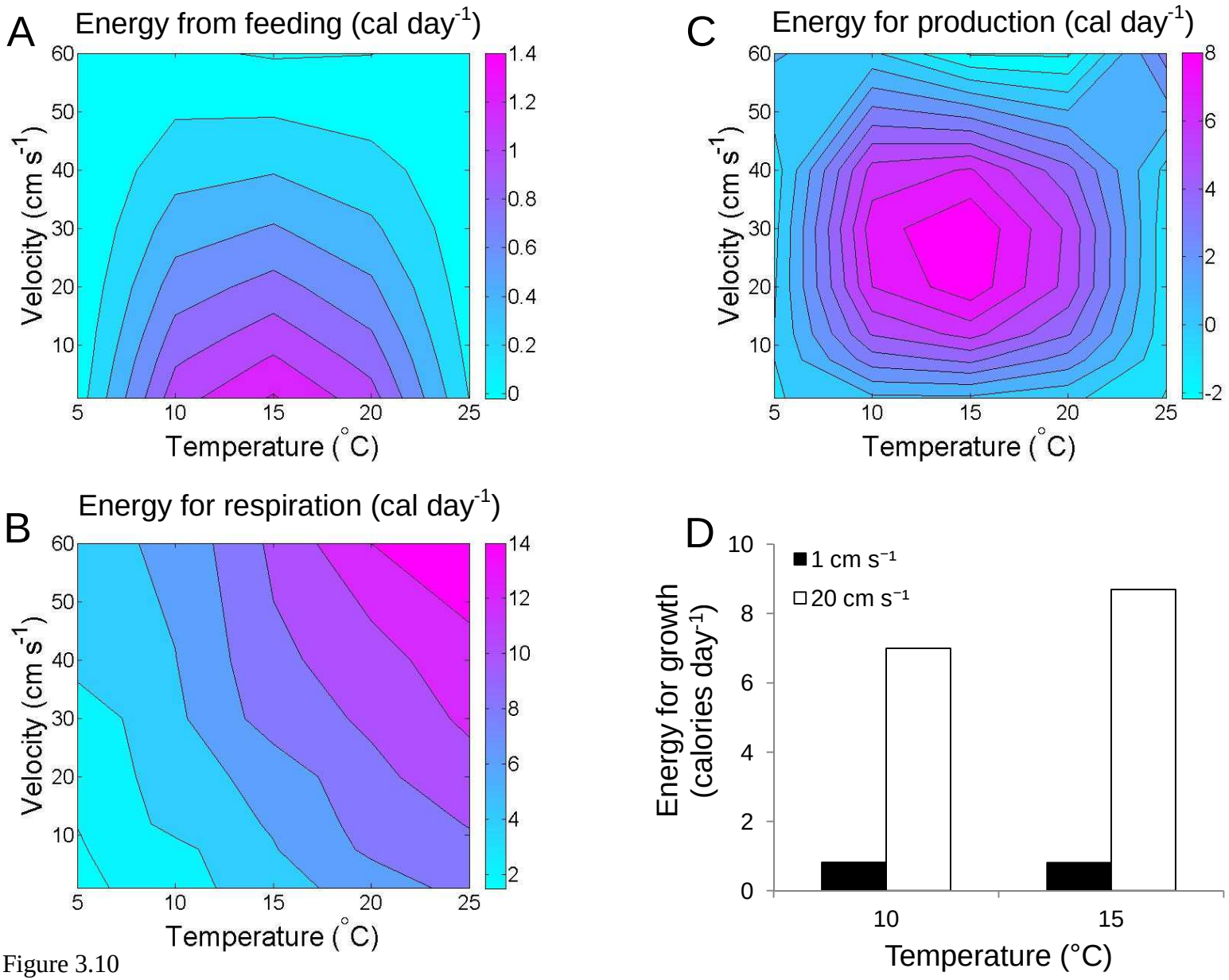
A
Energy from feeding (cal day-1)
Velocity (cm s-1)
Temperature (°C)
B
Energy for respiration (cal day-1)
Velocity (cm s-1)
Temperature (°C)
C
Energy for production (cal day-1)
Velocity (cm s-1)
Temperature (°C)
D
1 cm s⁻¹
20 cm s⁻¹
Energy for growth (calories day-1)
Temperature (°C)

Figure 3.10