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The effect of water temperature and flow on respiration in barnacles: patterns of mass transfer versus kinetic limitation.

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Summary

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, limitation by uptake reaction kinetics, when the biotic capacity of barnacles to absorb and process oxygen is slower than its physical delivery by mass transport, prevailed at high flows (40 to 150 cm s^{-1}) and low temperatures (5 to 15°C). Moreover, there are 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.

Key-words barnacles, intertidal, respiration, thermal stress, mass transfer limitation

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 (Bruce, 1926; Jansen et al., 2009; Matoo et al., 2013). 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, 1983).

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 et al., 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, 2010; 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 (Mass et al., 2010; Patterson et al., 1991). Thus, mass transfer between an organism and the water column should increase with faster flow. In contrast, the effects of water temperature on mass transfer rates are far less clear. Cooler waters contain more dissolved oxygen and diffusivity, 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 opposing processes, increases in diffusivity appear to have the most profound influence on oxygen mass transfer (Denny, 1993).

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. Barnacles possess a chitinous cuticle (Koulish and Klepal, 1981) that potentially slows oxygen uptake as diffusion through chitin is much slower (4%) than diffusion through water (Krogh, 1919). Haemolymph circulation represents an additional rate limiting step as haemolymph pressure in balanamorphs is low and circulation is largely driven by body/cirral movements as opposed to a heart (Waite and Walker, 1986). As respiratory pigments are generally absent in balanomorph barnacles (Southward, 1963; Waite and Walker, 1988), it is likely that diffusion across the cuticle and circulation represent the most important aspects of kinetic limitation.

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; Brown and Carpenter, 2013) 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, 2007; 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, 2007; 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.

Results

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).

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. 2). Respiration rates rose more rapidly from 5 to 15°C than 15 to 25°C (Temperature coefficients (Q_{10}) averaged over all flows (+/- SEM) = 2.69 ± 0.25 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. 2). 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. 2).

Sherwood number - Reynolds number analysis.

Sherwood numbers (Sh)-Reynolds number (Re) plots indicate that oxygen uptake was flow-dependent at slow water velocities (≤ 7.5 cm s^{-1}; Fig. 3), as evidenced by high Re exponents across all temperatures (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 relatively flow-independent. Alternatively, Sh derived from fluid transport processes displayed higher Re exponents at both low and high flows (1.01 ± 0.04 for velocities ≤ 7.5 cm s^{-1} and 0.98 ± 0.013 for velocities > 7.5 cm s^{-1}).

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. 4). 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.

Cirral beating behavior

At low temperature (5°C), the frequency of both abbreviated and extended beating behaviors were limited (< 23% and 26% respectively; Fig. 5). 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.

Discussion

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} = 2.69 ± 0.25 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 & 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 & McMahon 1981), and shore crabs (Q_{10} = 2.2 to 2.4; Greenaway et al. 1996).

For barnacles under kinetic limitation (Fig. 4), respiration rates had temperature coefficients (Q_{10}) = 2.92 from 5 to 15ºC and 1.42 from 15 to 25ºC. To what extent do these respiratory changes match known responses in biochemical/physiological processes? Oxygen dissociation rates for myoglobins in teleost fish have Q_{10} = 1.17 to 2.36 for temperatures ranging from 5 to 20ºC (Cashon et al., 1997). Diffusion of oxygen through peritoneum tissue

(vertebrate) had Q_{10} = 1.09 for temperatures ranging from 15 to 36ºC (Krogh, 1919). Similarly, activity rates of citrate synthase (CS), a pace-making enzyme in the Krebs Cycle, are also temperature-dependent. In crustaceans, CS Q_{10} ~ 1.78 to 1.84 between 5 and 15ºC and 1.56 to 1.61 between 20-30ºC (Salomon and Buchholz, 2000). Moreover, the relative magnitudes of our respiration rates appear to be generally consistent with the rate of known biochemical/physiological processes. 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. 2). 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. 4). 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 has been reported for coral respiration (Edmunds, 2005) where respiration rates were independent of flow (kinetic limitation) at low temperatures, but were dependent on flow (mass transfer limited) at high temperatures (Edmunds, 2005). A second example of kinetic limitation comes from measurements of nutrient uptake and photosynthesis in the freshwater plant, *Vallisneria americana* (Nishihara and Ackerman, 2009). The thermal maximum for photosynthesis 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.

Respiration under kinetically-limited conditions is potentially limited by both O_2 absorption/circulation rates and reduced metabolic cost. In decapod crustaceans, low temperatures (1°C) result in decreased oxygen haemolymph concentrations, lower circulation rates and increased anaerobic respiration (Frederich and Pörtner, 2000). This suggests that aerobic activity was limited by circulation rate rather than decreased metabolic demand. For

barnacles at low temperatures, slow cirral movement was observed, suggesting that low circulation rates may be a limiting factor.

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). Furthermore, if one considers a 1.5°C rise in sea surface temperature as is predicted over the next century (Meehl et al., 2011), the time that barnacles experience mass transfer limited conditions rises to 6.3%.

Although body temperatures do not directly correlate with air temperatures (Helmuth 1998, Denny and Harley 2006), it is likely that conditions of mass transfer limitation exist for many barnacles under field conditions.

Cirral behavior

Temperature influenced the proportion of barnacles displaying extended beating (i.e., thermal optima at 20°C; Fig. 5), consistent with many related species of barnacle (see Anderson & 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 & 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.

Materials and methods

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.

Measuring respiration rate.

Experiments were conducted in a closed, recirculating flow chamber of 600 mL volume (Fig. 6). 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.

Analysis

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

$$Q_{10} = \left(\frac{R_1}{R_2}\right)^{\left(\frac{10}{T_1 - T_2}\right)} \quad (1),$$

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).

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, 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 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 may underestimate the potential for advective mass transfer. For comparison, Sh numbers were calculated using a form of h_m that relates the ability of diffusion versus fluid flow to deliver oxygen (see β_{rough} in equation 7 in *Determining mass transfer vs. kinetic limitation*).

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

$$Sh = aRe^b \quad (4),$$

where *a* is an empirical coefficient that is dependent 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 4.

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 estimated according to Sanford and Crawford (2000) as,

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

Where F = flux (µmol m^{-2} s^{-1}), β is the mass transfer velocity (m s^{-1}), C_∞ is the bulk fluid concentration (µmol O_2 ml^{-1}) at a distance from the boundary and C_0 is the concentration at the boundary (µmol O_2 ml^{-1}). C_∞ was measured from the free stream portion of the flow chamber.

Flux over a rough surface (i.e., barnacles), however, can be calculated according to Bilger and Atkinson (1992) as,

$$F_{rough} = St_{m,\,rough}\, U_b\, (C_\infty - C_0) \quad (6),$$

Where $St_{m,\,rough}$ is the Stanton number (a non-dimensional ratio of O_2 flux to advection past an object) for mass transfer in flow over a rough surface and U_b is the bulk velocity of seawater in the flow chamber (cm s^{-1}).

Mass transfer velocity can be calculated as the product of the Stanton number and bulk velocity (Cussler, 2009; Sharma, 2007),

$$\beta_{rough} = St_{m,rough}\, U_b \quad (7),$$

with (Bilger and Atkinson, 1992),

$$St_{m,\,rough} = E\, St_{m,\,smooth} \quad (8),$$

Where E is the enhancement factor for a rough surface (nondimensional), which can be calculated as (Bilger and Atkinson, 1992),

$$E = 1.94\, Sc^{0.09}\, Re_{rough}^{-0.10} \quad (9),$$

Where Sc is the Schmidt number, a non-dimensional ratio between the diffusivity of momentum to that of molecules. Re_{rough} is the roughness Reynolds number for seawater flowing over barnacles (nondimensional; Bilger and Atkinson, 1992),

$$Sc = \nu / D \quad (10),$$

$$Re_{rough} = (u_* k') / \nu \quad (11),$$

Where D is O_2 diffusivity ($m^2\ s^{-1}$), u_* is friction velocity ($m\ s^{-1}$) and k' is the height of roughness elements (m). Friction velocity over barnacles was estimated at each velocity using the linear relationship between U and u_* based on values reported in the literature for water flowing over a rough surfaces (Fig. 7; $u_* = 0.1112U + 0.3448$). As the data are limited to 30 cm s^{-1}, a linear extrapolation was assumed at higher water velocities.

Stanton number for flow over a smooth surface can be calculated as (Bilger and Atkinson, 1992),

$$St_{m,\ smooth} = \left(\frac{c_f}{2}\right)^{-1/2} (0.0575\ Sc^{-2/3} + 0.1184\ Sc^{-1}) \quad (12),$$

with,

$$\left(\frac{c_f}{2}\right)^{-1/2} = -5.1 \log_{10}\left(\frac{6.9}{Re_{smooth}}\right) \quad (13),$$

Where c_f is the friction coefficient.

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

$$R = \frac{V_m C_0}{K_m + C_0} \quad (14),$$

where R is the reaction rate (μmol $O_2\ m^{-2}\ h^{-1}$), V_m is the maximum uptake rate (μmol $O_2\ m^{-2}\ h^{-1}$) and K_m is oxygen concentration at which the uptake rate is one half of its maximum (μmol $O_2\ m^{-3}$). 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 14,

$$R = \frac{V_m C_0}{K_m + C_0} = \beta(C_\infty - C_0) \quad (15),$$

A non-dimensional solution (see Sanford & Crawford 2000 for further details) is derived by dividing equation 15 by $V_m C_\infty / K_m$,

$$\frac{RK_m}{V_m C_\infty} = \left[\frac{C_\infty}{K_m} + \frac{1}{2}\left(\gamma + \sqrt{\gamma^2 + 4\frac{C_\infty}{K_m}}\right)\right]^{-1} \quad (16),$$

where

$$\gamma = 1 + \left(\frac{\beta K_m}{V_m}\right)^{-1} - \frac{C_\infty}{K_m} \quad (17),$$

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,

$$\frac{\beta K_m}{V_m} < \frac{0.25}{1 + \frac{C_\infty}{K_m}} \quad (18),$$

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

$$\frac{\beta K_m}{V_m} > \frac{4 + 0.8\frac{C_\infty}{K_m}}{(1 + \frac{C_\infty}{K_m})^2} \quad (19),$$

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.

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).

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List of Figures

Figure 1. Seasonal water temperatures from Argyle Creek, Washington, USA from June 2011 to August 2012. Water temperatures were sampled every 15 minutes.

Figure 2. Response in respiration rate as a function of water temperature and velocity for *Balanus glandula*. Colored lines represent different temperatures. Error bars represent one standard error, N = 3 plates.

Figure 3. Non-dimensional plot of Sherwood number as a function of Reynolds number. Values based on total oxygen consumption rates are represented by diamonds and crosses represent Sherwood numbers based on advective/diffusive transport.

Figure 4. Non-dimensional mass transfer coefficient ($\beta K_m/V_m$) plotted against non-dimensional oxygen saturation (C_∞/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 and the hatched line represents the lower limit for conditions of kinetic limitation. β is the mass transfer velocity (m s^{-1}), V_m is the maximum uptake rate (μmol O_2 m^{-2} h^{-1}), K_m is oxygen concentration at which the uptake rate is one half of its maximum (μmol O_2 m^{-3}) and C_∞ is oxygen concentration in the bulk flow.

Figure 5. Cirral behavior of barnacles under varying water temperatures and velocities. Behaviors, as described by Anderson & 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. Error bars represent one standard error. N=3 plates.

Figure 6. 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

773 barnacles was less than 0.6% h^{-1}. Barnacle behavior was recorded directly to a computer (PC2)
774 in digital video format. Dimensions are not to scale.

775 Figure 7. Friction velocity (u_*) as a function of free stream water velocities (U) over
776 aggregations of benthic aquatic invertebrates (corals, marine/freshwater mussels and oysters).
777 Data are from (Ackerman et al.; Finelli et al., 2007; Frechette et al.; Matoo et al., 2013 [inferred
778 from Figure 2]).

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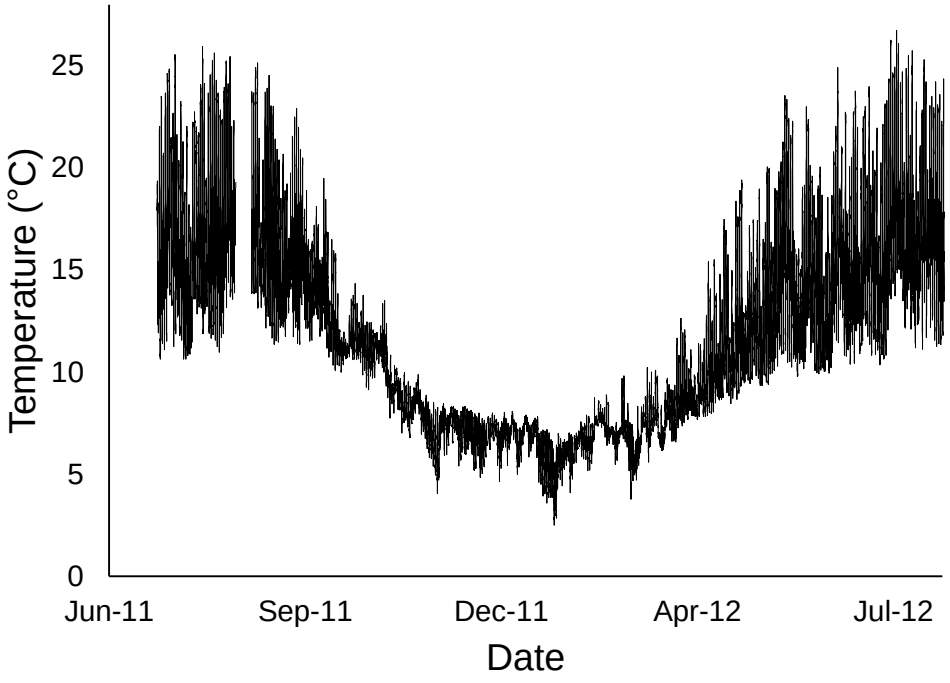

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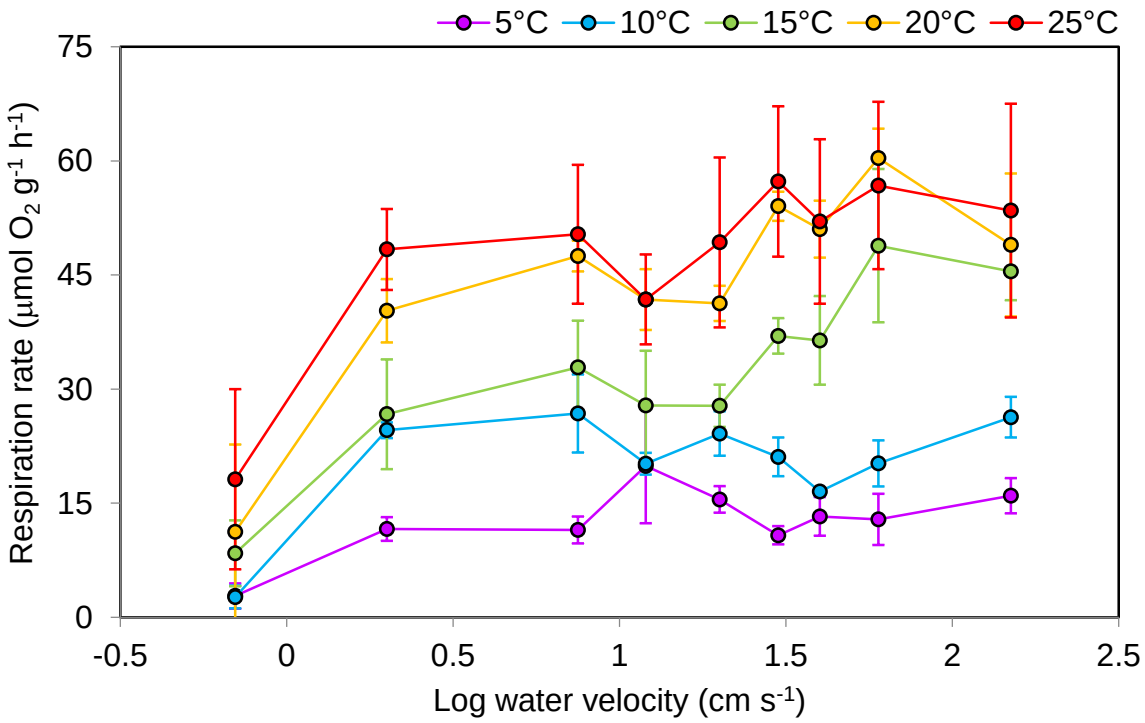

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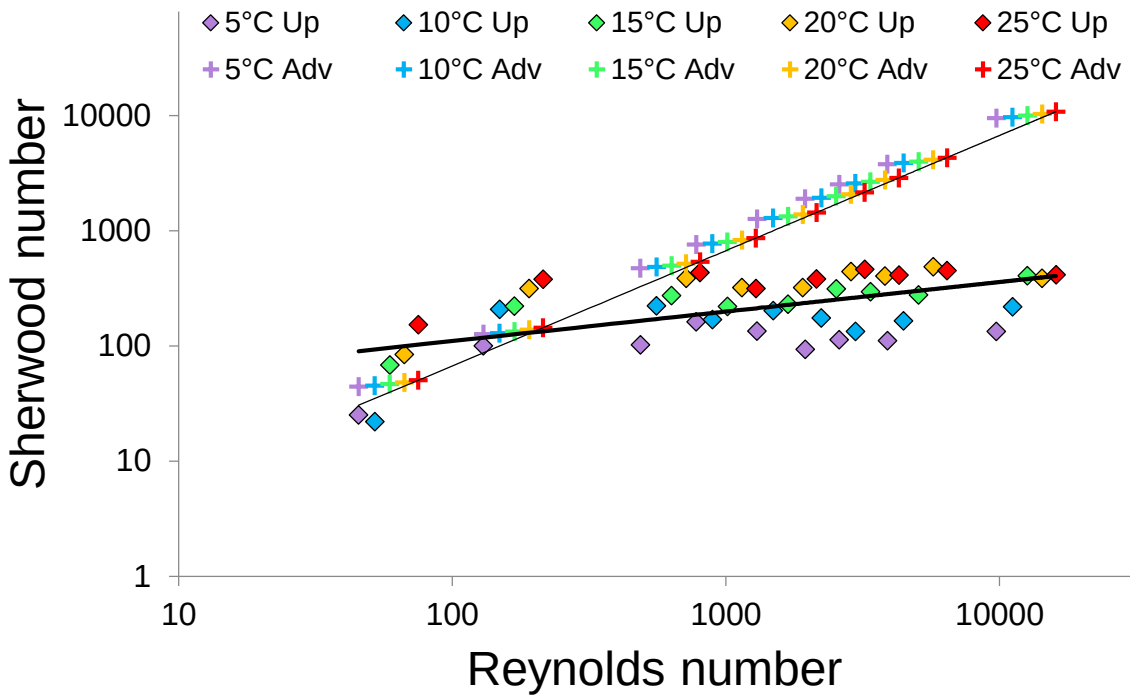

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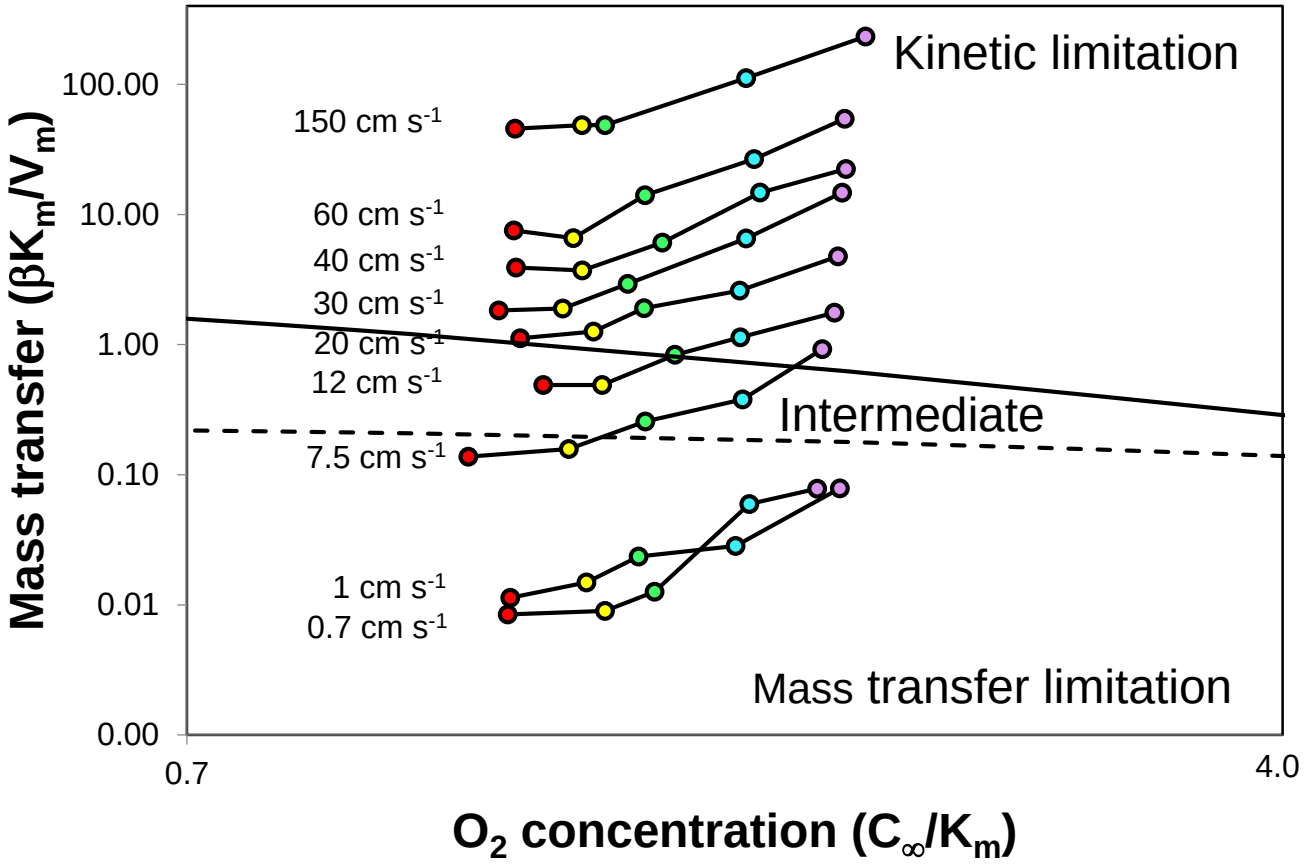

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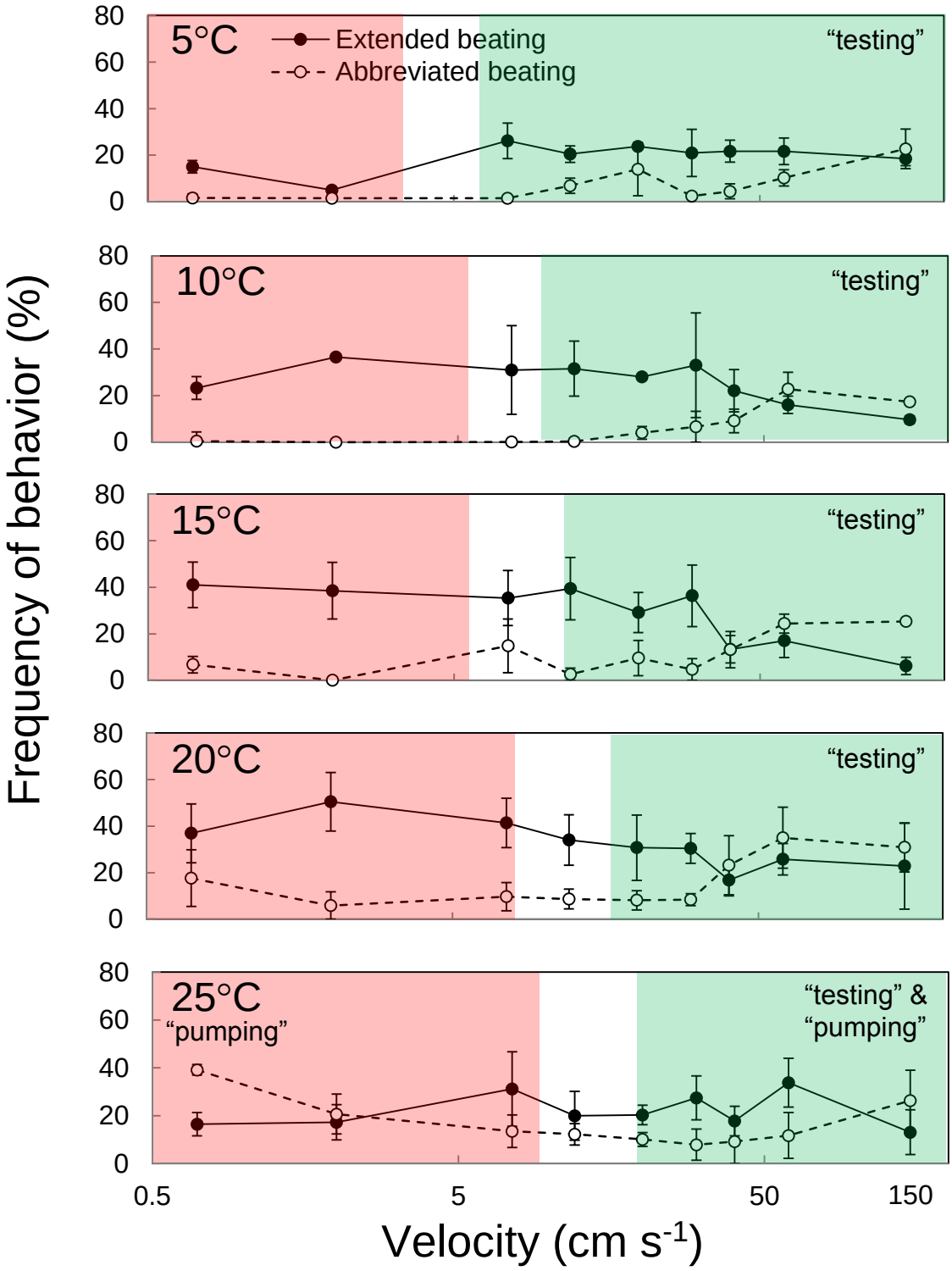

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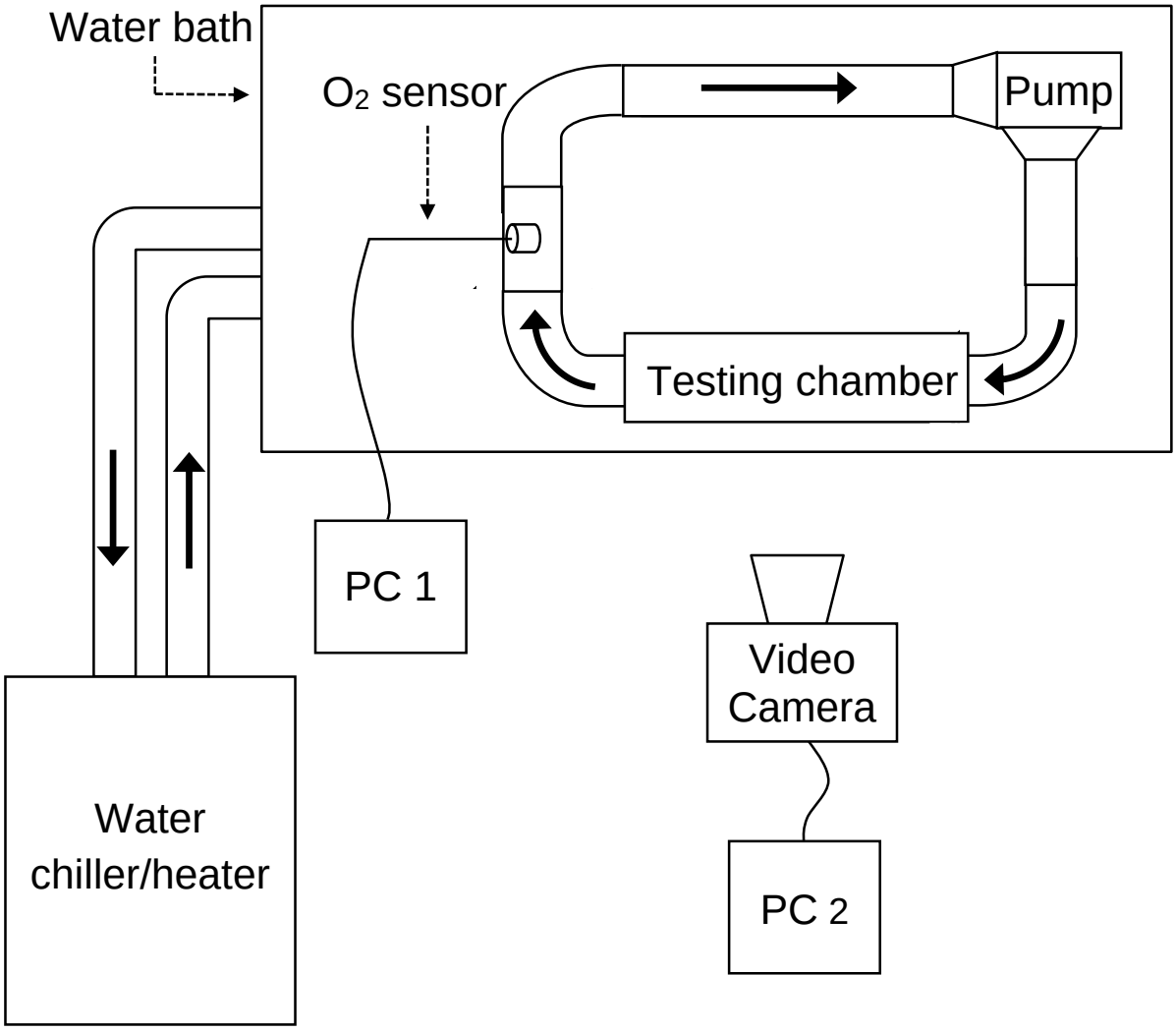

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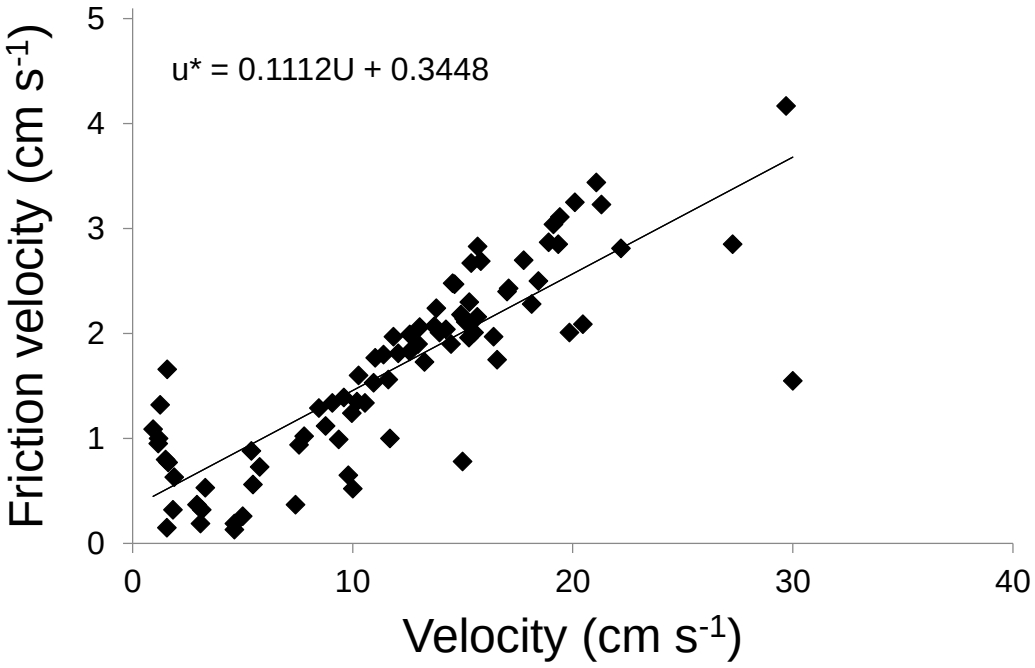

Figure 7.