Physiological Responses to Water Temperature and Flow in Three Marine Mussel Species

Ruairi Brown[1,2]

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[1] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

[2] Department of Biology, Carleton College, Northfield, MN 55057

Contact information: Ruairi Brown

Department of Biology

Carleton College

300 N College St.

Northfield, MN 55057

brownr2@carleton.edu

Abstract

Many marine organisms must contend with the physiological challenges associated with warming water temperatures. This is especially true in nearshore ecosystems where organisms often live near their thermal limits. However, physiological responses to thermal stress may vary depending on local flow conditions that can influence rates of gas exchange with surrounding waters. As such, I examined the influence of both water temperature and flow on respiration rate in *Mytilus californianus* and compared the responses to those found in two congeneric *M. trossulus,* and *M. galloprovinicalis*. Aquatic respiration rates were quantified at five different temperatures (5, 11, 17, 23, and 29 °C) and water velocities (2, 4, 6, 10, 20 cm s^{-1}) in a fully factorial design. Respiration was highest in *M. californianus,* followed by *M. trossulus,* and the lowest rates were measured in *M. galloprovincialis*. Respiration rates in *M. californianus* were relatively consistent under different temperatures and flows, whereas *M. trossulus* and *M. galloprovincialis* displayed optimal peak patterns. We discuss these physiological patterns in the context of their different ecologies.

Introduction

Since 1880, global temperatures have risen an average of 1.1°C (Dahlman et al., 2023). As a direct consequence, the ocean has absorbed at least ninety percent of the additive heat, causing global ocean temperatures to warm by approximately 0.8°C (Aral et al., 2016; IPCC, 2019). The combination of increased ocean temperatures and more frequent marine heatwaves pose extreme threats to marine life (IPCC, 2019). Organisms which experience these warm temperatures may react in one of several ways: 1) the organism may die (Sorte et al., 2016); 2) their distribution may shift (Barry et al., 1995; Sorte et al, 2016; Sunday et al., 2012;); 3) they may experience long-term evolutionary adaptations (Sunday et al., 2012) or; 4) they may experience short-term physiological change (Tang et al., 2018). Physiological performance, as a sublethal response, occurs on ecological scales pertinent to the individual. Increased temperatures significantly impact rocky intertidal organisms by altering key physiological processes including respiration, growth, reproduction, and overall life span (Ackerman and Nishizaki, 2004; Petes et al. 2008). For example, mussels are common in rocky intertidal ecosystems and often live near their upper thermal limits and endure higher temperatures during periods of low tide (Somero, 2002; Marshall et al., 2018). Consequently, it is necessary to understand the impact of thermal stress on the physiology of intertidal organisms.

A common approach to assess thermal stress in ectotherms involves the construction of thermal performance curves (Huey et al., 1989). Thermal performance curves are used to determine how an individual's physiological performance changes as a result of temperature variation (Figure 1). They depict a range of temperatures in which the organism can survive, often called the "tolerance zone" (Angilletta, 2006). There is an "optimal" temperature, where the organism's physiological performance is maximized (Huey et al., 1989). Studying the response of intertidal organisms to environmental stress through the use of thermal performance curves is critical in understanding the potential impacts of climate change on marine communities.

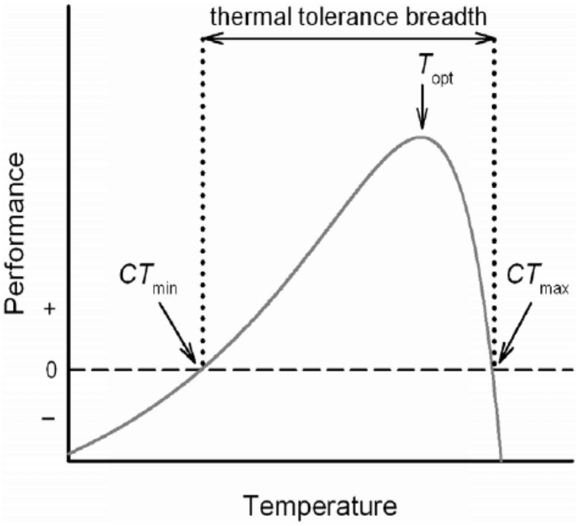

Figure 1. Thermal Performance Curve. CT_{min} and CT_{max} indicate the two critical temperatures beyond which the organism cannot survive. T_{opt} indicates the optimal temperature for the organism. (Krenek et al., 2012)

Mytilid mussels, found on rocky coasts throughout the world, serve as excellent model organisms due to their role as both foundation species and ecosystem engineers (LaBarre et al., 2023; Buschbaum et al., 2009). As foundation species, mytilids aggregate in beds which other marine organisms use as shelter from stresses related to waves and desiccation (Jurgens et al., 2018; Commito et al., 2005). In addition, mussels have filtering abilities which allows them to create a cleaner environment for themselves and other organisms (Tang et al., 2018). One mussel alone possesses the ability to filter approximately fifteen gallons of seawater per day (Hurlburt and Hurlburt, 1975). As mytilid mussels live in the intertidal zone, they experience a range of environmental conditions (Stillman, 2000). These characteristics underscore mytilid mussels' ecological importance, and it is necessary to investigate how rapidly changing environmental factors may or may not affect mussels' continuing existence in the age of climate change.

In addition to temperature, water motion also impacts a mussels' physiological performance (Leigh et al., 1987). In areas of high flow, mussels typically form aggregations to compensate for hydrodynamic forces associated with high water velocities (Carrington, 2008). These aggregations are not only beneficial to the mussels, as they increase the rate of fertilization, but also protect other organisms from predators (Christenson et al., 1970). In areas of low flow, however, there are less mussels and therefore less areas of shelter for other smaller species (Leonard et al., 1998). These areas of low flow also hinder mass transfer of dissolved oxygen between the surrounding water and the mussel (Figure 2). The area directly outside of the mussel aggregation is also characterized as an area of lower flow, called the boundary layer (Figure 2). Aerobic respiration decreases dissolved oxygen concentrations in the water, and it is increasingly difficult for dissolved oxygen to diffuse inside the boundary layer because of low flow conditions (Vogel, 1983). Furthermore, during low tide, intertidal mussels may experience a combination of low flow and warmer temperatures, which is demanding for their respiratory systems. It is still unclear, however, whether increased water flow outside of the boundary layer could aid the mussels by allowing more oxygen to diffuse inside the boundary layer and remove the mussels' respiration product, dissolved carbon dioxide, from the mussels' vicinity.

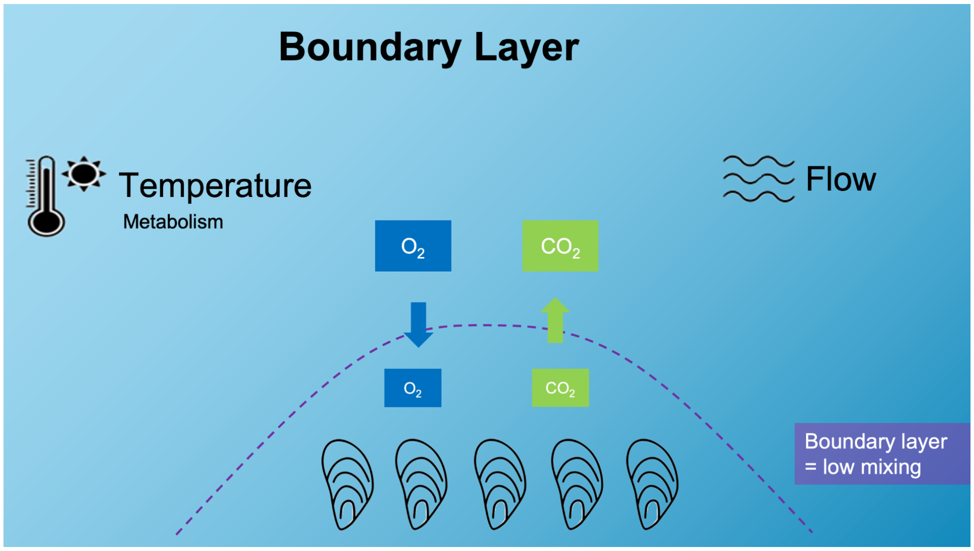

Figure 2. Boundary layer outside of mussel aggregation. Characterized by lower flow conditions.

The temperature of the water can have a direct effect on mussel respiration, or mussels' oxygen intake. The mussels' respiration typically has a directly proportional linear relationship to temperature, until they reach a critical temperature where they are no longer able to absorb the amount of oxygen they need, and their respiration decreases (SW et al., 2009). By plotting the relationship between water temperature and mussel respiration, the thermal performance curves can be used to determine both the thermal breadth and optimal temperatures of different species.

As these environmental stressors continue to increase as a result of climate change, it is necessary to quantify exactly how these stressors impact the physiological performance of organisms such as mussels. As many other physiological studies have focused on marine organisms' reactions to singular environmental stressors, it is crucial to take into account the many factors which influence the organisms' overall physiological performance. Through this experiment, the respiration rate of the California mussel, *Mytilus californianus*, was ascertained in relation to a variety of temperatures and water velocities. *M. californianus* is native to the west coast of North America, ranging from the Aleutian Islands to Baja California (Paggeot et al., 2022). In this study, thermal performance curves were produced to compare the effects of temperature and water velocity on physiological activity. This data will be used to compare with existing data for closely related species (*M. trossulus, M. galloprovincialis*). As *M. trossulus* is found in the Pacific Northwest and typically experiences cooler temperatures, we hypothesized that *M. californianus* has a higher optimal temperature and a wider range of thermal tolerance (Figure 4).

Materials and Methods

Animal Collection

M. californianus Conrad, 1837 were obtained from Cattle Point in Friday Harbor, WA (48° 28' 6.888" N, 123° 0' 30.9168" W). The mussels were maintained in seatables (32 × 66 × 135 cm) with a constant flow of seawater. Mussels were fed twice daily with a total of approximately 10 mL of Shellfish Diet 1800 TM (Reed Mariculture, Campbell, CA).

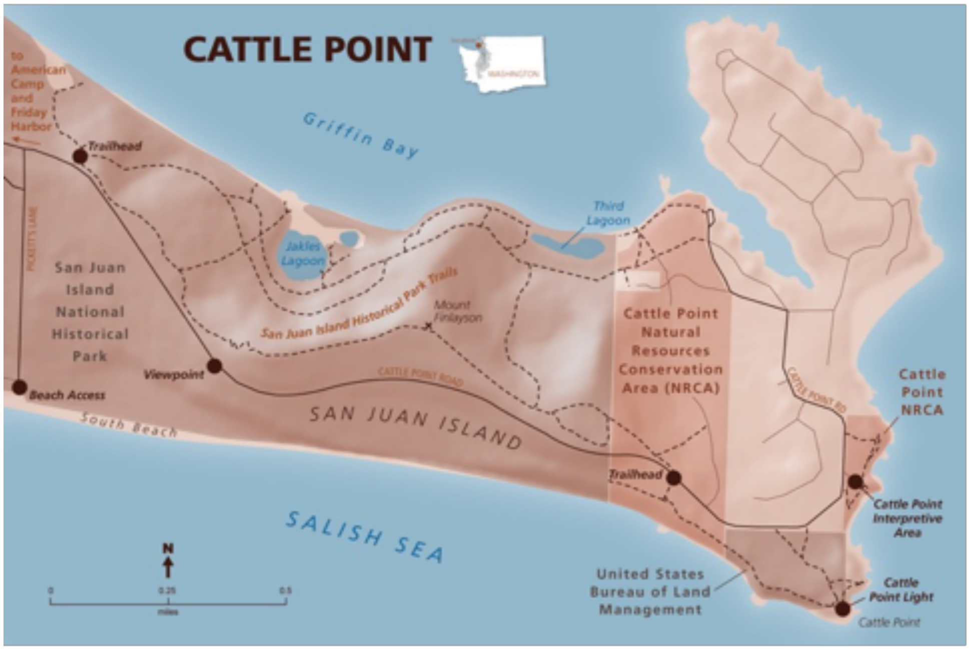

Figure 3. Cattle Point Map (wa100.dnr.wa.gov)

Measuring Respiration Rates

Respiration trials were performed in a test chamber connected to a submersible pump (500 GPH, Model 25D, Rule Industries, Gloucester, MA, USA) via PVC pipe (25.4 mm i.d.) and high-density polyethylene connector fittings (Figure 5). A cooler (Xtreme Marine Coolers, Coleman, Chicago, IL) was filled with 50 L of freshwater, and the temperature was controlled to 5, 11, 17, 23, and 29°C with a programmable, water heater and chiller (RTE 10 Circulating Chiller, Thermo, Neslab, MA). These temperatures were chosen based on summer temperatures seen in the intertidal zone (Figure 4).

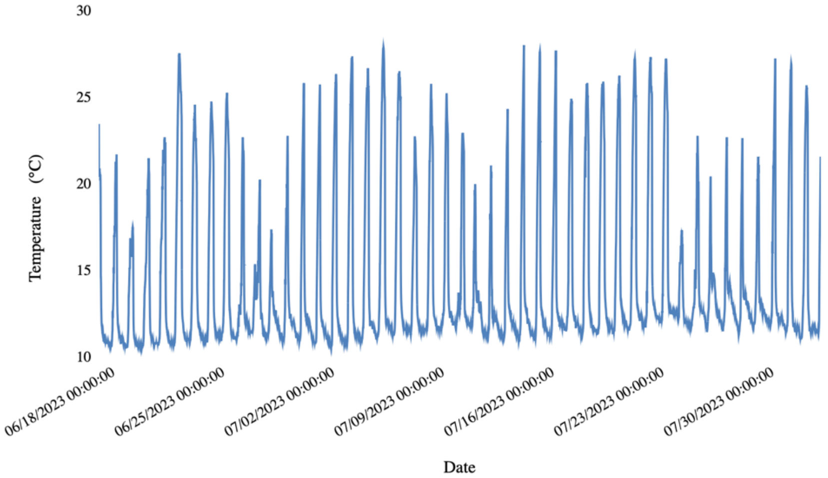

Figure 4. Cattle Point Tide Pool Summer Temperatures. Temperatures were recorded via a Onset HOBO® Bluetooth Pendant Temperature Data Logger sampled every 10 minutes.

Individual mussels were tested in a respiration chamber with sea water filtered to 1 micron. The chamber was then sealed and placed in the bottom of the cooler and completely immersed in fresh water. A series of water velocities (2, 4, 6, 10, 20 cm s^{-1}) were maintained by a submersible pump connected to a variable power supply (SPS3010, Kungber DC Power Supply Variable, location). Three replicates were conducted of each temperature-velocity combination. Changes in oxygen concentration were measured using an optical sensor system (either NeoFox Oxygen Sensing System, Ocean Optics, Dunedin, FL and FOSPOR-R RedEye patch, Ocean Optics, Dunedin, FL). Neofox software was used to record the data (NeoFox Viewer v.2.4, Ocean Optics, Dunedin, FL). Trials were run for a minimum of two hours to ensure that there was a steady linear decline in oxygen concentration.

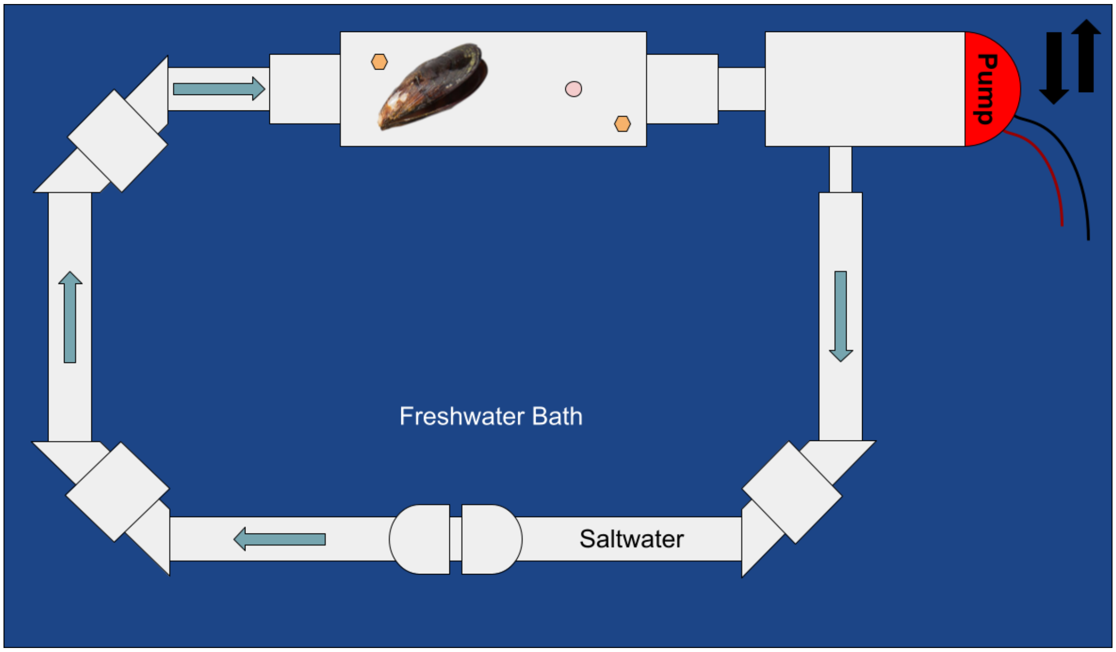

Figure 5. Recirculating respiration flow chamber (not to scale). The red semi-circle indicates the pump. The red and black lines illustrate the wires attached to the pump and power supply. Red circle on the chamber designates the sensor dot where the oxygen probe measures oxygen concentration. The orange hexagons specifies the short hexagon bolts which seal the chamber.

The blue arrows represent the flow of the water through the chamber via the pump and the two black arrows indicate the flow of water in and out of the cooler via the chiller.

Data Analysis

Each mussel's biomass was dried post-trial for at least 48 hours at 60°C in a Precision® convection oven. The R package *respR* was used to analyze the respiration data (Harianto, 2019). Respiration rates were standardized by the dry biomass of the mussels. A three-way ANOVA determining the influence of water temperature, water velocity, and mussel species on the respiration rate was performed.

Results

Both environmental stressors, water temperature and velocity, affected the *M. californianus'* respiration rate. Respiration rates increased from 5°C to 11°C and 17°C to 23°C, but decreased from 11°C to 17°C and 23°C to 29°C (Figure 6). Respiration rates were typically higher at extreme velocities as well, as average respiration rates across all temperatures were highest at 2 and 20 cm s^{-1} (Figure 6).

There was a significant effect of temperature on the mussels' respiration rates ($F_{(4,4)} = 9.36$, $p < 0.001$). Respiration rates typically peaked at both 11°C and 23°C (Figure 6). Flow also had a significant effect on the respiration rate of the *M. californianus* ($F_{(4,2)} = 2.64$, $p=0.0351$). The interaction between temperature and velocity was not significant ($p = 0.122$).

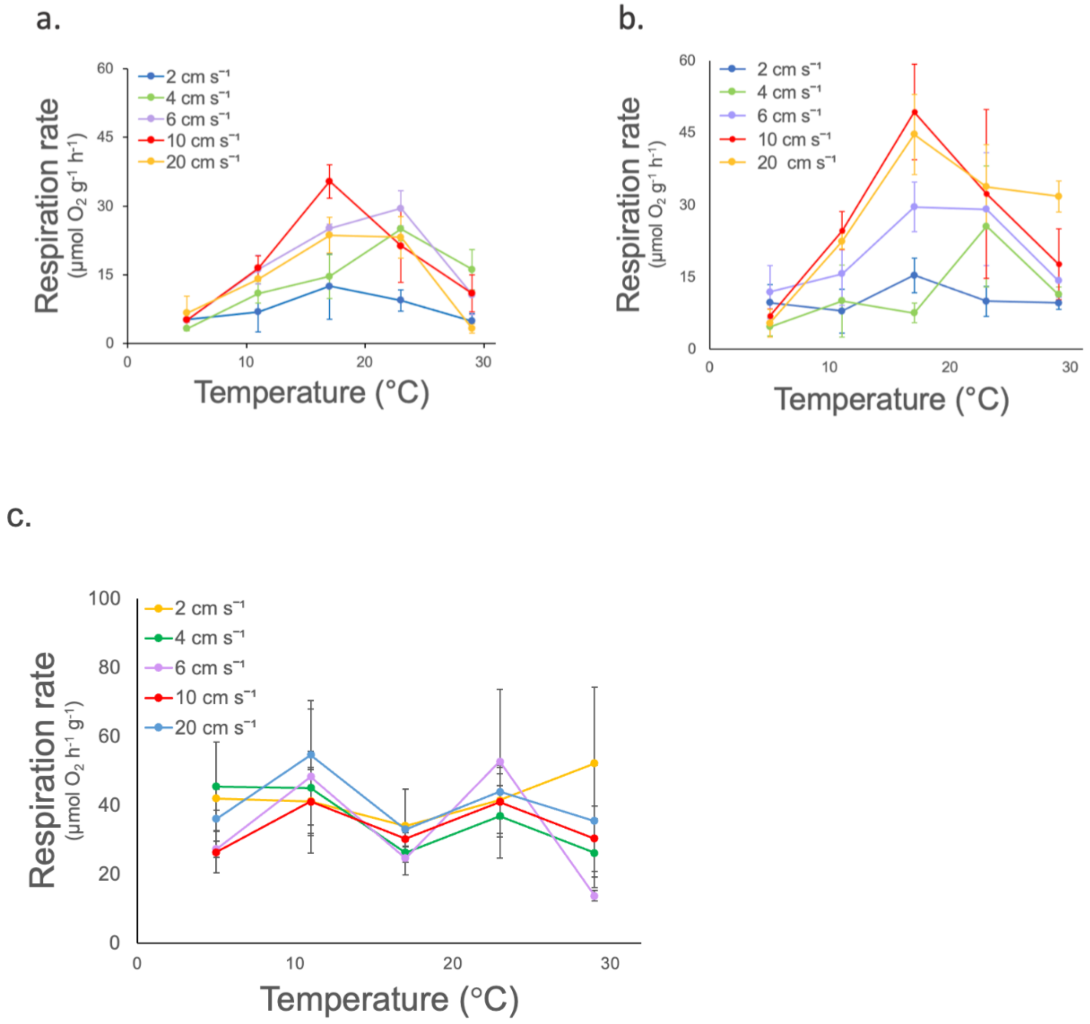

Figure 6. Respiration rates in mytilid mussels. A) *Mytilus trossulus* B) *M. galloprovincialis*. C) *Mytilus californianus.* Each dot represents 3 trials. Error bars indicate one standard error.

Comparing M. californianus, M. galloprovincialis and M. trossulus

Although *M. galloprovincialis* and *M. trossulus* demonstrated similar patterns according to their responses to the two environmental stressors, *M. californianus*' response to water temperature and velocity varied significantly. Unlike the other two species, *M. californianus*' respiration rates peaked bimodally at 11°C and 23°C and notably dipped at 17°C (Figure 6). A statistically significant effect of temperature on the respiration rate of *M. galloprovincialis, M.*

trossulus, and *M. californianus* was seen ($F_{(8,8)} = 3.16$, $p = 0.0021$). There was also a statistically significant effect of water velocity on the respiration rates of the three mussels ($F_{(8,8)} = 2.36$, $p = 0.0187$). The average respiration rates of the three species were calculated across all treatments. *M. californianus'* average respiration rate was 37.451 ± 2.145 µmol O_2 h^{-1} L^{-1} g^{-1}, *M. tross*' average respiration rate was 20.675 ± 1.823 µmol O_2 h^{-1} L^{-1} g^{-}, and *M. galloprovicinalis*' average respiration rate was 14.107 ± 1.183 µmol O_2 h^{-1} L^{-1} g^{-1}.

Discussion

It is necessary to determine how organisms' physiological performance may or may not be affected by rising temperatures associated with climate change. Environmental stressors such as water temperature and velocity can negatively affect intertidal organisms' physiological performance (Ackerman and Nishizaki, 2004). This study explored the impacts of these two environmental stressors on the California mussel, *M. californianus*. This project also compared the effects of these stressors on three different mytilid mussels: *M. californianus, M. galloprovincialis and M. trossulus.*

Ectotherms' physiological performance can be greatly influenced by temperature (Petes et al. 2008). This relationship was seen through the respiration rates of the *M. cali* mussels, where performance peaked at two temperatures (11°C and 23°C). Although the velocity of the water's flow also had a significant impact on the performance of the mussels, there were no clear trends among which velocities were more beneficial to organisms.

When using all treatments to compare respiration rates among species, we found that *M. californianus* had the highest average respiration rates, followed by *M. trossulus*, and lastly *M. galloprovinicalis*. The difference in respiration rates is possibly due to the species' different geographic locations. *M. californianus*' location stretches along the entire West Coast, which exposes the species to a broad spectrum of temperatures. Furthermore, due to *M. californianus'* relatively higher location in the intertidal zone, they may have longer periods of emersion, and presumably build up greater oxygen debt. When the mussels are immersed in the tide, they may be more motivated to open and pump water to replenish oxygen levels. As a result, the *M. californianus*' physiological performance might be less sensitive to temperature or flow conditions.

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Literature Cited

Ackerman, J. D. and Nishizaki, M. T. (2004). The effect of velocity on the suspension feeding and growth of the marine mussels Mytilus trossulus and M. californianus: implications for niche separation. *Journal of Marine Systems* **49**, 195–207.

Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology* **31**, 541–545.

Aral, M. M. and Guan, J. (2016). Global Sea Surface Temperature and Sea Level Rise Estimation with Optimal Historical Time Lag Data. *Water* **8**, 519.

Barry, J. P., Baxter, C. H., Sagarin, R. D., & Gilman, S. E. (1995). Climate-related, long-term faunal changes in a California rocky intertidal community. *Science*, *267*(5198), 672-675.

Buschbaum, C., Dittmann, S., Hong, J.-S., Hwang, I.-S., Strasser, M., Thiel, M., Valdivia,

Carrington, E., Moeser, G. M., Thompson, S. B., Coutts, L. C. and Craig, C. A. (2008).Mussel attachment on rocky shores: the effect of flow on byssus production. *Integrative and Comparative Biology* **48**, 801–807.

Christensen, H. T., Dolmer, P., Hansen, B. W., Holmer, M., Kristensen, L., Poulsen, L. K., Stenberg, C., Albertsen, C. M. and Støttrup, J. (2015). Aggregation and attachment responses of blue mussels, Mytilus edulis—impact of substrate composition, time scale and source of mussel seed. *Aquaculture* **435**, 245–251.

Commito, J., Celano, E., Celico, H., Como, S. and Johnson, C. (2005). Mussels matter: Postlarval dispersal dynamics altered by a spatially complex ecosystem engineer. *Journal of Experimental Marine Biology and Ecology* **316**, 133–147.

Dahlman, R. L. A. L. (2023). Climate change: Global temperature. *NOAA Climate.gov*.

Huey, R. B. and Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol Evol* **4**, 131–135.

Hurlburt, C. G. and Hurlburt, S. W. Blue Gold: Mariculture of the Edible Blue Mussel (*Mytilus edulis*).

Jansen, J., Hummel, H. and Wendelaar Bonga, S. (2009). The respiratory capacity of marine mussels (*Mytilus galloprovincialis*) in relation to the high temperature threshold. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* **153**, 399–402.

Jurgens, L. J. and Gaylord, B. (2018). Physical effects of habitat-forming species override latitudinal trends in temperature. *Ecol Lett* **21**, 190–196.

Krenek, S., Petzoldt, T. and Berendonk, T. (2012). Coping with Temperature at the Warm Edge – Patterns of Thermal Adaptation in the Microbial Eukaryote Paramecium caudatum. *PloS one* **7**, e30598.

LaBarre, A., Konar, B. and Iken, K. (2023). Influence of Environmental Conditions on Mytilus trossulus Size Frequency Distributions In Two Glacially Influenced Estuaries. *Estuaries and Coasts* **46**, 1253–1268.

Leigh Jr, E. G., Paine, R. T., Quinn, J. F., & Suchanek, T. H. (1987). Wave energy and intertidal productivity. *PNAS*, *84*(5), 1314-1318.

Leonard, G. H., Levine, J. M., Schmidt, P. R. and Bertness, M. D. (1998). Flow-driven variation in intertidal community structure in a Maine estuary. *Ecology* **79**, 1395–1411.

Marshall, D. J., Brahim, A., Mustapha, N., Dong, Y., & Sinclair, B. J. (2018). Substantial heat tolerance acclimation capacity in tropical thermophilic snails, but to what benefit?. *Journal of Experimental Biology*, *221*(22), jeb187476.

N., Yoon, S.-P. and Reise, K. (2009). Mytilid mussels: global habitat engineers in coastal sediments. *Helgol Mar Res* **63**, 47–58.

Paggeot, L. X., DeBiasse, M. B., Escalona, M., Fairbairn, C., Marimuthu, M. P. A., Nguyen, O., Sahasrabudhe, R. and Dawson, M. N. (2022). Reference genome for the California ribbed mussel, Mytilus californianus, an ecosystem engineer. *J. Hered.* **113**, 681–688.

Petes, L. E., Mouchka, M. E., Milston-Clements, R. H., Momoda, T. S. and Menge, B. A. (2008). Effects of environmental stress on intertidal mussels and their sea star predators. *Oecologia* **156**, 671–680.

Somero, G. N. (2002). Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living1. *Integrative and Comparative Biology* **42**, 780–789.

Sorte, C. J. B., Davidson, V. E., Franklin, M. C., Benes, K. M., Doellman, M. M., Etter, R. J., Hannigan, R. E., Lubchenco, J. and Menge, B. A. (2017). Long-term declines in an intertidal foundation species parallel shifts in community composition. *Global Change Biology* **23**, 341–352.

Stillman, J. H. and Somero, G. N. (2000). A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol Biochem Zool* **73**, 200–208.

Sunday, J., Bates, A. and Dulvy, N. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change* **2**, 686–690.

Tang, B. and Riisgård, H. U. (2018). Relationship between oxygen concentration, respiration and filtration rate in blue mussel *Mytilus edulis*. *J. Ocean. Limnol.* **36**, 395–404.

Vogel, Steven. 1983. *Life in Moving Fluids: The Physical Biology of Flow - Revised and Expanded Second Edition*. *Princeton: Princeton University Press*.