The Effects of Multiple Environmental Stressors on the Respiration Rate of *Mytilus galloprovincialis*

Abdisalan K. Hawadle 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:
Abdisalan Hawadle
Department of Biology
Carleton College
300 N College St.
Northfield, MN 55057
hawadlea@carleton.edu

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Abstract

Intertidal organisms are subject to environmental variations that may influence their physiological performance. As processes such as respiration depend on gas exchange between organisms and their environment, they are potentially affected by water temperature and velocity. In this study, we compare the effects of multiple environmental stressors (temperature and flow velocity) on the respiration rate in two mytilids, the Mediterranean mussel, *Mytilus galloprovincialis* and the temperate bay mussel *M. trossulus*. Thermal performance curves (5, 11, 17, 23, and 29 °C) for respiration rate were quantified at five different flow velocities (2, 4, 6, 10, 20 cm s^{-1}) in a fully crossed design. Well-defined thermal performance curves were present at moderate to high water velocities, whereas, at the lowest velocity (2 cm s^{-1}) respiration rates remained low across all temperatures. Although Mediterranean mussels displayed higher thermal optima than Bay mussels under moderate flow speeds (4-6 cm s^{-1}), those differences were absent at higher flow velocities (>10 cm s^{-1}). These results highlight the importance of considering hydrodynamic conditions when estimating thermal tolerance in marine mussels.

Introduction

Climate change is increasingly challenging the marine environment and threatening species that live in ocean environments around the world. The global average temperature has been rising at a rate of 1.7°C per century since 1970, and by 2100, it is projected that the ocean is likely to warm by two to seven times as much compared with the observed changes since 1970 (IPCC, 2019). Such changes can have significant effects on many nearshore organisms and thus, it is critical to understand how marine organisms will respond physiologically to these changes.

In the intertidal zone, marine organisms are exposed to environmental variation that can limit physiological performance (Arribas et al., 2014; Barbariol and Razouls, 2000). Although many marine physiology studies focus on the effects of single environmental stressors, organisms are exposed to multiple stressors in their natural environment which can affect growth, respiration, survival, and larval dispersal (Ackerman and Nishizaki, 2004; Todgham and Stillman, 2013). This is especially true for mytilid mussels given the fluctuating nature of the intertidal zone, making it essential to study the impacts of climate change-related stressors and their interaction.

Mussels in the genus Mytilus occur worldwide, inhabiting temperate coasts characterized by periods of strong tidal and wave-induced currents (Arribas et al., 2014; Widdows et al., 2002). These benthic suspension-feeding bivalves are ecologically and economically important members of the rocky intertidal zone for their role as ecosystem engineers (Arribas et al., 2014; Widdows et al., 2002). They are foundational species that form multilayered beds offering protection against waves and providing shelter for small organisms (Arribas et al., 2014; Commito et al., 2008). They also shape local environments by altering biotic and abiotic factors such as filtering the surrounding water to remove particles (Borthagaray and Carranza, 2007; Commito et al., 2008; Jorgensen, 1990).

In these environments, mussels provide ecosystem services by altering local habitats, biomass, and biodiversity (Commito et al., 2005; Commito et al., 2008). Furthermore, as an important aquaculture species, mussels are economically important as marine bivalves have a total market value of $23 billion (Smaal et al., 2019). The majority of marine bivalve production comes from aquaculture which is responsible for half of the global seafood production (Fuentes-Santos et al., 2021; Smaal et al., 2019). Understanding how benthic organisms like mussels cope physiologically to the harsh conditions present in nearshore habitats is important in predicting their distribution and abundance both today and in the future. Moreover, water temperature and velocity are two key factors when considering processes like respiration that depend on the uptake of oxygen. In this study, we examine the effects of water temperature and water velocity on *Mytilus galloprovincialis*, a Mediterranean species that is invasive to the Pacific Northwest, to understand how this species copes with environmental stressors in a non-native environment.

In mussels, respiration rate increases with increasing temperatures in a linear matter (Barbariol and Razouls, 2000; Bayne, 1976), however, depending on the temperature conditions tested, respiration rates in mussels can increase until a thermal optimum is reached, but begin to decrease as the metabolic demand of the mussel cannot be met beyond a temperature range. This would suggest there is a range of temperatures an organism can function and a range of temperatures where performance is maximal (Sinclair et al., 2016). As marine environments experience the effects of climate change, intertidal organisms can be pushed beyond their thermal optimum.

Another factor that can affect the physiological performance of mussels is flow velocity which can affect species distribution, feeding, and growth (Ackerman and Nishizaki, 2004; Leigh, 1987; Leonard et al., 1998). Areas of high flow are characterized by dense mussel beds that support higher consumer densities, whereas low flow sites are less mussel and predator dense (Leonard et al., 1998). The intertidal zone is subject to variable flow velocities which are important for transporting larvae nutrients, and gasses to benthic habitats (Leonard et al., 1998). In the areas of low flow, mussels continue to respire and decrease the amount of dissolved oxygen available for carrying out metabolic processes. This is a challenge for mussels because as temperatures increase, low flow can intensify the effects of temperature on performance as mussels will have a higher energetic demand which cannot be met in low flow environments.

To predict climate change's impact on mussels, it is essential to look at how organisms respond to multiple environmental stressors. Physiological studies involving multiple environmental stressors will likely produce outcomes that are more predictive of ecological consequences of climate change. In this study, the respiration rate of *M. galloprovincialis*, a Mediterranean mussel, to varying water temperatures and flow velocities was determined experimentally. As an intertidal organism, *M. galloprovincialis* experiences strong variable water temperatures (Kamermans and Saurel, 2022) and velocities (Widdows et al., 2002). This study looked at multiple factors in respiratory physiology to produce performance curves in a fully crossed design and compared *M. galloprovincialis* and *Mytilus trossulus*, a species native to the Pacific Northwest, using *M. trossulus* data obtained from a study examining the effects of temperature and flow velocity on the respiration rate of *M. trossulus*.

Materials and Methods

Specimen Collection

M. galloprovincialis Lamarck, 1819 were obtained from Penn Cove Shellfish (Coupeville, WA). Mussels were kept in seatables (135 × 66 × 32 cm, H×W×L) receiving a continuous and strong flow of natural seawater through plastic tubing connected to the Friday Harbor Laboratories (Friday Harbor, Washington) seawater system, which draws unfiltered seawater from a depth of 10 meters. The mussels were fed twice daily with XX mL of Shellfish Diet 1800TM (Reed Mariculture, Campbell, CA).

Respiration

Respiration trials were conducted 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. A 50 L cooler (Xtreme Marine Coolers, Coleman, Chicago, IL) was filled with water, and the temperature was maintained at 5, 11, 17, 23, or 29°C with a programmable, water heater/chiller (AP07R-20-A11B, Polyscience, Niles, IL, USA).

Individual mussels were placed in the airtight flow chamber filled with 1 micron filtered seawater (Fig. 1). The chamber was submerged in the temperature-controlled cooler. A submersible pump was connected to a programmable power source (9130 Triple Output Programmable DC Power Supply, B&K Precision, Yorba Linda, CA, USA) to control flow velocity.

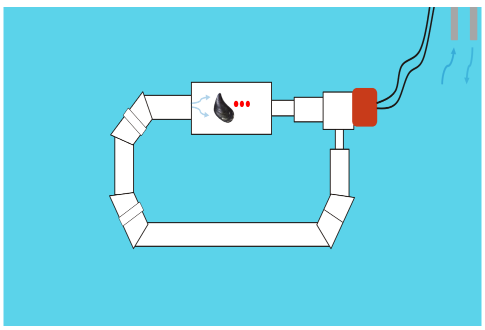

Figure 1. Schematic of closed-recirculating flow chamber. Blue arrows indicate water flow, red represents the submersible pump that generates the flow in the test chamber, and the three red dots indicate sensor patches for the optical sensing system to measure oxygen concentration.

Oxygen concentrations and temperature were measured via an optical sensing system (NeoFox Oxygen Sensing System, Ocean Optics, Dunedin, FL and FOSPOR-R RedEye patch, Ocean Optics, Dunedin, FL). Data was recorded using NeoFox Viewer software (NeoFox Viewer v.2.4, Ocean Optics, Dunedin, FL). Each respiration trial was conducted for two hours or until a stable respiration rate was reached. Three replicates of combinations of water temperature (5, 11, 17, 23, 29°C) and flow (2, 4, 6, 10, 20 cm s^{-1}) were conducted.

Analysis

Mussel dry mass was taken after each trial (Sartorius Electronic Analytical Balance, Sartorius, Göttingen, Germany) and dried for 48 hours at 60°C in a Precision® convection oven. Respiratory data for trials were analyzed in R software and the R package respR (Harianto, 2019). Respiration rates were standardized to the mussel tissue dry mass and the volume of the flow chamber. The effect of water temperature and velocity on respiration rate were analyzed with two-way ANOVA and comparisons between species were made using a three-way ANOVA. Thermal performance curves were created for each flow velocity for *M. galloprovincialis* and *M. trossulus* to determine maximum respiration rate, thermal optimum, and performance breadth using the R package rTPC (Padfield and O'Sullivan, 2021).

Results

Respiration rates changed in response to temperature and flow velocity. Respiration rates were low at 5°C and rose until reaching maximal respiration rate at 17°C or 23°C before decreasing (Fig. 2A). There was a general increase in respiration at lower temperatures before decreasing at higher temperatures. Respiration rates faced an overall increase corresponding with increasing flow velocity (Fig. 2).

There was a significant effect of temperature on respiration rates ($F_{(4,49)}=17.81$, $p<0.001$). At lower temperatures (5-11°C), respiration rates increased whereas, at higher temperatures (17-29°C), respiration rates generally decreased (Fig. 2). There was also a significant effect of flow on respiration rate ($F_{(4,49)}=5.77$, $p<0.001$). At low flow velocities, flow had little influence on respiration rate whereas flow had a much larger effect on respiration at higher velocities. There was no interacting effect of temperature and flow on respiration rate.

Comparing *M. galloprovincialis* and *M. trossulus*

Respiration rates in both species showed similar responses to temperature and water flow velocities. For both species, respiration rates were lower at low temperatures and increased until rates peaked between 17°C or 23°C before decreasing at higher temperatures (Fig. 3). Respiration rates generally increased with increasing water velocity (Fig. 3).

There was a statistically significant effect of temperature on the respiration rate in both *M. galloprovincialis* and *M. trossulus* ($F_{(4,141)}=14.41$, $p<0.001$). There was a significant effect of flow on respiration rate for both species ($F_{(4,141)}$=6.17, p=0.0001). Respiration rates were lower at low flow velocities and highest at high flow velocities (Fig. 3). Respiration rates were significantly different between species ($F_{(1,141)}$=7.01, p=0.009) with *M. galloprovincialis* having lower respiration rates than *M. trossulus*.

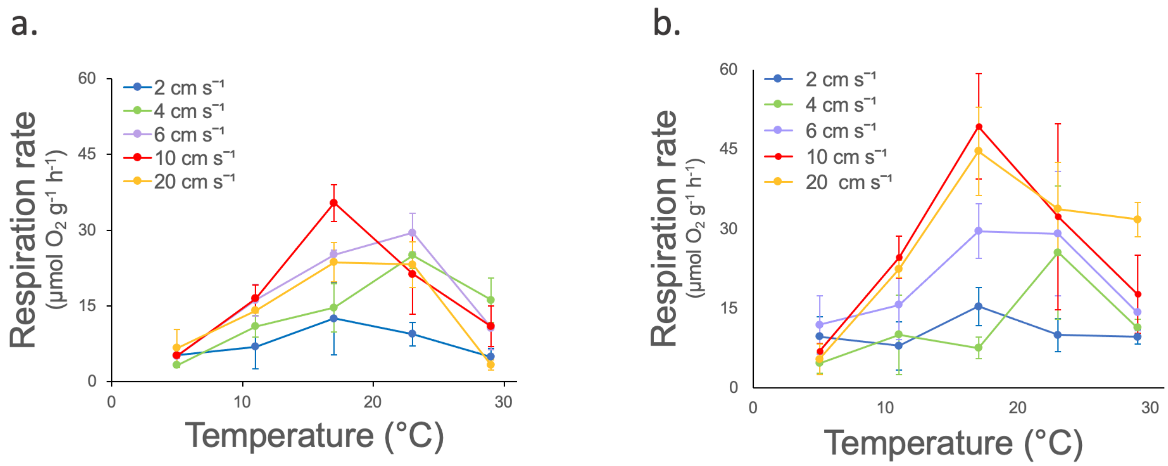

Figure 2. Respiration rate under different water temperatures and velocities for M. galloprovincialis (a) and M. trossulus (b). Colored lines represent different flow velocity. Error bars represent one standard error.

R_{max} were generally high in M. trossulus than in M. galloprovincialis, though those differences were not significant (P > 0.05; Fig. 3). There were no significant differences detected in thermal optimum or breadth between the two species (p > 0.05).

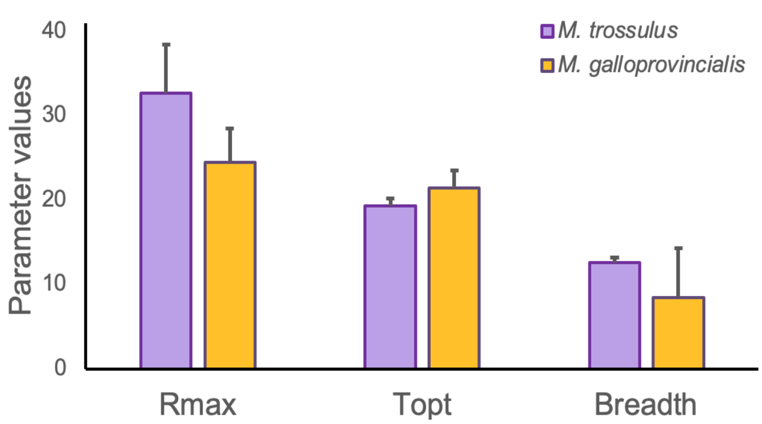

Figure 3. Estimated parameters for thermal performance curves for *M. galloprovincialis* and *M. trossulus*. Maximum respiration rate (R_{max}), thermal optimum (T_{opt}), and thermal breadth. Each bar represents mean of the different velocity treatments (N=5) and each replicate was an estimate from a mean of 5,000 bootstrap runs. Error bars represent one standard error.

Discussion

In the face of global warming, tolerance to thermal extremes is fundamentally important to understand. For nearshore organisms that inhabit the rocky-intertidal zone, environmental stressors can limit physiological performance (Arribas et al., 2014; Barbariol and Razouls, 2000). In this study, I examined the impacts of climate change-related stressors and their interaction on the Mediterranean mussel, *M. galloprovincialis*, to understand how these mussels cope physiologically to multiple environmental stressors. This work also serves as a species comparison between *M. galloprovincialis* and *M. trossulus*, a species native to the Pacific Northwest.

Temperature has a significant effect on the fitness and performance of many ectotherms (Huey and Kingsolver, 1989). This is true for mussels, where respiration rates displayed a non-linear response, increasing from 5°C to 17°C or 23°C before decreasing. Peak performance for *M. galloprovincialis* was achieved at either 17°C or 23°C, indicating that the elevated metabolic demand at higher temperatures could not be met (Matoo et al., 2021).

Although temperature had a significant effect on the respiration rate of *M. galloprovincialis*, flow also had a significant effect, as oxygen uptake depends on the transport of gasses from the water column to the organism (Sebens et al., 1997). We found that higher respiration rates corresponded with higher flow velocities because greater oxygen availability allows for higher oxygen uptake under those conditions.

In comparing *M. galloprovincialis* and *M. trossulus*, we found that respiration rates differed between species. This is likely due to the geographic origins of the species. *M. galloprovincialis* are native to the Mediterranean and have a global distribution as an invasive species (Smart et al., 2021). As such, this species is exposed to a wide range of temperatures as it has expanded from its native range of the Mediterranean to both the northern and southern hemispheres. When considering the respiration rates between the two species, it is possible that the *M. galloprovincialis* which are used to higher temperatures had suppressed respiration rates as a result of being kept at cold temperatures in the seatables.

Our results also indicated that the *M. galloprovincialis* and *M. trossulus* have the same thermal optimum which likely is due to the phenotypic plasticity of mussels, as intertidal mussels generally display high plasticity and adaptation to environmental fluctuations (Smart et al., 2021).

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SUPPORTING MATERIALS

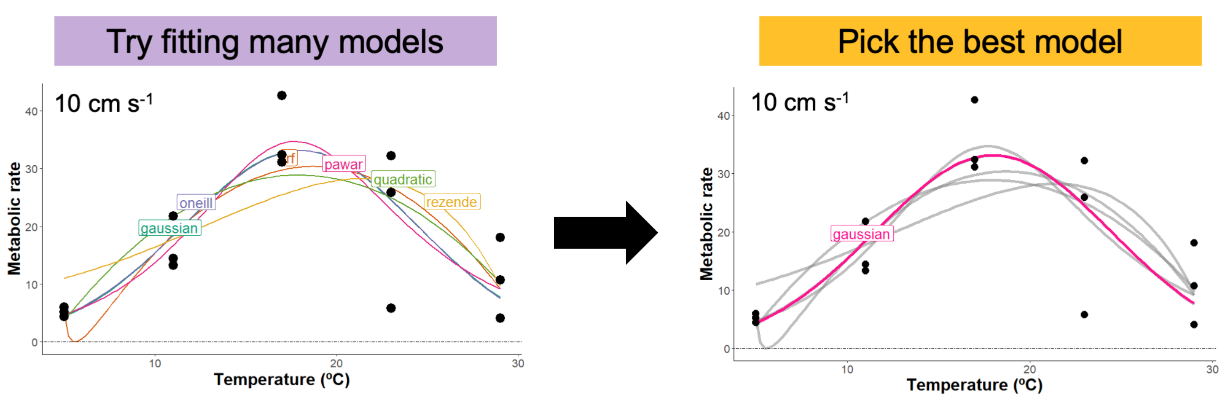

Fig. S1. Example of workflow for best non-linear model selection. Models ranked by AICc values.