Temperature and flow effects on mussel gaping behavior

Nina Michael, Kindall Murie

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Friday Harbor Laboratories, University of Washington

Abstract

Anthropogenic induced thermal stress is a major driver of change, specifically within marine coastal ecosystems. Mussel beds provide important structures and chemical functions to surround marine ecosystem. Mussel behavior is known to be influenced by environmental factors, but less has been explored about how behavior might influence their role in chemical manipulation. In this study, we developed a system to monitor gaping distance of *Mytilus* (*californianus* and *trossulus*) across time under different temperature and flow speed conditions. We documented increases in *Mytilus californianus* gaping frequencies and decreases in *Mytilus trossulus* gaping frequencies as water was warmed. Although *Mytilus trossulus* often inhabit low flow areas, there was not a significant change observed in time spent open when placed in higher flow. Although further replication is desired, temperature and flow are seen to affect gaping across species, providing implications for marine warming created shifts.

Introduction

The increasing temperatures observed due to climate change are key influences on ecosystems and the organisms within. Heatwaves in marine ecosystems have been observed to occur more frequently and at higher intensities over time (Smith et al., 2023). At the organismal level, as temperature increases within a species tolerance range, physiological processes are known to shift (He and Silliman, 2019). Increasing thermal stress threatens the success of important marine species, which can likely be detrimental to the entire ecosystem.

Mussels are key players within coastal habitats as they provide structure, as well as being an economically important species. Mussels are known to be ecosystem engineers, organisms that alter properties of their environment (Ninokawa et al., 2019). Mussel beds not only form habitats within their interstitial space, but chemically alter the seawater within. Respiration and calcification increase CO_2, strengthening the chemical gradient. Economically, many mussel species are largely utilized in bivalve aquaculture.

Less is known on whether behavior has an influence on a mussel's ability to change its chemical environment. As mussels gape, they respire, and the water flow brings food through. But it is thought that the actions are not synonymous, and that filtration might not always be occurring while gaped. This is important because filtration mixes the seawater, lessening the chemical

gradient. Additionally, behavior may differ between species due to morphological and environmental factors.

To test the influence on mussel behavior, our study uses laboratory flume experiments to observe the effects of temperature and water flow speed on gaping. We collected gape data for two species, *Mytilus californianus* (MC) and *Mytilus trossulus* (MT), under two temperature levels and two flow speeds and examined the proportions of time each mussel spent open. Considering the generally larger size of MC and its open coast habitat, they should be able to tolerate higher flow and remain gaped to acquire sufficient nutrients. MT are smaller in size and generally found in protected bays and seas. As they are exposed to lower flow, we should observe a smaller proportion of time spent open during the high flow trials. MT are found at higher temperature environments and therefore should show higher proportions of time spent open during the high temperature trials, where MC might contract due to less frequent exposure to comparable conditions.

Methods

Sourcing and maintenance of experimental organisms

The experiment was conducted at Friday Harbor Labs (FHL), San Juan Island, WA. The mussels, *Mytilus californianus* and *Mytilus trossulus,* were ordered from Penn Cove Shellfish (Coupeville, WA). Mussels were held from June 2021 through April 2022 in a live box off the FHL dock. Once the experiment began, the mussels were maintained in a flow-through tank in Lab 6 at FHL. While in the tanks, the mussels were held at temperatures consistent with current natural conditions. Mussels were fed in the flow-through tanks throughout the duration of the experiment.

Preparation prior to experimental trials

To measure gaping behavior, we used Hall Effect gape sensors (Miller and Dowd, 2017). These sensors utilize the Hall Effect to detect a magnetic field and output a voltage value. The magnitude of the output varies with the strength of the field. Sensors were placed on the posterior end of the shell, next to the excurrent siphon. A small stack of magnets was placed opposite the sensor (Figure 1A) so that as the mussel gapes, the magnetic field between its shell weakens. The mussels were placed in an upright position to emulate those found in a natural bed. Each mussel was affixed to an individual base of plastic with a pole in the center. The sensors, magnets and mussels were adhered using Z-Spar two-part epoxy (Pettit Paint Z-Spar A-788 Splash Zone Compound). Placing the mussels on their poles, adhering the sensors and subsequently, the magnets, took approximately 48 hours due to a required hardening time between each step, as well as prior to taking any sensor readings. This was to allow for the epoxy to harden between each step, particularly before recording any sensor readings.

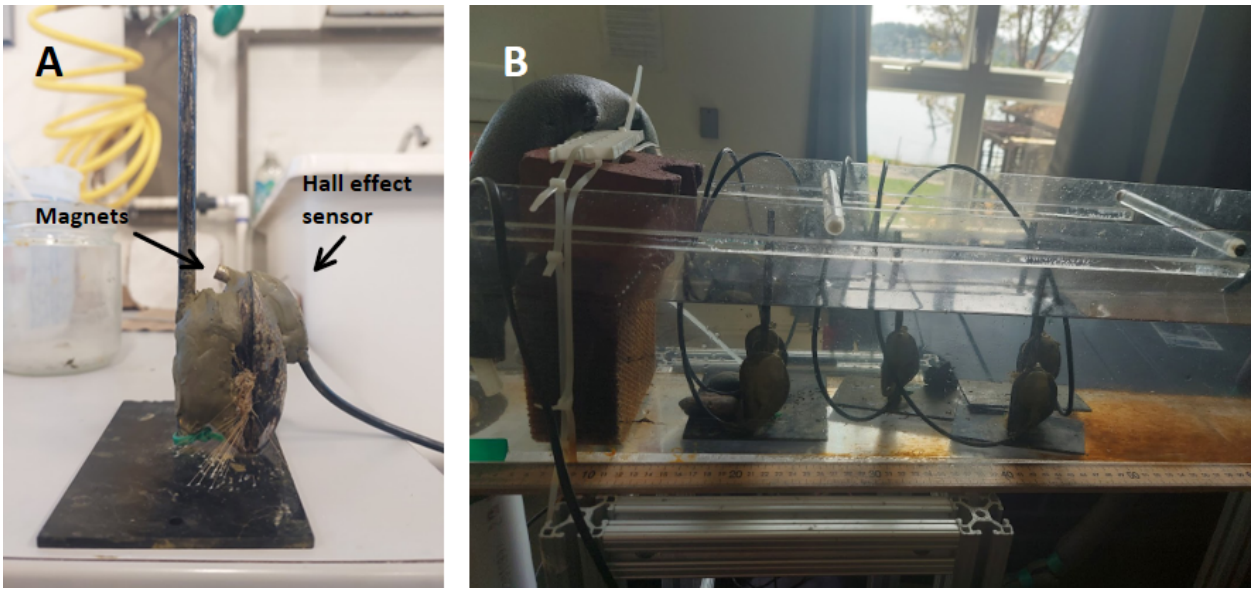

Figure 1. Images of mussel sensor and flume set up. (A) Hall effect sensor and magnet placement on mussel valves. The mussel is glued upright to a plastic pole and base. (B) Flume set up of a trial. Five mussels with attached sensors and magnets are placed in the flume with excurrent siphon facing downstream. Bases of poles are staggered to allow for equal flow.

Prior to experimental trials, we took sensor reading data before and after applying the magnets. The before magnet data was taken to ensure the sensors were at an appropriate baseline level. The sensor data taken after the magnets were placed verified that they had hardened in an acceptable position. The optimal voltage of the after-magnet readings is at least 100mV lower than the gape sensor baseline with no magnets. A larger range between the before and after sensor readings allows for more specific voltage data after calibration. This data provides a baseline for each mussel at its closed position and is used to verify calibration data at the end of each trial in case of possible sensor drift.

Experimental design

Trials ran for 48 hours and were conducted in the small flume in Lab 6 in April and May of 2023. This experiment is a full factor design with two temperatures (12°C, 18°C) and two flow speeds (0 cm/s, 6 cm/s). Five mussels per species were placed in the flume under each temperature (Figure 1B) and exposed first to 0 cm/s flow and then to 6 cm/s flow, each for 48 hours (MC 12°C n = 5, 18°C n = 4, MT at 12°C n = 5, 18°C n = 5). Due to the size of the flume, each trial of five individuals was conducted separately. In between temperature shifts, both the mussels and the water in the flume were changed out.

Gape sensor data throughout the duration of each trial was collected using an Arduino board and computer setup that connected to each sensor. The board was housed in a watertight box during the experiment to avoid water damage from flume or sensor cables.

The temperature in the flume was held consistent with a chiller (Model MC-1/13HP, AquaEuroUSA) and heater (JAS 300W Submersible Aquarium Heater, Jetsu). A temperature logger (HOBO Pendant MX2202, Onset) was kept in the flume to ensure consistency during each trial (Table 1). As the flume was not a flow-through system, an air stone was placed downstream of the mussels to provide oxygen throughout the trials.

Species	Target temp (°C)	Flow (cm/s)	Average temp ± s.d.
MC	12	0	11.44 ± 0.08
MC	12	6	11.33 ± 0.16
MT	12	0	11.65 ± 0.2
MT	12	6	11.78 ± 0.48
MC	18	0	18.02 ± 0.38
MC	18	6	18.05 ± 0.33
MT	18	0	18.05 ± 0.34
MT	18	6	18.01 ± 0.33

Table 1. Average temperature over time throughout duration of each trial. The average and standard deviation values were calculated from the temperature data collected by the logger placed in the flume during every trial.

Calibration and conversion to millimeters

After each set of mussels were exposed to both flow treatments, they were forced open through dissection to calibrate each sensor. Calibration took place after every trial, and for every mussel, as they can vary in shell thickness. Shell thickness can influence the voltage readings of the sensors, which requires each mussel to have its own calibration curve. Calipers were used to open the mussel from close to 20 mm, in one mm increments. The gape distance was measured between the sensor and magnet, and the voltage was noted for each increment. The baseline voltage (at zero) was subtracted from each value, and the resulting numbers were plotted and fitted with an exponential curve. The resulting equation was applied to the raw voltages for each mussel to convert the gape data to millimeters.

The converted data was scaled by the minimum value to adjust for any negative values. This offset occurs since the true zero value varies between mussels. Additionally, the zero value when the mussel is contracted differs from the zero value when the mussel is held closed during calibration. After scaling, the data set was standardized by the maximum gape value to allow for comparison. All mussels, regardless of species, seem to differ in preferred gape distance and exhibit individual patterns. Standardization put the data on the same plane, which allowed comparison both within and across species.

To calculate the proportion of time spent at maximum gape distance, we calculated the frequency of each gape distance by 0.1 mm bins. Any value less than 0.1 was considered “functionally closed.”

Statistical analyses

All analyses were performed using R 4.3.0. A two-way ANOVA test was conducted to analyze the influence of temperature and flow on the proportion of time spent open. A separate test was run for each of the species due to their morphological differences. Additionally, a three-way ANOVA test was also conducted for proportion of time spent open with temperature, flow, and species as factors.

Results

Summary statistics of the average, minimum, and maximum gape distance for each treatment is provided below in Table 2. Of the four temperature bins, the *Mytilus trossulus* at the lower temperature showed the highest average gape, 2.066 mm at the higher flow. Regardless of temperature, MT spanned a larger range when gaping, showing the highest maximum values.

		Mytilus californianus (MC)		*Mytilus trossulus* (MT)	
12°	Flow (cm/s)	0	6	0	6
	Average (mm)	1.42641	1.23180	2.04962	2.06610
	Minimum (mm)	0.00000	0.00000	0.08247	0.08247
	Maximum (mm)	4.76701	4.76701	8.77831	9.12256
18°	Flow (cm/s)	0	6	0	6
	Average (mm)	1.45291	1.31998	0.73150	1.06383
	Minimum (mm)	0.18917	0.18917	0.00000	0.00000
	Maximum (mm)	4.66995	4.07680	4.79545	7.70111

Table 2. Summary statistics of scaled gape data for each species and temperature trial. The average, minimum, and maximum value were calculated from the gape sensor data collected from all sensors in each trial. This was done after the raw voltages were converted to (mm) and scaled by the minimum value. The trials observed to have minimum values of exactly 0.0, MC 12, and MT 18, included sensors that output negative values and required scaling.

Frequency distribution

Frequency distributions (Figure 2) of the proportion of time spent open indicates a lack of difference between the higher flow trials within both species. Between the two MC trials at 6cm/s and the two MT trials at 6cm/s, there is an observed overlap in the data distribution and median value, both within species and across. Distribution trends are more varied in lower flow data. In MC, mussels spent more time open at 18° than at 12°. Conversely, in MT, mussels spent a substantially smaller fraction of their time open at 18° than when compared at 12°. This indicates interaction, where gaping by both species at high and low flow is dependent on the temperature (e.g., seen particularly within MT data; Table 3). Many of the treatments exhibit at least one outlier in the dataset, decreasing the already small sample size.

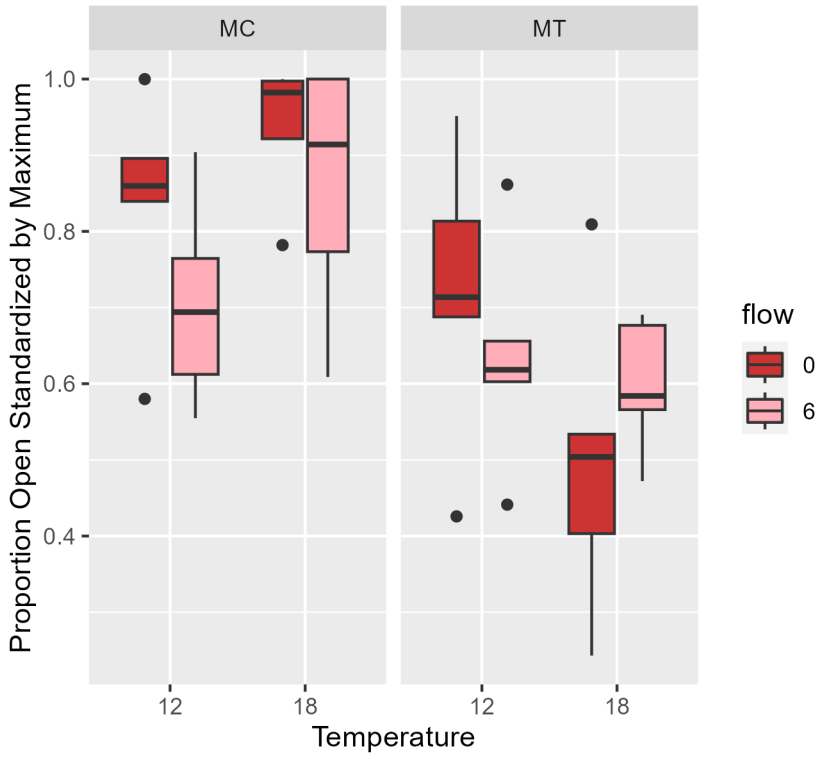

Figure 2. Distribution of proportion of time spent open for each trial. The red colored plots indicate trials run at 0 cm/s flow speed and the pink at 6 cm/s flow. Outliers are represented by black dots plotted to the side of boxplots.

To examine the proportion data more closely, the following graphs (Figure 3) visualize the proportion of time the mussels spent at each gape distance, using averages of all sensor data collected within each trial and standardizing by the maximum value. Regardless of temperature, MT is seen to spend the largest proportion of time at only ten percent of the maximum gape distance and smaller, more equal portions at all other gape distances. At both temperatures, 12° and 18°, the average proportion of time MT spent at 0.1 or less of maximum gape was between 0.28 and 0.5. Every increment following was only observed for ≤ 0.1 of the trial. MC data differs, both between species and within temperature treatments. Like MT, MC data from the low temperature trials show a larger average proportion spent at the first ten percent of maximum gape, particularly seen in the 6cm/s treatment where 27 percent of time was spent at 0.1 of maximum gape or less. These trends are no longer observed when analyzing MC gaping during the high temperature trials. A low proportion was spent at only ten percent of maximum gape (0.06 at 0 cm/s, 0.14 at 6 cm/s). Trendlines for both flow speeds peak at 10 to 30 percent of maximum gape distance, where over 30 percent of time was spent between these increments. The data set at 12° is bimodal, with a second, lower, peak from 0.5 to 0.6 of the maximum gape distance.

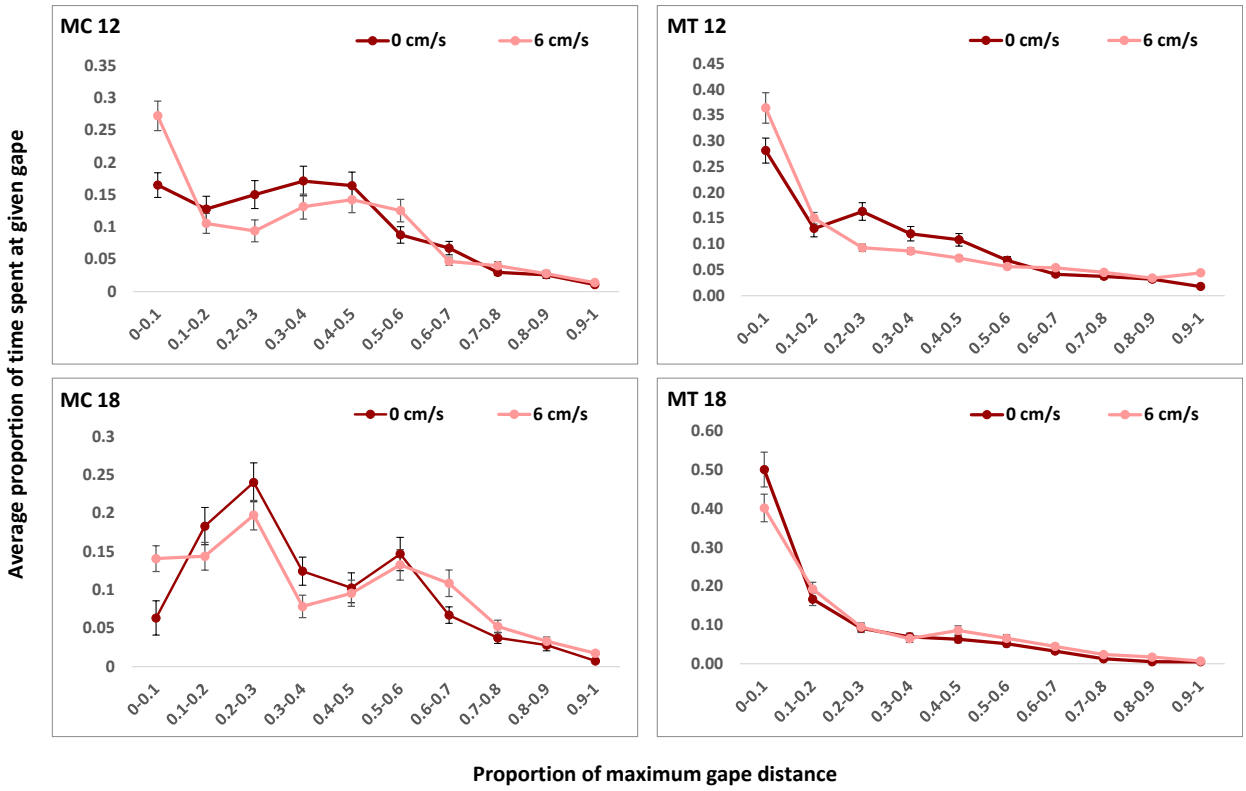

Figure 3. Average proportion of time spent at given proportions of maximum gape distance, for each temperature trial. Red colored lines correspond to trials conducted at 0 cm/s flow speed and pink colored lines to those at a 6 cm/s flow speed. Averages were calculated from the data collected from ~5 sensors per trial. Frequencies calculated using the gaping dataset standardized by the maximum value were used to find the proportion of maximum gape distance.

A summary of the three-way ANOVA test (Table 3) indicates two P-values that fall under the significance level $\alpha = 0.05$. All factors evaluated in the ANOVA resulted in large P-values ($p > 0.05$) apart from species ($p = 0.000249$) and temperature: species ($p = 0.018724$).

	Df	F-value	P-value
temperature	1	0.154	0.697971
flow	1	0.798	0.378870
species	1	17.264	0.000249
temp: flow	1	1.515	0.227985
temp: species	1	6.179	0.018724
flow: species	1	1.095	0.303773
temp: flow: species	1	0.397	0.533285

Table 3. Three-way ANOVA results. The highlighted rows indicate statistically significant results ($p < 0.05$).

Discussion

Gape distance monitoring of two mussel species confirmed likelihood that temperature and water flow factors influence gaping behavior. We observed increased gaping in MC at higher temperature which indicates the possibility of the species being less sensitive to thermal stress than initially hypothesized. Similarly, gaping in MT decreased at the higher temperature, indicating the possibility for less of a tolerance than was initially hypothesized. These observations were made at the 0 cm/s flow speed level, where differences between temperature treatments were substantially more apparent. Gaping was not significantly different in either species between temperatures when at 6 cm/s. The higher flow seemed to be a main influence, masking any gaping behavior induced by temperature changes.

Two statistically significant values were calculated from the proportion open data set and support the previously described trends. P-values under 0.05 were reported for species and temp: species, indicating that the differences observed in proportion of time spent open between MC and MT are statistically significant, as are the differences in which the species react to thermal stress. There is an interaction between temperature level and species, where when temperature increases, gaping shifts differently depending on species.

Although all other factors cannot be determined to be statistically significant, it is important to note that the sample size should be increased for more supported conclusions to be established. With the small number of replicates as well as the many outlying values, it is difficult to obtain representative data. Regardless, the trends observed indicate that further experimentation and replication would result in more confident differences in gaping caused by shifts in temperature and flow speeds.

Our findings demonstrate the need for future replication of mussel gaping experimentation and possibly the addition of more variables. Much is unknown about intertidal mussel gaping behaviors and patterns (Miller and Dowd, 2017) particularly in the context of climate change impacts. Using this experimental design as a base, not only should more replicates be conducted, but the addition of pH or respiration as measures would allow for observations on interactions between temperature, flow, behavior, and chemical modification. The ability of mussel beds to modify the surrounding physical environment is essential to the plethora of marine life that reside within the interstitial space. The combination of temperature and flow produces variation in mussel behavior, subsequently influencing the surrounding chemical gradients. These interactions create potential consequences for mussel function, individually and in the context of the mussel bed ecosystem, particularly as climate change continues to drive marine heating.

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References

He, Q., & Silliman, B. R. (2019). Climate change, human impacts, and coastal ecosystems in the anthropocene. *Current Biology*, *29*(19), R1021–R1035. https://doi.org/10.1016/j.cub.2019.08.042

Miller, L. P., & Dowd, W. W. (2017). Multimodal *in situ* datalogging quantifies inter-individual variation in thermal experience and persistent origin effects on gaping behavior among intertidal mussels (*Mytilus californianus*). *Journal of Experimental Biology*, jeb.164020. https://doi.org/10.1242/jeb.164020

Ninokawa, A., Takeshita, Y., Jellison, B. M., Jurgens, L. J., & Gaylord, B. (2020). Biological modification of seawater chemistry by an ecosystem engineer, the California mussel, *Mytilus californianus*. *Limnology and Oceanography*, *65*(1), 157–172. https://doi.org/10.1002/lno.11258

Smith, K. E., Burrows, M. T., Hobday, A. J., King, N. G., Moore, P. J., Sen Gupta, A., Thomsen, M. S., Wernberg, T., & Smale, D. A. (2023). Biological impacts of marine heatwaves. *Annual Review of Marine Science*, *15*(1), 119–145. https://doi.org/10.1146/annurev-marine-032122-121437