

UC Davis

UC Davis Previously Published Works

Title

Sirtuin-dependent recovery from aerial heat shock: The effects of food ration, thermal history, and sirtuin inhibition on clearance rates and valve gape activity of the California mussel, *Mytilus californianus* (Conrad)

Permalink

<https://escholarship.org/uc/item/5gr5f6rp>

Journal

Journal of Experimental Marine Biology and Ecology, 536

ISSN

0022-0981

Authors

May, Melissa A
Feezell, Maya K
Gonzalez, Silvano J
[et al.](#)

Publication Date

2021-03-01

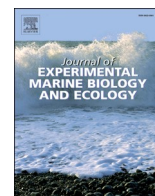
DOI

10.1016/j.jembe.2020.151510

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed



Sirtuin-dependent recovery from aerial heat shock: The effects of food ration, thermal history, and sirtuin inhibition on clearance rates and valve gape activity of the California mussel, *Mytilus californianus* (Conrad)

Melissa A. May^{a,*}, Maya K. Feezell^a, Silvano J. Gonzalez^a, M. Christina Vasquez^{a,b}, Anne E. Todgham^c, Lars Tomanek^a

^a California Polytechnic State University, Biological Sciences Department, 1 Grand Avenue, San Luis Obispo, CA 93407-0401, USA

^b Loyola Marymount University, Biology Department, 1 LMU Drive, Los Angeles, CA 90045, USA

^c University of California, Davis, Department of Animal Science, 2205 Meyer Hall, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Keywords:

Clearance rate
Valve gape activity
Mytilus californianus
Sirtuins
Food availability
Acute aerial heat shock

ABSTRACT

Intertidal mussels regularly experience seasonal fluctuations in food availability and heat stress over the course of a tidal cycle, yet little is known about the biochemical mechanisms linking food ration and thermal tolerance, despite evidence that food enhances stress tolerance at the organismal level. We hypothesized that sirtuins (food-dependent protein regulators of cellular homeostasis), which may regulate the cellular stress response of mytilids during heat stress, also affect organismal performance during recovery from heat stress. As part of a broader study, we investigated the effects of acclimation food ration, acclimation emersion (air) temperature, and sirtuin inhibitors on the feeding behavior of the California mussel, *Mytilus californianus*, during recovery from acute aerial heat shock. Mussels were acclimated for 3 wk. to either a low (0.25% algae-g mussel dry wt⁻¹) or high (1.5% algae-g mussel dry wt⁻¹) algal ration and low (20 °C) or high (30 °C) emersion temperatures, in tidal simulator tanks capable of maintaining standard circadian and circatidal rhythms. Following acclimation, mussels were exposed to two sirtuin inhibitors (nicotinamide and suramin) or no inhibitors for one high tide cycle. During the subsequent low tide cycle, all mussels were exposed to an acute aerial heat shock (33 °C) and then allowed to recover in their respective acclimation conditions. Every 12 h during the 48 h before sirtuin inhibition and 48 h following acute heat shock (in the corresponding high tide periods), we recorded clearance rates and valve gape activity. Mussels acclimated to a low food ration significantly reduced clearance rates with heat shock and sirtuin inhibition, but not with heat shock alone, relative to pre-treatment values regardless of emersion temperature. Mussels acclimated to a low food ration and high emersion temperature showed significant reductions in valve gape activity following an acute heat shock following sirtuin inhibition, but not in any of the other acclimation groups. Unexpectedly, heat shock alone reduced clearance rates of mussels acclimated to high food and high emersion temperatures only, without any additional effect by heat shock and sirtuin inhibition. Together, these findings suggest an important role for sirtuins at the organismal level during recovery from heat shock following acclimation to a low food ration.

1. Introduction

The intertidal zone is a highly variable physical environment and mussels inhabiting the rocky intertidal routinely experience seasonal and daily fluctuations in body temperature, food availability, salinity, and oxygen (Elvin and Gonor, 1979; Morris and Taylor, 1983; Menge, 1992; Helmuth and Hofmann, 2001; Helmuth et al., 2006; Denny et al., 2011; Dowd et al., 2013). The California mussel (*Mytilus californianus*) is

a dominant space holder in rocky intertidal and nearby subtidal communities along the Pacific coast of North America (Paine, 1974), where they form dense aggregations that provide habitat (Suchanek, 1985), thermal refuge (Lathlean et al., 2017), and a source of food (Landenberger, 1968; Garza, 2005) for other intertidal organisms. Mussels also help maintain ecosystem health (Shinen and Morgan, 2009) and contribute to benthic-pelagic coupling (Loo and Rosenbberg, 1996) by filtering the water column at rates of up to 6 l·h⁻¹·mussel⁻¹ (Rao, 1953).

* Corresponding author at: Florida Gulf Coast University, Marine and Earth Sciences, 10501 FGCU Boulevard South, Fort Myers, FL 33965, USA.
E-mail address: mmay@fgcu.edu (M.A. May).

Thus, persistence of mussel populations is an integral component of maintaining coastal community structure and function. Climate change models predict that the extreme conditions experienced by intertidal mussels may be exacerbated, including not only due to an increase in the frequency of high degree days (Harley et al., 2006; Morak et al., 2013; Seuront et al., 2019), but also due to a decrease in the availability and quality of nearshore phytoplankton (Winder and Sommer, 2012), which serve as their primary food source. Alterations in the feeding behavior of mussels affects the growth and fitness of individual mussels (Dahlhoff and Menge, 1996; Dahlhoff et al., 2002; Bayne, 2004), which indirectly influences food available for mussel predators and the composition of plankton communities (Norén et al., 1999). Numerous studies have evaluated the effects of environmental variables, such as food availability and water temperature, on feeding rates of marine mussels (for examples, see Schulte, 1975; Riisgård et al., 2006; Saurel et al., 2007), but little is known about controls of filtration and how feeding behavior shifts temporally after stress under different food ration (Maire et al., 2007).

Sensitivity to aerial temperatures during emersion is thought to be the main driver of mussel distributions in the intertidal zone (Harley and Helmuth, 2003) and mussels in the higher intertidal are generally more tolerant to extreme conditions than lower intertidal mussels (Sorte et al., 2019). Increased tolerance seemingly comes at a cost, as high intertidal mussels show decreases in growth, body size (Connor and Robles, 2015), and reproduction, as well as increased mortality relative to their lower intertidal counterparts (Petes et al., 2007, 2008). The stress of living in the intertidal zone may be one factor driving reductions in growth and reproductive output, which may be compounded by reductions in time available for feeding (Blanchette et al., 2007). Under stressful conditions, such as high aerial temperature exposure, mytilids in wild populations reduce their valve gape activity (Kramer et al., 1989; Dowd and Somero, 2013; Gleason et al., 2017; Miller and Dowd, 2017, 2019) during periods of reimmersion. However, other studies have suggested that mussels may not use feeding as a recovery mechanism following emersion (Bayne et al., 1988), leaving the direct relationship between feeding behavior and aerial heat shock unclear. In this study, we specifically investigated whether mussels respond to acute thermal stress by altering their feeding behavior when reimmersed by measuring clearance rates and valve gape activity during multiple high tide periods, before and after an acute heat shock.

This study was undertaken as part of a broader project that aims to understand how previous food ration and thermal history alter the ability of *M. californianus* to respond to aerial heat stress across multiple levels of biological organization. Both previous food availability and thermal history are known to affect feeding behavior (Schulte, 1975; Riisgård et al., 2003, 2006; Saurel et al., 2007; Cheng et al., 2018) and thermal tolerance (Lesser et al., 2010; Schneider et al., 2010; Fitzgerald-Dehoog et al., 2012; Dowd et al., 2013), yet the interactive effects of previous caloric intake and thermal history on feeding behavior and thermal sensitivity are not known and likely complex. For instance, mussels (Schneider et al., 2010; Fitzgerald-Dehoog et al., 2012) and barnacles (Gilman and Rognstad, 2018) fed high algal rations show higher rates of survival and growth when exposed to heat stress than those with a poor diet. Yet, perhaps contradictorily, both high algal abundances (Schulte, 1975; 2013; Riisgård et al., 2006, 2013; Pascoe et al., 2009) and acute heat stress (Gleason et al., 2017) are known to reduce feeding and valve gape, respectively. Repeated aerial exposures to elevated temperatures may cause significant reductions in feeding effort, which could negate the benefits of having high food availability. As such, we compared the feeding response to acute heat shock in mussels that had been acclimated to different aerial temperature and algal abundance conditions, following the acclimation conditions used by Fitzgerald-Dehoog et al. (2012). We predicted that mussels that were well-fed and acclimated to higher emersion temperatures would be less affected by an acute heat shock than those fed lower algal concentrations or acclimated to lower aerial temperatures, and would

consequently, recover more quickly from the stress (i.e., resume normal feeding behavior).

To investigate the underlying biochemical mechanisms linking food availability and thermal sensitivity at the organismal level (i.e., clearance rate and valve activity), we examined the role of sirtuins, which are important regulators of cellular homeostasis during stress at the level of the proteome (Vasquez and Tomanek, 2019). One of the grand challenges in physiology is linking phenotypic responses across levels of biological organization (Mykles et al., 2010) and often involves searching for cellular and molecular mechanisms that correspond to changes observed at higher levels (i.e., organ or organism). Our previous research suggests that sirtuins (silent information regulator proteins; Loo and Rine, 1995) are involved in the heat shock response and, therefore, thermal sensitivity in mussel congeners (Tomanek and Zuzow, 2010; Vasquez et al., 2017; Vasquez et al., 2020). Sirtuins are protein deacetylases, responsible for activating numerous proteins involved in metabolism, oxidative stress, and protein homeostasis (Rodgers et al., 2005; Han et al., 2013) and their activity varies with caloric intake in other species (Houtkeeper et al., 2010; Kapahi et al., 2017; Vasquez and Tomanek, 2019). Thus, we hypothesized that the observed linkage between caloric intake and thermal sensitivity in mussels is regulated by this protein family. To understand the role of sirtuins and their downstream targets in the response to aerial heat stress, we exposed mussels to two sirtuin inhibitors, nicotinamide and suramin (Lawson et al., 2010; Kumar and Lombard, 2018), prior to aerial heat shock exposure in each of our acclimation groups, as previous research in our lab has shown that suramin alters the proteome of mytilids 24 h after application of the inhibitor (Vasquez et al., 2017). Furthermore, recent evidence suggests that sirtuin inhibition reduced feeding behavior and food intake in planarians (Ziman et al., 2020), but it is not clear how food ration and heat stress modify this effect on feeding in mussels. We predicted that inhibition of sirtuins would increase thermal sensitivity in mussels compared to uninhibited mussels, and would therefore, reduce feeding for longer periods of time than the acute heat shock alone.

2. Methods and materials

2.1. Mussel collection and experimental design

Adult *Mytilus californianus* (45–65 mm length) were collected from the intertidal zone at Hazard Canyon Reef, Montano de Oro State Park, Los Osos, CA (35.27° N, 120.90° W) and cleared of all epibionts. Mussels were acclimated for 3 wk. at the California Polytechnic State University Center for Coastal Marine Studies (Avila, CA) in flow-through, 'tidal-simulator' tanks that mimicked a 12-h circadian rhythm, semi-diurnal tide, and allowed for control of food ration and water (immersion) and aerial (emersion) temperatures. Briefly, mussels experienced daylight from 06:00 to 18:00 PST and two 6-h high (00:00 and 12:00) and low (06:00 and 18:00) tide cycles per day (water temperature: 16 °C, filtered to 1 µm), with 30 min intertidal 'splash' periods to indicate tidal transitions. During the daytime low tides, the aerial temperature was raised to either 20 °C or 30 °C at a rate of 0.1 °C·min⁻¹ using heat lamps controlled by 'robomussels' (Harley and Helmuth, 2003) placed within the mussel bed; thus, the emersion temperatures reflect mussel body temperature. The nighttime low tide temperature was ambient (18 °C). Shellfish Diet 1800® (Reed Mariculture, Campbell, CA) was pulsed into the tanks once an hour during every high tide cycle at either a low (0.25% algae·g mussel dry wt⁻¹) or high (1.5% algae·g mussel dry wt⁻¹) food ration, which were determined based on guidelines for broodstock conditioning (Helm et al., 2004 and Vasquez et al., 2020). Thus, each tank represented one combination of food ration and maximal daily aerial temperature (Fig. 1a), yielding four different acclimation regimes, following those used in Fitzgerald-Dehoog et al. (2012). Within each tank, mussels were held in two separate baskets to minimize handling stress for subsequent treatments ($n = 200$ per

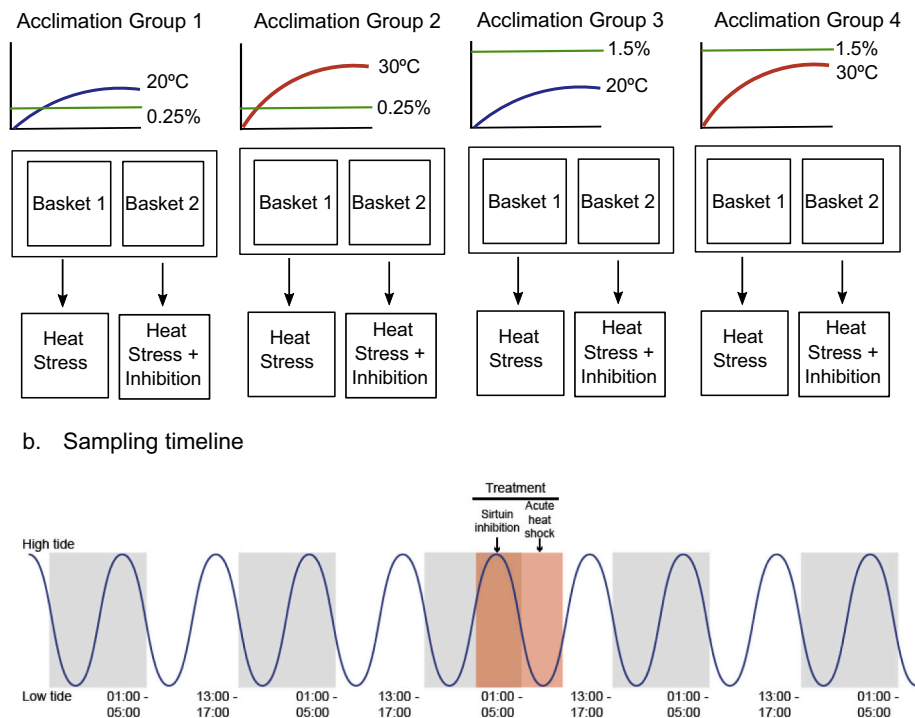


Fig. 1. Experimental design to test the combined effects of acclimation emersion temperature and diet, as well as, sirtuin inhibition on the feeding physiology of mussels before and after acute heat stress. Mussels were acclimated to either a low emersion temperature (20 °C; blue lines) and a low food ration (0.25% Shellfish Diet-g dry mussel weight⁻¹·day⁻¹; lower green line) or high food ration (1.5% Shellfish Diet-g dry mussel weight⁻¹·day⁻¹; upper green line) for 3 wk. (Fig. 1a). At the end of the acclimation period, we measured basal clearance rates and valve gape activity for each group during four high tide periods (Fig. 1b). Following acclimation, the half of the mussels from each acclimation tank were exposed to sirtuin inhibitors, while the other half were placed in filtered seawater, and then all of the mussels were then exposed to an acute, aerial heat stress (33 °C) in the subsequent low tide period. We then measured clearance rates and valve gape activity for each group following treatment to monitor recovery over the next four high tide periods. Gray bars indicate sampling periods that occurred during the nighttime high tides, from 0100 to 0500 PST (Fig. 1b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

basket).

To test the role of sirtuins in the response to food ration and acute heat stress, one basket of mussels from each tidal simulator was placed into separate tanks containing filtered seawater and two sirtuin inhibitors, nicotinamide (25 mmol·l⁻¹) and suramin (100 μmol l⁻¹) or into tanks containing only filtered seawater for 5 h immediately following acclimation (see Fig. 1b for timeline). All mussels were then rinsed with filtered seawater, placed back into the tidal simulators for the intertidal, high-to-low tide transition, and exposed to an acute aerial heat shock (33 °C) during the subsequent low tide (ramp rate: 0.1 °C·min⁻¹), as previous studies have shown that acute exposure to 33 °C has adverse effects on mussel growth (Fitzgerald-Dehoog et al., 2012). For the following 48 h, the mussels recovered from stress under their respective acclimation conditions.

2.2. Clearance rate measurements

Clearance rate was measured during the high tide periods of the final 48 h of acclimation preceding heat shock and the 48-h recovery following acute heat shock, beginning at 6, 18, 30, or 42 h before or after acute heat shock (Fig. 1b). One mussel from each treatment was selected at random and placed into a 1 l beaker on a stir plate containing filtered seawater (16 °C). Mussels were fed an aliquot of Shellfish Diet 1800® at an equivalent concentration to their acclimation feeding regime (18.1 μl algae·l⁻¹ for the low food ration and 108.8 μl algae·l⁻¹ for the high food ration) and the initial *in vivo* chlorophyll concentration from each beaker, measured as relative fluorescence at 460 nm (RFU¹) using an AquaFlor handheld fluorometer (Turner Designs, San Jose, CA), was recorded. After 30 min, a final chlorophyll reading was taken, the mussel was returned to its respective treatment group, and another mussel was selected, and this procedure repeated. There were a total of four mussels per treatment used throughout this study and measurements from these mussels were taken during each high tide cycle of the acclimation and recovery periods (i.e., repeated measurements every 12 h). At the end of

the experiment, we sacrificed the mussels and recorded their wet tissue weight.

Steady-state clearance rate (CR), in l·h⁻¹, was determined using the following equation described by Fuller (1937) and Beggel et al. (2017):

$$CR = \frac{V \cdot (\ln C_o - \ln C_f)}{t}$$

where *V* is the volume of water in each beaker, *C_o* is the blank-corrected, initial chlorophyll reading, *C_f* is the corrected, final chlorophyll reading, and *t* is the duration of time elapsed between the initial and final measurements. Clearance rate was then standardized to the wet tissue weight of each mussel prior to statistical analysis, as size is known to affect pumping rates in mytilids (Sukhotin et al., 2003).

2.3. Valve gape measurements

Measurements of valve gape activity were collected concurrently with clearance rate for each individual. Above each of the 8 beakers where clearance rate was being measured, we mounted a Nikon DSLR HD camera (model D5500 with 18–55 mm lens) and recorded video for 30 min. To ensure the mussels remained in the field of view, we affixed Velcro to their shells prior to the 3-wk acclimation period and mounted the mussels near the top of the beaker (still fully submerged). As with clearance rate, one mussel from each treatment was selected and the valve movements were recorded for approximately 30 min. This process was repeated until the high tide cycle ended.

To determine the percent of time mussels spent with their valves open, videos were imported into ImageJ (Schneider et al., 2012) and analyzed frame-by-frame (60 fps²) to look for changes in valve state (i.e., valves visibly open or closed). We then calculated the number of frames where the valves were open and frames where the valves were closed to determine the percentage of time open as a metric for valve gape activity, as described by the equation below.

¹ Relative Fluorescence Units

² FPS, frames per second

Percentage of Time Open = 100 × $\frac{\text{\#of frames open}}{\text{Total\#of frames}}$

The percentage of time the mussels spent open during the observation period was then scaled to the wet tissue weight of the individual to account for size-dependent variation in valve activity (MacDonald et al., 2009).

2.4. Statistical analysis

To test the interactive effects of acclimation conditions and exposure to acute heat shock and sirtuin inhibition on the clearance rates of *M. californianus*, we ran a 3-way full-factorial mixed GLM³ in JMP® Pro (Version 14.0, SAS Institute Inc., Cary, NC). In this model, we tested acclimation conditions, treatment, and before and after treatment as main effects (see Table 1 for descriptions of variables), with mussel as a repeated measure, as an adaptation of the BACI method (Underwood, 1992). Additionally, we nested sampling time period within the before and after variable and tested the interactions of sampling time (before and after treatment) with acclimation conditions and treatment. We used then Tukey's HSD⁴ post-hoc analysis for pairwise comparisons within an acclimation tank to determine differences in clearance rates before and after acute heat shock or acute heat shock and sirtuin inhibition. To adjust for a non-normal distribution, we first transformed clearance rates to log₁₀(clearance rate + 1). The same mixed GLM was used to test the interactive effects of each factor on valve gape activity (reported as % time open·g⁻¹ wet tissue weight). As with the clearance rate model, pairwise comparisons among the fixed effects were made using Tukey's HSD.

3. Results

The combined effects of a) acclimation to different food rations and emersion temperatures, b) acute heat shock, and c) sirtuin inhibition on the clearance rates and valve gape activity of *M. californianus* are complex. We observed variation in the response of mussels exposed to acute heat shock alone or a heat shock following sirtuin inhibition depending on acclimation ration and emersion temperature conditions.

3.1. Reduction of clearance rates following acute heat shock

As predicted, we observed a significant effect of sampling time period on clearance rates of *M. californianus* ($F_{2,198.2} = 28.5, p < 0.001$).

Table 1
Summary of the four experimental parameters tested.

Experimental Parameter
Before or after treatment
• Before (baseline)
• After
Sampling time period
4 high tide periods before or after treatment (Fig. 1)
Acclimation conditions
• Low ration and low emersion temperature
• Low ration and high emersion temperature
• High ration and low emersion temperature
• High ration and high emersion temperature
Treatment
• Heat shock only
• Heat shock and inhibition

³ General Linear Model
⁴ Honestly Significant Difference

Because circadian and tidal rhythms are known to affect physiological processes in mytilids (see Connor and Gracey, 2011; Miller and Dowd, 2017), there is little biological significance in comparing clearance rates at specific time points before or after treatment. We were more interested in the overall impact of heat shock ± sirtuin inhibition on the clearance rates that we collectively sampled prior to and following the application of stress. In general, we found that clearance rates overall tended to decline following exposure to acute heat shock or exposure to acute heat shock and sirtuin inhibition (Fig. 2; also see next paragraph) in all acclimation groups. Additionally, there was some evidence of diurnal variation in the feeding behavior of *M. californianus* following

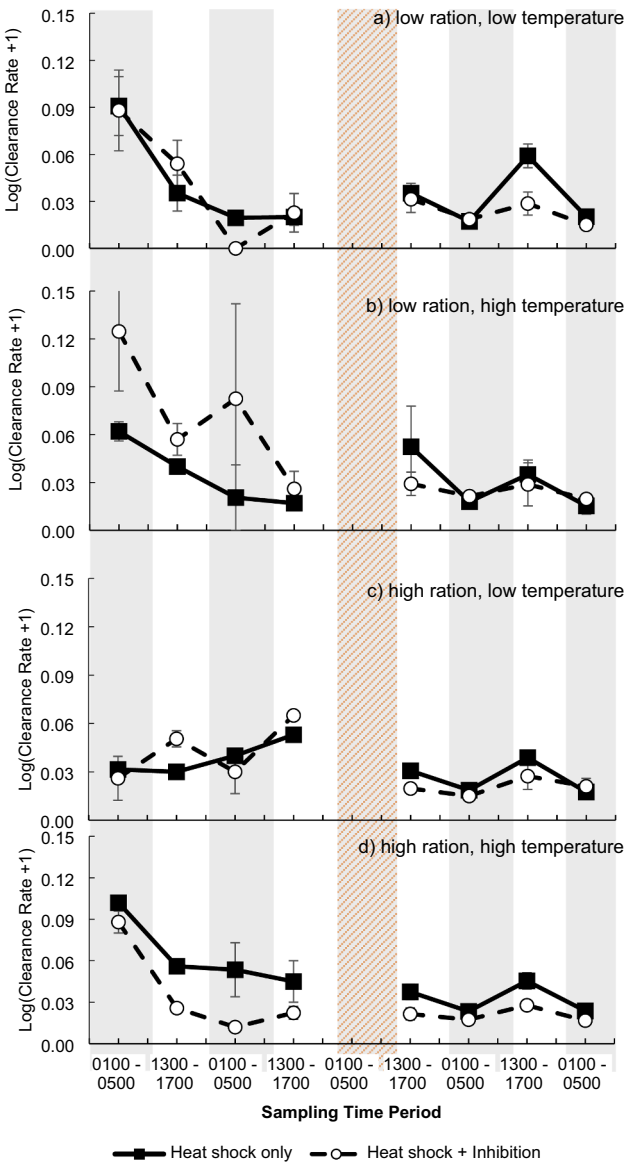


Fig. 2. Data plots showing temporal variation in clearance rates of *M. californianus* (plotted as the mean of the log₁₀(clearance rate + 1) ± SEM for each of the four acclimation conditions and before or after acute heat shock ± sirtuin inhibitors. Each graph shows clearance rates for mussels exposed to heat shock only (solid squares and line) or heat shock and sirtuin inhibition (open circles and dashed line) under various acclimation regimes: a) low ration and low emersion temperature; b) low ration and high emersion temperature; c) high ration and low emersion temperature; and d) high ration and high emersion temperature. The sampling time periods are shown on the x-axis and include samples taken before or after treatment (orange bar) during the daytime (open bars) and nighttime (gray bars) high tide periods.

treatment (Fig. 2), where clearance rates were generally higher when taken during the daytime high tides (i.e., 13:00–17:00 sampling periods) compared to those taken at night (i.e., 01:00–5:00 sampling periods). Our sample sizes were lower during the baseline measurements from problems with our fluorometer during the first day of measurements, so more data would need to be collected to see if these patterns persist.

We also observed a significant three-way interaction between acclimation conditions, before and after treatment, and treatment (acute heat shock $\pm$ sirtuin inhibition) on the clearance rates of the California mussel ($F_{3,199.7} = 4.30, p = 0.006$). Overall, both the application of acute heat shock and acute heat shock following sirtuin inhibition decreased the clearance rates of *M. californianus*, but this trend depended on the conditions the mussels had been acclimated to (Fig. 3). In the low emersion temperature and low ration tank, we found a significant reduction in the clearance rates of mussels exposed to both sirtuin inhibitors and acute heat shock, but not in those exposed to acute heat shock only, relative to the baseline measurements (Fig. 3a). We observed a similar trend in mussels acclimated to a low food ration and high emersion temperature, with a significant reduction in clearance rates before and after acute heat shock and inhibition, but not if the mussels had been exposed to only an acute heat shock (Fig. 3b). For mussels acclimated to a high ration, we found a significant reduction in clearance rates if mussels acclimated to a high ration and high emersion temperature were exposed to an acute heat shock only, but not if there was an additional application of sirtuin inhibitors (Fig. 3d). This was surprising because we hypothesized that these mussels would be the most tolerant of an acute heat shock. Finally, we did not observe any significant changes in clearance rates in the mussels acclimated to a high food ration and a low emersion temperature in either treatment (Fig. 3c).

3.2. Decreased valve gape activity following acute heat shock

As expected, valve gape activity, measured as the percentage of time the mussels spent with their valves open scaled to wet tissue weight, also

significantly varied over the sampling time period ($F_{2,190.3} = 5.39, p = 0.005$). The trends in valve gape activity over time were not as evident as they were for the clearance rate data (Fig. 4). During our experiment, mussels spent most of the time with their valves open to some degree but increased the frequency of valve closure following treatment. Similar to clearance rate, we found that exposure to sirtuin inhibition followed by acute heat shock reduced the amount of time the mussels spent filtering, but also may have shown signs of recovery to pre-treatment values towards the end of the recovery period we monitored, depending on the acclimation conditions.

We observed a significant three-way interaction between food ration and aerial temperature acclimation conditions and treatment (acute heat shock $\pm$ sirtuin inhibition) on the valve gape activity before and after treatment in *M. californianus* ($F_{3,193.0} = 3.69, p = 0.02$). The valve gape activity was variable among the four acclimation groups (Fig. 5) and we only observed a significant reduction in the valve gape activity of mussels acclimated to a low ration and high emersion temperature following sirtuin inhibition and acute heat shock, similar to the reduction in clearance rate at the same acclimation condition.

4. Discussion

This study was undertaken to evaluate the relationship between previous thermal history, food ration, and sirtuins on the thermal sensitivity of the California mussel, measured as changes in feeding behavior following exposure to acute heat shock during emersion. Global climate models predict both shifts in food availability and the frequency of high temperature events (Harley et al., 2006; Winder and Sommer, 2012) and an adequate understanding of the link between food availability and thermal tolerance is necessary for predictions of the effects of climate change on intertidal invertebrates. We found that response to acute heat shock or acute heat shock and sirtuin inhibition varied among our acclimation groups, both clearance rate and valve gape activity. Feeding behavior was generally reduced over multiple tidal cycles following acute heat shock if the mussels were first exposed

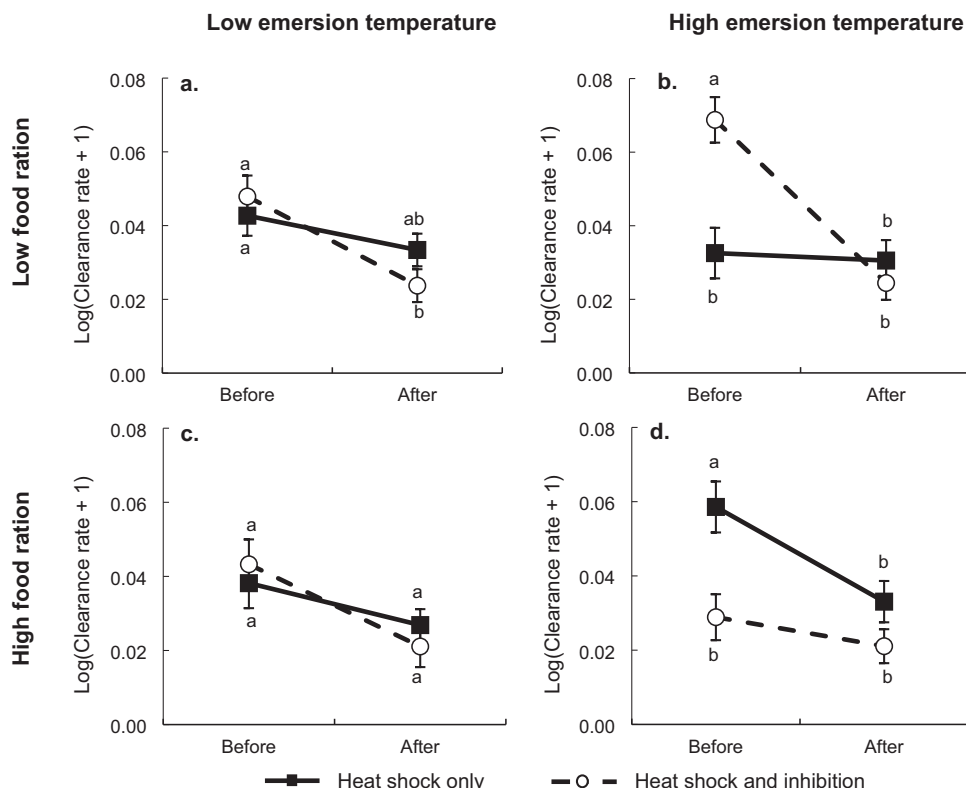


Fig. 3. The interactive effects of acclimation conditions, before and after treatment, and treatment (acute heat shock $\pm$ sirtuin inhibition) on the clearance rates of *M. californianus* ($F_{3,199.7} = 4.30, p = 0.006$). Each plot signifies the response of mussels to acute heat shock (solid squares) or acute heat shock and sirtuin inhibition (open circles) for each acclimation tank, with low emersion temperatures on the left, high emersion temperatures on the right, low ration on the top, and the high ration on the bottom. Clearance rates ($\text{l}\cdot\text{h}^{-1}\cdot\text{g wet tissue weight}^{-1}$), are reported as the least squares mean of $\log_{10}(\text{clearance rate} + 1) \pm \text{SEM}$. The letters indicate statistically significant differences in clearance rates before or after heat shock or heat shock following inhibition within each acclimation tank, estimated using Tukey's HSD ($\alpha = 0.05$).

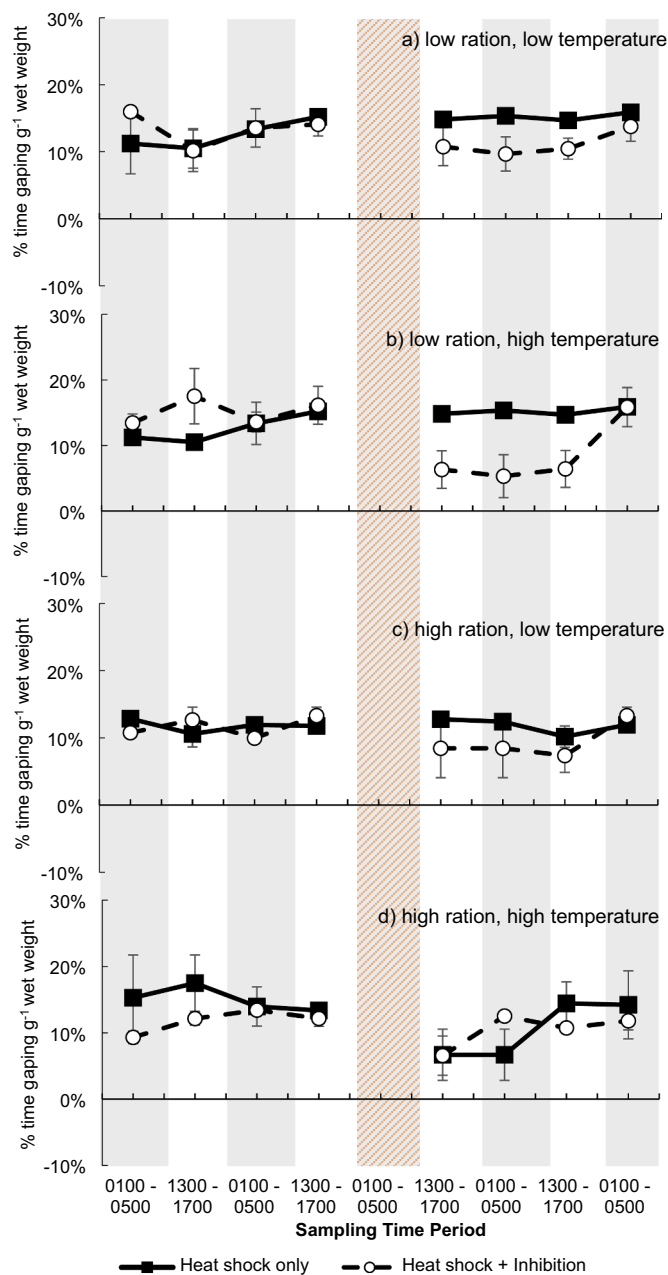


Fig. 4. Data plots showing temporal variation in valve gape activity of *M. californianus* (plotted as the mean percent time gaping scaled to wet tissue weight $\pm$ SEM for each of the four acclimation conditions and before or after acute heat shock acute heat shock $\pm$ sirutin inhibitors. Each graph shows valve gape activity for mussels exposed to heat shock only (solid squares and line) or heat shock and sirutin inhibition (open circles and dashed line) under various acclimation regimes: a) low ration and low emersion temperature; b) low ration and high emersion temperature; c) high ration and low emersion temperature; and d) high ration and high emersion temperature. The sampling time periods are shown on the x-axis and include samples taken before or after treatment (orange bar) during the daytime (open bars) and nighttime (gray bars) high tide periods.

to sirutin inhibitors and mussels acclimated to a low food ration were more sensitive than those in the high food rations, suggesting a strong linkage between food ration, feeding behavior, thermal sensitivity, and sirutins.

4.1. Food ration and previous thermal history influences thermal sensitivity

While most studies evaluate the response to acute heat stress at discrete time points, our experimental design allowed us to monitor changes in the feeding behavior of *Mytilus californianus* for several subsequent high tide periods. We predicted that acute heat shock would result in initial reductions in clearance rate and valve gape as an energy-saving mechanism and that filtration would return to pre-treatment values during the first or the subsequent high tide of the recovery period, similar to recovery from heat shock in other intertidal organisms (Tomanek and Somero, 2000). Furthermore, we hypothesized that well-fed mussels, acclimated to elevated aerial temperatures (30 °C), would be less affected by a sublethal, acute heat shock of 33 °C compared to mussels acclimated to a lower food ration and milder aerial temperatures (20 °C) and would, therefore, resume normal feeding and valve gaping upon reimmersion. Contrary to our predictions, mussels that had been acclimated to a high ration and high emersion temperature were the only group that showed significant reductions in clearance rates following heat shock (Fig. 3d) and large, but non-significant, decreases in valve gape activity (Fig. 5d). This was surprising as it has been shown that both high food ration and emersion temperature confer high thermal tolerance at the organismal level (i.e., survival) following an acute heat shock of 33 °C and 36 °C (Fitzgerald-Dehoog et al., 2012). While, the former study did not investigate the role of clearance rate in higher rates of survival following acute heat shock or the underlying mechanisms that contribute to increased thermal tolerance, it is possible that declines in clearance rates, even temporarily, may reduce energy expenditures associated with feeding (Bayne et al., 1976a), allowing for a reallocation of energy from higher levels of organization (organ) to the cellular stress response (Buckley et al., 2001; Halpin et al., 2004; Tomanek, 2008) to survive heat stress. Additional studies are required to test this hypothesis.

Variation in feeding behavior during recovery from extreme emersion temperatures has not been well studied in mussels, however, there are known physiological changes that occur during recovery from aerial exposure (without a concomitant thermal stress), such as increased oxygen consumption (Widdows and Shick, 1985; Ober et al., 2019) and heat dissipation (Shick et al., 1988), which result from the oxygen debt acquired during hypoxia. These increased metabolic rates reported in *M. edulis* occurred quickly following reimmersion and mussels returned to basal metabolic rates within a few hours (Shick et al., 1988). While one might predict that increased metabolic demands could be met by increasing energy consumption through feeding, Bayne et al. (1976b) did not observe such increases in filtration in *M. californianus* and concluded that intertidal mussels do not compensate by increasing feeding rates following reimmersion (Bayne et al., 1988). These findings, with the relatively high cost of feeding in filter-feeding bivalves (Bayne et al., 1976a), would suggest that increasing filtration as a means of recovering from the stressors associated with aerial exposure would be prohibitively expensive. Therefore, it may be energetically beneficial to reduce feeding following aerial exposure, at least as a short-term response.

As mentioned above, the only group that showed significant reductions in feeding following acute aerial heat shock were mussels acclimated to high emersion temperatures and a high ration (Fig. 3). While a reduction in feeding may be an indication of stress, it is also possible that these reductions may benefit the organisms by allowing the mussels to divert energy from feeding towards combatting stress, which may explain the increases in survival reported in this group by Fitzgerald-Dehoog et al. (2012). In this case, reduced feeding may be an indication of the mussels' resilience to acute heat shock, but further studies would need to be conducted to test this hypothesis. To our knowledge, only one other study has specifically tested how acute thermal stress in air influences the feeding rates following reimmersion and found that a tropical, intertidal oyster (*Isognomon nucleus*) initially

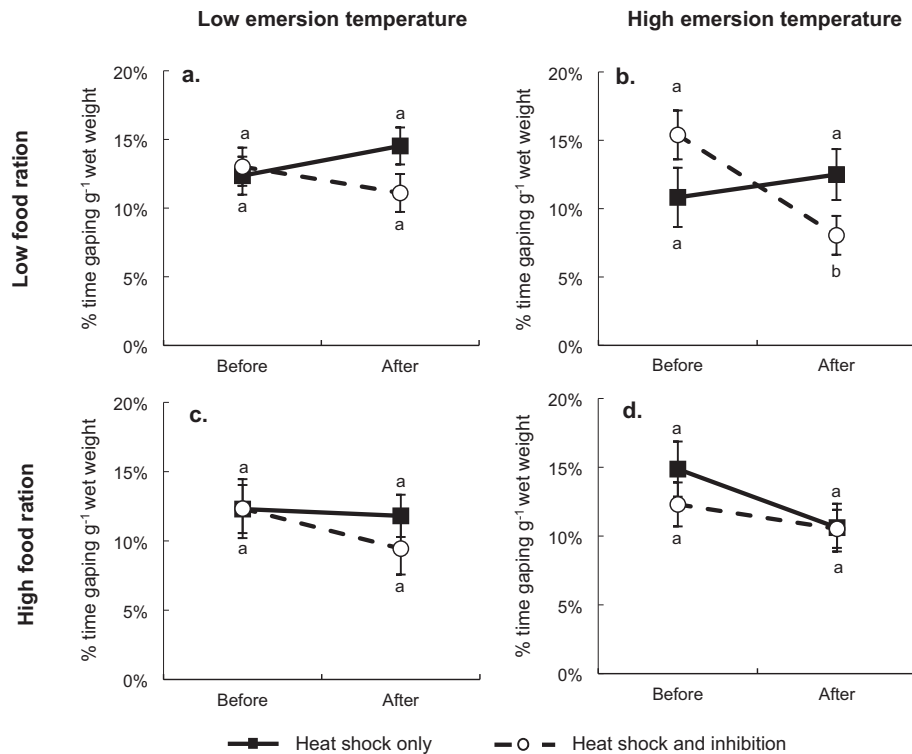


Fig. 5. The interactive effects of acclimation conditions, before and after treatment, and treatment (acute heat shock $\pm$ sirtuin inhibition) on the valve gape activity of *M. californianus* ($F_{3,193.0} = 3.37$, $p = 0.0197$). Each plot signifies the response of mussels to acute heat shock (solid squares) or acute heat shock and sirtuin inhibition (open circles) for each acclimation tank, with low emersion temperatures on the left, high emersion temperatures on the right, low ration on the top, and the high ration on the bottom. Valve gape activity, in % of time spent gaping $\cdot$ g wet tissue weight $^{-1}$ only significantly declined in mussels that were acclimated to a low ration and high emersion temperature following exposure to both acute heat shock and sirtuin inhibitors (mean = 0.080, SEM = 0.014) relative to the pre-treatment values (mean = 0.108, SEM = 0.022). The letters indicate statistically significant differences in valve gape activity before or after heat shock or heat shock following inhibition within each acclimation tank, estimated using Tukey's HSD post-hoc analysis ($\alpha = 0.05$).

decreased clearance rates but showed signs of recovery within a few hours of immersion (Giomi et al., 2016). Furthermore, the 'unresponsiveness' with respect to feeding following thermal stress in our other acclimation groups may be the underlying cause of slower growth rates and increased mortality that have been documented in intertidal mussels (Fitzgerald-Dehoog et al., 2012), intertidal oysters (Bishop and Peterson, 2006), and clams (Peterson and Black, 1987) or alterations to reproductive cycles observed in intertidal barnacles (Kasten and Flores, 2013) following heat shock. Further studies should be undertaken to better understand the long-term implications of reduced feeding resulting from exposure to sub-lethal aerial temperatures.

In a long-term monitoring study of *M. californianus* along the central coast of California, mussels from high intertidal sites showed reductions in the proportion of time gaping widely (i.e., valve angle of $>20^\circ$) during subsequent high tide periods (Gleason et al., 2017; Miller and Dowd, 2017) for up to 24 h after a high-temperature day (Miller and Dowd, 2019) compared to mussels inhabiting the lower intertidal zone. These studies suggest that the recovery from exposure to high aerial temperatures may not occur as rapidly as previous studies suggested. Interestingly, we observed high variation in the valve gape response of mussels following acute heat shock depending on their acclimation conditions (Fig. 4, solid lines) and only mussels acclimated to a high food ration and high emersion temperature showed any declines in valve gape activity (Fig. 5B). While these reductions in valve gape activity occurred initially (Fig. 4d), the mussels resumed normal valve gape activity after 24 h following an acute heat shock relative to the pre-treatment values. While we observed similar trends for both valve gape activity and clearance rate in the high ration and high emersion temperature group, other studies have shown that valve gape activity (measured as percentage of time open or degree of gaping) correlate poorly with feeding behavior (Maire et al., 2007; MacDonald et al., 2009).

The interactive effects of food ration and acute heat shock on the clearance rates of *M. californianus* is noteworthy. There is an abundance of research showing that both clearance rates and valve gape activity are influenced by diet and that mussels fed a low ration generally have

higher rates of filtration than those fed a high ration (Schulte, 1975; Pascoe et al., 2009; Riisgård et al., 2013). While we found no evidence for ration-induced variation in feeding before treatment, there was evidence that mussels in the high food rations tended to show greater decreases in clearance rate (Fig. 3, solid lines) and valve gape activity (Fig. 5, solid lines) than the low food groups following acute heat shock. While it is difficult to compare our feeding rations with threshold values found in other studies (chlorophyll *a* measurements of Shellfish Diet 1800® can be unreliable; Reed Mariculture, pers. comm.), the high food groups were fed quantities recommended for broodstock conditioning of hatchery mussels (Helm et al., 2004), and likely exceeded threshold values that reduce filtration found in other studies. Mussels from the low food ration, on the other hand, had access to roughly $1.5 \mu\text{g} \cdot \text{l}^{-1}$ dry weight Shellfish Diet, which is similar to what is observed in their natural habitat along the California coastline (Blanchette et al., 2007). Consistent with our hypothesis above, for mussels fed a high ration, a decrease in feeding is probably of little physiological importance, as the ration was likely in excess of what mussels require to meet their energy demands.

In mussels acclimated to a low ration, increased pumping rates are necessary to compensate for lower algal densities (Jørgensen et al., 1986), so a similar reduction in feeding, confounded by lower algal abundances, would equate to a roughly a 4-fold decrease per hour in the food intake by mussels in the low ration groups relative to mussels in the high ration groups following acute heat shock. Thus, by maintaining clearance rates following acute heat shock, our findings for the low food ration groups support other studies that propose food availability may be more important than previous thermal history for combatting aerial temperature stress using various metabolic enzymes as indicators of stress activation at the cellular level (Elvin and Gonor, 1979; Schneider et al., 2010; Dowd et al., 2013; Jimenez et al., 2016). Mussels fed a high food ration may be less impacted energetically by emersion temperature stress than those mussels that are fed a low ration, but further research is needed to directly quantify food intake and assimilation during recovery from aerial heat shock in mussels exposed to varying algal concentrations.

4.2. The role of sirtuins and thermal sensitivity

Another component of this study was to evaluate the role of sirtuins in the response to acute heat shock in mussels acclimated under different food and low tide temperatures. Sirtuins generally increase in abundance during caloric restriction in mammals (Houtkeeper et al., 2010) and invertebrates (Kapahi et al., 2017) and have been implicated as regulators of heat stress (Westerheide et al., 2009; Liu et al., 2014), oxidative stress (Qiu et al., 2010), and metabolic state (Choudhary et al., 2014). Additionally, studies have observed changes in sirtuin gene expression and protein abundance during heat shock in other marine invertebrates, including intertidal limpets (Han et al., 2013; Huang et al., 2015; Dong and Zhang, 2016) and mytilid congeners (Tomanek and Zuzow, 2010; Vasquez et al., 2017, 2020). As part of our broader study, we predicted that sirtuins play a role linking food ration and thermal tolerance in *M. californianus*. While it is difficult to hypothesize how protein inhibitors of cellular homeostasis will affect organismal-level functions such as feeding, we expected that mussels that were exposed to both acute heat shock and sirtuin inhibitors would reduce feeding more and recover slower than mussels exposed to heat shock only due to the role of sirtuins in maintaining homeostasis during stress. We found that exposure to sirtuin inhibitors caused significant decreases in both clearance rates (Fig. 3) and valve gaping activity (Fig. 5) during recovery from aerial heat shock, depending on the acclimation conditions, and as expected, that these decreases were more pronounced than when mussels were exposed to heat shock without inhibition.

We had also predicted that food ration would alter the response to sirtuin inhibitors, as sirtuins have been shown to decrease in abundance during caloric excess in mammals (Chalkiadaki and Guarente, 2012). More specifically, we expected that mussels from the high ration groups, exposed to sirtuin inhibitors (Fig. 1) would show greater reductions in feeding relative to the low ration groups, due to presumed differences in sirtuin levels (with sirtuin inhibitors having presumably a greater effect on lower levels of sirtuins found in well-fed mussels). In contrast to our predictions, mussels acclimated to low food rations, regardless of acclimation emersion temperature showed significant reductions in clearance rate following both sirtuin inhibition and acute heat shock, but not an acute heat shock alone, suggesting that sirtuins likely play a role in the thermal sensitivity of *M. californianus* when food availability is low. We are currently working to delineating pathways that may comprise the molecular food phenotype at the level of the transcriptome and proteome following acute heat shock and sirtuin inhibition. Our previous and current studies indicate that sirtuins affect the canonical cellular and the more specific oxidative stress response in *Mytilus* (Vasquez et al., 2017, 2020; May et al., in prep) but further research is needed to understand whether food ration has different implications for increased heat tolerance at the cellular versus organismal levels.

4.3. Evidence for circadian variation in feeding

Studies have documented circatidal and circadian rhythms in the transcriptome (Place et al., 2012; Connor and Gracey, 2011; Gracey and Connor, 2016), metabolome (Connor and Gracey, 2012), proteome (Elowe and Tomanek, in review), and more recently, in the gaping behavior (Miller and Dowd, 2017, 2019) of *M. californianus*. While not the focus of this particular study, sampling clearance rates and valve gaping activity over multiple day and night high tide cycles permitted us to account for circadian variation in the feeding behavior of mussels. We found a significant effect of time relative to treatment on both clearance rates (Fig. 2) and percentage of time spent filtering (Fig. 4), but these trends varied depending on metric and over the course of the experiment. Feeding in these mussels was highly variable (Fig. 2) and sample sizes during the acclimation period were reduced because of equipment error, but there is evidence that clearance rates were higher during our daytime measurements following treatment (Fig. 2), but possibly higher during the nighttime prior to treatment. Other studies have found

evidence of diurnal variation in the feeding behavior of mytilids, but found that feeding (Robson et al., 2010) and gape angle (Wilson et al., 2005) increased during the night or varied with tidally-driven variation in available food (Saurel et al., 2007). Our mussels were fed the same concentrations throughout the experiment and held at a constant water temperature during measurements, so our findings may be more indicative of endogenous rhythms, perhaps driven by circadian variation at the molecular level, that are masked under more natural conditions. Future studies should account for endogenous rhythms during stress using kinetic experiments, as biological rhythms can drive molecular processes with direct effects at higher phenotypic levels.

4.4. Conclusions

In this study, we observed significant reductions in the valve gaping activity and clearance rate of *M. californianus* following exposure to acute heat stress and sirtuin inhibition over a 48-h recovery period. These reductions in filtration were influenced by acclimation food ration and thermal history during low tide periods, consistent with other studies of this species (Elvin and Gonor, 1979; Dowd et al., 2013). Following sirtuin inhibition and sequential acute heat stress during aerial exposure, mussels in the low food treatment reduced filtration significantly, which suggests that sirtuins may be an integral regulator for these mussels to replace energy reserves following environmental stress. In the natural environment, mussels that live in the upper intertidal zone are more susceptible to acute exposure to high-temperature days than those in the lower intertidal (Harley et al., 2006). Mussels from the high intertidal zone also have less time submerged for feeding during recovery periods. If reduced feeding time is compounded by a reduction in feeding rates, higher intertidal mussels may be more at risk to warming and will likely show reductions in growth and reproduction and increases in mortality driven by caloric restriction. Future research efforts attempting to understand the effects of aerial temperature stress on the fitness of mytilids should more directly test changes in feeding and assimilation. Finally, we found that sirtuins play an important role in the response to acute heat shock at the organ and organismal level, suggesting that the molecular effects of sirtuins previously identified may affect higher levels of biological organization (Vasquez et al., 2017). The actual signaling pathways of these upstream effects are unknown and part of our ongoing investigation on the integration of molecular level changes on tissue and organismal performance.

Funding

This work was supported by the National Science Foundation to Lars Tomanek (IOS-1557500) and Anne Todgham (IOS-1557496). Additional funding for student support was provided by the Frost Foundation and the Biology Department at California Polytechnic State University. This work was also supported by the California Agricultural Experimental Station of the University of California Davis (CA-D-ASC-2252-H to AET).

Author contributors

LT, AET, MAM, and MCV were responsible for conceptualization, while all authors participated in data curation. MAM did the formal analyses and writing - original draft. All authors contributed to writing - review & editing. LT and AET obtained funding and provided supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the numerous students who assisted with data collection, including O. Carmo, A. Kretschmar, and J. T. Fang. We would also like to thank Dr. A. Shaffner for statistical advice and R. Brewster, D. Brewster, T. Moylan, and J. Felton for their logistical support with designing and maintaining the tidal simulators at the Cal Poly Pier. And, finally, thank you to our reviewers for making helpful suggestions and overall improving the data analyses and manuscript.

References

- Bayne, B.L., 2004. Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve molluscs. *Integr. Comp. Biol.* 44, 425–432.
- Bayne, B.L., Bayne, C.J., Carefoot, T.C., Thompson, R.J., 1976a. The physiological ecology of *Mytilus californianus* Conrad. 1. Metabolism and energy balance. *Oecologia* 22, 211–228.
- Bayne, B.L., Bayne, C.J., Carefoot, T.C., Thompson, R.J., 1976b. The physiological ecology of *Mytilus californianus* Conrad 2. Adaptations to low oxygen tension and air exposure. *Oecologia* 22, 229–250.
- Bayne, B.L., Hawkins, A.J.S., Navarro, E., 1988. Feeding and digestion in suspension-feeding bivalve molluscs: the relevance of physiological compensations. *Am. Zool.* 28, 147–159.
- Beggel, S., Hinzmann, M., Mahado, J., Geist, J., 2017. Combined impact of acute exposure to ammonia and temperature stress on the freshwater mussel *Unio pictorum*. *Water* 9, 455.
- Bishop, M.J., Peterson, C.H., 2006. Direct effects of physical stress can be counteracted by indirect benefits: oyster growth on a tidal elevation gradient. *Oecologia* 147, 426–433.
- Blanchette, C.A., Helmuth, B., Gaines, S.D., 2007. Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at point conception, California, USA. *J. Exp. Mar. Biol. Ecol.* 340, 126–148.
- Buckley, B.A., Owen, M.-E., Hofmann, G.E., 2001. Adjusting the thermostat: the threshold induction temperature for the heat shock response in intertidal mussels (genus *Mytilus*) changes as function of thermal history. *J. Exp. Biol.* 204, 3571–3579.
- Chalkiadaki, A., Guarente, L., 2012. Sirtuins mediate mammalian metabolic responses to nutrient availability. *Nat. Rev. Endocrinol.* 8, 287–296.
- Cheng, M.C.F., Sará, G., Williams, G.A., 2018. Combined effects of thermal conditions and food availability on thermal tolerance of the marine bivalve, *Perna viridis*. *J. Therm. Biol.* 78, 270–276.
- Choudhary, C., Weinert, B.T., Nishida, Y., Verdin, E., Mann, M., 2014. The growing landscape of lysine acetylation links metabolism and cell signaling. *Nat. Rev. Mol. Cell Biol.* 15, 536–550.
- Connor, K.M., Gracey, A.Y., 2011. Circadian cycles are the dominant transcriptional rhythm in the intertidal mussel *Mytilus californianus*. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16110–16115.
- Connor, K.M., Gracey, A.Y., 2012. High-resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R103–R111.
- Connor, K.W., Robles, C.D., 2015. Within-site variation of growth rate and terminal sizes in *Mytilus californianus* along wave exposure and tidal gradients. *Biol. Bull.* 228, 39–51.
- Dahlhoff, E.P., Menge, B.A., 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. *Mar. Ecol. Prog. Ser.* 144, 97–107.
- Dahlhoff, E.P., Stillman, J.H., Menge, B.A., 2002. Physiological community ecology: variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. *Integr. Comp. Biol.* 42, 862–871.
- Denny, M.W., Dowd, W.W., Bilir, L., Mach, K.J., 2011. Spreading the risk: small-scale body temperature variation among intertidal organisms and its implications for species persistence. *J. Exp. Mar. Biol. Ecol.* 400, 175–190.
- Dong, Y.-W., Zhang, S., 2016. Ecological relevance of energy metabolism: transcriptional responses in energy sensing and expenditure to thermal and osmotic stresses in an intertidal limpet. *Funct. Ecol.* 30, 1539–1548.
- Dowd, W.W., Somero, G.N., 2013. Behavior and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater. *J. Exp. Biol.* 216, 502–514.
- Dowd, W.W., Felton, C.A., Heymann, H.M., Kost, L.E., Somero, G.N., 2013. Food availability, more than body temperature, drives correlated shifts in ATP-generating and antioxidant enzyme capacities in a population of intertidal mussels (*Mytilus californianus*). *J. Exp. Mar. Biol. Ecol.* 449, 171–185.
- Elowe, C., Tomanek, L., Sept, 2019. Circadian and circatidal rhythms of protein abundance in the California mussel (*Mytilus californianus*). *Molec. Ecol.* 1–26. Submitted for publication.
- Elvin, D.W., Gonor, J.J., 1979. The thermal regime of an intertidal *Mytilus californianus* Conrad population on the Central Oregon coast. *J. Exp. Mar. Biol. Ecol.* 39, 265–279.
- Fitzgerald-Dehoog, L., Browning, J., Allen, B.J., 2012. Food and heat stress in the California mussel: evidence for an energetic trade-off between survival and growth. *Biol. Bull.* 223, 205–216.
- Fuller, J.L., 1937. Feeding rate of *Calanus finmarchicus* in relation to environmental conditions. *Biol. Bull.* 72, 233–246.
- Garza, C., 2005. Prey productivity effects on the impact of predators of the mussel, *Mytilus californianus* (Conrad). *J. Exp. Mar. Biol. Ecol.* 324, 76–88.
- Gilman, S.E., Rognstad, R.L., 2018. Influence of food supply and shore height on the survival and growth of the barnacle *Balanus glandula* (Darwin). *J. Exp. Mar. Biol. Ecol.* 498, 32–38.
- Giomì, F., Mandaglio, C., Ganmanee, M., Han, G.-D., Dong, Y.-W., Williams, G.A., Sará, G., 2016. The importance of thermal history: costs and benefits of heat exposure in a tropical, rocky shore oyster. *J. Exp. Biol.* 219, 686–694.
- Gleason, L.U., Miller, L.P., Winnikoff, J.R., Somero, G.N., Yancey, P.H., Bratz, D., Dowd, W.W., 2017. Thermal history and gape of individual *Mytilus californianus* correlate with oxidative damage and thermoprotective osmolytes. *J. Exp. Biol.* 220, 4292–4304.
- Gracey, A.Y., Connor, K., 2016. Transcriptional and metabolic characterization of spontaneous metabolic cycles in *Mytilus californianus* under subtidal conditions. *Mar. Genomics* 30, 35–41.
- Halpin, P.M., Menge, B.A., Hofmann, G.E., 2004. Experimental demonstration of plasticity in the heat shock response of the intertidal mussel *Mytilus californianus*. *Mar. Ecol. Prog. Ser.* 276, 137–145.
- Han, G., Zhang, S., Marshall, D.J., Ke, C., Dong, Y., 2013. Metabolic energy sensors (AMPK and SIRT1), protein carbonylation and cardiac failure as biomarkers of thermal stress in an intertidal limpet: linking energetic allocation with environmental temperature during aerial emersion. *J. Exp. Biol.* 216, 3272–3282.
- Harley, C.D., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J., Thorneber, C. S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change on coastal marine systems. *Ecol. Lett.* 9, 228–241.
- Harley, C.D.G., Helmuth, B.S.T., 2003. Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective short level on patterns of intertidal zonation. *Limnol. Oceanogr.* 48, 1498–1508.
- Helm, M.M., Bourne, N., Lovatelli, A., 2004. Hatchery Culture of Bivalves: A Practical Manual. FAO Fisheries Technical Paper 471, Rome, p. 177.
- Helmuth, B., Broitman, B.R., Blanchette, C.A., Gilman, S., Halpin, P., Harley, C.D.G., O'Donnell, M.J., Hofmann, G.E., Menge, B., Strickland, D., 2006. Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecol. Monogr.* 76, 461–479.
- Helmuth, B.S.T., Hofmann, G.E., 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol. Bull.* 201, 374–384.
- Houtkeeper, R.H., Williams, R.W., Auwerx, J., 2010. Metabolic networks of longevity. *Cell* 142, 9–14.
- Huang, X., Wang, T., Ye, Z., Han, G., Dong, Y., 2015. Temperature relations of aerial and aquatic physiological performance in a mid-intertidal limpet, *Cellana toreuma*: Adaptation to rapid changes in thermal stress during emersion. *Integr. Zool.* 10, 159–170.
- Jimenez, A.G., Alves, S., Dallmer, J., Njoo, E., Roa, S., Dowd, W.W., 2016. Acclimation to elevated emersion temperature has no effect on susceptibility to acute, heat-induced lipid peroxidation in an intertidal mussel (*Mytilus californianus*). *Mar. Biol.* 163, 55.
- Jørgensen, C.B., Møhlenberg, F., Sten-Knudsen, O., 1986. Nature of relation between ventilation and oxygen consumption in filter feeders. *Mar. Ecol. Prog. Ser.* 29, 73–88.
- Kapahi, P., Kaeblerlein, M., Hansen, M., 2017. Dietary restriction and lifespan: Lessons from invertebrate models. *Ageing Res. Rev.* 39, 3–14.
- Kasten, P., Flores, A.A.V., 2013. Disruption of endogenous tidal rhythms of larval release linked to food supply and heat stress in an intertidal barnacle. *Mar. Ecol. Prog. Ser.* 472, 185–198.
- Kramer, K.J.M., Jenner, H.A., de Zwart, D., 1989. The valve movement response of mussels: a tool in biological monitoring. *Hydrobiologia* 188 (/189), 433–443.
- Kumar, S., Lombard, D.B., 2018. Functions of the sirtuin deacetylase SIRT5 in normal physiology and pathobiology. *Crit. Rev. Biochem. Mol. Biol.* 53, 311–334.
- Landenberger, D.E., 1968. Studies on selective feeding in the Pacific starfish *Pisaster* in Southern California. *Ecology* 49, 1062–1075.
- Lathlean, J.A., Seuront, L., Ng, T.P.T., 2017. On the edge: the use of infrared thermography in monitoring responses of intertidal organisms to heat stress. *Ecol. Indic.* 81, 567–577.
- Lawson, M., Uciechowska, U., Schemies, J., Rump, T., Jung, M., Sippl, W., 2010. Inhibitors to understand molecular mechanisms of NAD⁺-dependent deacetylases (sirtuins). *BBA—Gene Regul. Mech.* 1799, 726–739.
- Lesser, M.P., Bailey, M.A., Merselis, D.G., Morrison, J.R., 2010. Physiological response of the blue mussels *Mytilus edulis* to differences in food and temperature in the Gulf of Maine. *Comp. Biochem. Physiol. A* 156, 541–551.
- Liu, D.J., Hammer, D., Komlos, D., Chen, K.Y., Firestein, B.L., Liu, A.Y.-C., 2014. Sirt1 knockdown promotes neural differentiation and attenuates the heat shock response. *J. Cell. Physiol.* 229, 1224–1235.
- Loo, L.-O., Rosenberger, R., 1996. Production and energy budget in marine suspension feeding populations: *Mytilus edulis*, *Cerastoderma edula*, *Mya arenaria*, and *Amphipura filiformis*. *J. Sea Res.* 35, 199–207.
- Loo, S., Rine, J., 1995. Silencing and heritable domains of gene expression. *Ann. Rev. Cell Develop. Biol.* 11, 519–548.
- MacDonald, B.A., Robinson, S.M., Barrington, K.A., 2009. Evaluating the use of exhalant siphon area in estimating feeding activity of blue mussels, *Mytilus edulis*. *J. Shellfish Res.* 28, 289–297.
- Maire, O., Amouroux, J.-M., Duchêne, J.-C., Grémare, A., 2007. Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. *Mar. Biol.* 152, 1293–1307.
- Menge, B.A., 1992. Community regulation: Under what conditions are bottom-up factors important on rocky shores. *Ecology* 73, 755–765.
- 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*). J. Exp. Biol. 220, 4305–4319.
- Miller, L.P., Dowd, W.W., 2019. Repeatable patterns of small-scale spatial variation in intertidal mussel beds and their implications for responses to climate change. Comp. Biochem. Physiol. A 236, 110516. <https://doi.org/10.1016/j.cbpa.2019.06.016>.
- Morak, S., Hegerl, G.C., Christidis, N., 2013. Detectable changes in the frequency of temperature extremes. J. Clim. 26, 1561–1574.
- Morris, S., Taylor, A.C., 1983. Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. Estuar. Coast. Shelf S. 17, 339–355.
- Mykles, D.L., Ghalambor, C.K., Stillman, J.H., Tomanek, L., 2010. Grand challenges in comparative physiology: Integration across disciplines and across levels of biological organization. Integr. Comp. Biol. 50, 6–16.
- Norén, F., Haamer, J., Lindahl, O., 1999. Changes in the plankton community passing a *Mytilus edulis* mussel bed. Mar. Ecol. Prog. Ser. 191, 187–194.
- Ober, G.T., Rognstad, R.L., Gilman, S.E., 2019. The cost of emersion for the barnacle, *Balanus glandula*. Mar. Ecol. Prog. Ser. 627, 95–107.
- Paine, R.T., 1974. Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia 15, 93–120.
- Pascoe, P.L., Parry, H.E., Hawkins, A.J.S., 2009. Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish. Aquat. Biol. 6, 181–190.
- Peterson, C.H., Black, R., 1987. Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. Limnol. Oceanogr. 32, 143–166.
- Petes, L.E., Menge, B.A., Murphy, G.D., 2007. Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. J. Exp. Mar. Biol. Ecol. 351, 83–91.
- Petes, L.E., Menge, B.A., Harris, A.L., 2008. Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. Ecol. Monogr. 78, 387–402.
- Place, S.P., Menge, B.A., Hofmann, G.E., 2012. Transcriptome profiles link environmental variation and physiological responses of *Mytilus californianus* between Pacific tides. Funct. Ecol. 26, 144–155.
- Qiu, X., Brown, K., Hirschey, M.D., Verdin, E., Chen, D., 2010. Calorie restriction reduces oxidative stress by Sirt3-mediated SOD2 activation. Cell Metab. 12, 662–667.
- Rao, K.P., 1953. Rate of water propulsion in *Mytilus californianus* as a function of latitude. Biol. Bull. 104, 171–181.
- Riisgård, H.U., Kittner, C., Seerup, D.F., 2003. Regulation of opening state and filtration rate in filter-feeding bivalves (*Cardium edule*, *Mytilus edulis*, *Mya arenaria*) in response to low algal concentration. J. Exp. Mar. Biol. Ecol. 284, 105–127.
- Riisgård, H.U., Lassen, J., Kittner, C., 2006. Valve-gape response times in mussels (*Mytilus edulis*)—effects of laboratory preceding-feeding conditions and in situ tidally induced variation in phytoplankton biomass. J. Shellfish Res. 25, 901–911.
- Riisgård, H.U., Pleissner, D., Lundgreen, K., Larsen, P.S., 2013. Growth of mussels *Mytilus edulis* at algal (*Rhodomonas salina*) concentrations below and above saturation levels for reduced filtration rate. Mar. Biol. Res. 9, 1005–1017.
- Robson, A.A., Garcia de Leaniz, C., Wilson, R.P., Halsey, L.G., 2010. Effect of anthropogenic feeding regimes on activity rhythms of laboratory mussels exposed to natural light. Hydrobiologia. 655, 197–204.
- Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M., Puigserver, P., 2005. Nutrient control of glucose homeostats through a complex of PGC-1 α and SIRT1. Nature 434, 113–118.
- Saurel, C., Gascoigne, J.C., Palmer, M.R., Kaiser, M.J., 2007. In situ mussel feeding behavior in relation to multiple environmental factors: regulation through food concentration and tidal conditions. Limnol. Oceanogr. 52, 1919–1929.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Meth. 9, 671–675.
- Schneider, K.R., Van Thiel, L.E., Helmuth, B., 2010. Interactive effects of food availability and aerial body temperature on the survival of two intertidal *Mytilus* species. J. Therm. Biol. 35, 161–166.
- Schulte, E.H., 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. Mar. Biol. 30, 331–341.
- Seuront, L., Nicastro, K.R., Zardi, G.I., Goberville, E., 2019. Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel *Mytilus edulis*. Sci. Rep. 9 (17), 498.
- Shick, J.M., Widdows, J., Graiger, E., 1988. Calorimetric studies of behavior, metabolism and energetics of sessile invertebrate animals. Am. Zool. 28, 161–181.
- Shinen, J.S., Morgan, S.G., 2009. Mechanisms of invasion resistance: competition among intertidal mussels promotes establishment of invasive species and displacement of native species. Mar. Ecol. Prog. Ser. 383, 187–197.
- Sorte, C.J.B., Bernatchez, G., Milan, K.A.S., Pandori, L.L.M., Silbiger, N.J., Wallingford, P.D., 2019. Thermal tolerance limits as indicators of current and future zonation patterns in a diverse mussel guild. Mar. Biol. 166, 6.
- Suchanek, T.H., 1985. Mussels and their role in structuring rocky shore communities. In: Moore, P.G., Seed, R. (Eds.), The Ecology of Rocky Coasts. Hodder and Stoughton Press, London, pp. 70–96.
- Sukhotin, A.A., Lajus, D.L., Lesin, P.A., 2003. Influence of age and size on pumping activity and stress resistance in the marine bivalve *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 284, 129–144.
- Tomanek, L., 2008. The importance of physiological limits in determining biogeographical range shifts due to global climate change: The heat shock response. Physiol. Biochem. Zool. 81, 709–717.
- Tomanek, L., Somero, G.M., 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (Genus *Tegula*) from different tidal heights. Physiol. Biochem. Zool. 73, 249–256.
- Tomanek, L., Zuzow, M.J., 2010. The proteomic response of the mussel congeners *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. J. Exp. Biol. 213, 3559–3574.
- Underwood, A.J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. J. Exp. Mar. Biol. Ecol. 161, 145–178.
- Vasquez, M.C., Tomanek, L., 2019. Sirtuins as regulators of the cellular stress response and metabolism in marine ectotherms. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 236, 110528 <https://doi.org/10.1016/j.cbpa.2019.110528>.
- Vasquez, M.C., Beam, M., Blackwell, S., Zuzow, M.J., Tomanek, L., 2017. Sirtuins regulate proteomic responses near thermal tolerance limits in the blue mussels *Mytilus galloprovincialis* and *Mytilus trossulus*. J. Exp. Biol. 220, 4515–4534.
- Vasquez, M.C., Martinez, D.A., Tomanek, L., 2020. Multiple stressor responses are regulated by sirtuins in *Mytilus* congeners. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 246 (110), 719.
- Westerheide, S.D., Anckar, J., Stevens Jr., S.M., Sistonen, L., Morimoto, R.I., 2009. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science 323, 1063–1066.
- Widdows, J., Shick, J.M., 1985. Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. Mar. Biol. 85, 217–232.
- Wilson, R., Reuter, P., Wahl, M., 2005. Muscling in on mussels: new insights into bivalve behavior using vertebrate remote-sensing technology. Mar. Biol. 147, 1165–1172.
- Winder, M., Sommer, U., 2012. Phytoplankton response to a changing climate. Hydrobiologia 698, 5–16.
- Ziman, B., Karabinis, P., Barghouth, P., Oviedo, N.J., 2020. Sirtuin-1 regulates organismal growth by altering feeding behavior and intestinal morphology in planarians. J. Cell Sci. 133, jcs239467.