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RESEARCH ARTICLE

Behavior and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater

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SUMMARY

Coping with environmental stress may involve combinations of behavioral and physiological responses. We examined potential interactions between adult mussels' simple behavioral repertoire – opening/closing of the shell valves – and thermal stress physiology in common-gardened individuals of three *Mytilus* congeners found on the West Coast of North America: two native species (*M. californianus* and *M. trossulus*) and one invasive species from the Mediterranean (*M. galloprovincialis*). We first continuously monitored valve behavior over three consecutive days on which body temperatures were gradually increased, either in air or in seawater. A temperature threshold effect was evident between 25 and 33°C in several behavioral measures. Mussels tended to spend much less time with the valves in a sealed position following exposure to 33°C body temperature, especially when exposed in air. This behavior could not be explained by decreases in adductor muscle glycogen (stores of this metabolic fuel actually increased in some scenarios), impacts of forced valve sealing on long-term survival (none observed in a second experiment), or loss of contractile function in the adductor muscles (individuals exhibited as many or more valve adduction movements following elevated body temperature compared with controls). We hypothesize that this reduced propensity to seal the valves following thermal extremes represents avoidance of hypoxia–reoxygenation cycles and concomitant oxidative stress. We further conjecture that prolonged valve gaping following episodes of elevated body temperature may have important ecological consequences by affecting species interactions. We then examined survival over a 90 day period following exposure to elevated body temperature and/or emersion, observing ongoing mortality throughout this monitoring period. Survival varied significantly among species (*M. trossulus* had the lowest survival) and among experimental contexts (survival was lowest after experiencing elevated body temperature in seawater). Surprisingly, we observed no cumulative impact on survival of 3 days relative to 1 day of exposure to elevated body temperature. The delayed mortality and context-specific outcomes we observed have important implications for the design of future experiments and for interpretation of field distribution patterns of these species. Ultimately, variation in the catalog of physiological and behavioral capacities among closely related or sympatric species is likely to complicate prediction of the ecological consequences of global change and species invasions.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/3/502/DC1

Key words: bivalve, ecophysiology, Hall effect, intertidal, *Mytilus californianus*, *Mytilus galloprovincialis*, *Mytilus trossulus*, valve gaping.

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INTRODUCTION

Organisms may respond to episodic environmental stress through physiological mechanisms, behavioral responses or some combination of the two (Huey et al., 2009; Kearney et al., 2009). The interactions among physiological responses to environmental stress, behavioral responses and ecological interactions may ultimately play an important role in communities' reactions to environmental variability, directional environmental change and species invasions (e.g. Nicastro et al., 2010; Schneider et al., 2005). In some cases, behavioral responses might obviate the need for mounting a robust physiological response to what would otherwise be 'stressful' conditions (Breuner and Hahn, 2003; Kidder, 1997), while in other scenarios behavior might exacerbate the physiological consequences of exposure to novel types or degrees of environmental stress (Schlaepfer et al., 2002). Yet, questions pertaining to these interactions remain largely understudied, in part due to their inherent complexity.

The physiological and ecological consequences of environmental stress among inhabitants of intertidal and sublittoral habitats have been of ongoing interest, particularly in the context of anthropogenic global change and its compounding impacts on these communities (e.g. Helmuth et al., 2006; Mislan et al., 2009; Somero, 2002). These dynamic habitats pose a suite of physiological challenges to organisms, among the most important of which are periodic extreme excursions in body temperature when midday low tides coincide with high solar irradiance and high air temperature (Denny et al., 2011; Helmuth, 1998). The frequency and intensity of these episodes of elevated body temperature are projected to increase over the next century (Diffenbaugh and Ashfaq, 2010; Parry et al., 2007), with largely unknown consequences for intertidal organisms and their communities.

Mussels, dominant competitors for primary space in intertidal and sublittoral zones, possess a limited behavioral repertoire that provides few options for coping with exposure to environmental

extremes. Individual mussels attach strongly to the substrate and to neighbors using robust byssal threads. Byssal threads protect mussels from dislodgment during water accelerations (such as crashing waves) (Carrington, 2002), but they also prevent individuals from voluntarily moving more than a few centimeters over relevant time scales (although juvenile *Mytilus edulis* appear more mobile than adults) (Allen et al., 1976). Thus, adult mussels' principal behavior is control of the degree of openness of the shell valves, known as the valve gape.

This simple behavior may have important ecological and physiological consequences. Sealing of the valves represents mussels' primary anti-predator behavior (Robson et al., 2010), especially against sea stars, yet valve closure may have negative consequences for the organism as well. When a mussel's valves are sealed closed, the individual no longer enjoys access to food particles and oxygenated seawater (SW). Metabolism shifts to less efficient anaerobic pathways (although they are more efficient than in most animals as a result of alternative end products) (Hochachka and Somero, 2002; Zandee et al., 1985), total metabolic output consequently decreases (Shick et al., 1986), and the ability to excrete wastes is hindered. At the other behavioral extreme, opening of the valves ('gaping') during episodes of elevated body temperature in air has been proposed to ameliorate the effects of high temperature *via* evaporative cooling in some bivalves (Lent, 1968), but studies to date have failed to demonstrate an effect of this gaping behavior on body temperature in *Mytilus* mussels (Fitzhenry et al., 2004). Rather, when it occurs, valve gaping in air appears to function more in the maintenance of mantle cavity fluid oxygenation to support aerobic metabolism (Bayne et al., 1976; Coleman, 1973). Meanwhile, limits to the degree of valve gaping in air are imposed by the risk of tissue desiccation (Hicks and McMahon, 2003; Nicastro et al., 2010). Based on these varied considerations, context-specific tradeoffs should exist in mussels' modulation of the valve gape in response to thermal extremes.

While several studies have measured valve gape in response to emersion and/or elevated temperature in mussels (Ameyaw-Akumfi and Naylor, 1987; Coleman and Trueman, 1971; Lent, 1968; Nicastro et al., 2010; Shick et al., 1988; Wilson et al., 2005), questions remain regarding the role of this behavior across closely related species or among different environmental contexts (e.g. in air *versus* in SW). For example, gaping behavior has been implicated in the differential success of native *versus* invasive mussel species at different tidal heights (Nicastro et al., 2010). Recent modeling and empirical work has also demonstrated that episodes of elevated body temperature (i.e. episodes capable of leading to thermal stress) in intertidal organisms tend to cluster into multi-day windows (Denny et al., 2011; Miller et al., 2009). Yet, a majority of thermal stress experiments conducted in the laboratory have exposed individuals to a single episode of elevated body temperature. Thus, studies to date have left unanswered a number of crucial questions. How does gaping behavior contribute to different mussel species' overall strategy of coping with repeated thermal challenges? Are the consequences of thermal stress additive over successive days, or are these consequences independent of an individual's recent thermal history?

We addressed these questions in three congeneric mussel species (the ribbed sea mussel *Mytilus californianus* Conrad and two blue mussels: *M. galloprovincialis* Lamarck and *M. trossulus* Gould). These three species all occur along the west coast of North America, but they have experienced different evolutionary thermal histories. *Mytilus galloprovincialis* evolved in the warmer Mediterranean Sea and invaded southern California waters sometime in the twentieth century, while the other two species are native to the region (reviewed by Lockwood and Somero, 2011). The physiological consequences of these divergent evolutionary histories include different thermal performance characteristics of certain metabolic enzymes (Fields et al., 2006; Lockwood and Somero, 2012), differences in the thermal limits of cardiac function (Braby and Somero, 2006b), and disparate transcriptomic and proteomic responses to a single episode of elevated body temperature in SW between the blue mussels (Lockwood et al., 2010; Tomanek and Zuzow, 2010). These biochemical, physiological and molecular differences all are consistent with interspecific differences in susceptibility to thermal stress. Field surveys also suggest a key role of temperature in establishing biogeographic patterning and in outcomes of interspecific competition in areas where distributions of the blue mussel species overlap. *Mytilus galloprovincialis* has displaced *M. trossulus* from most of southern California, but the latitudinal location of the hybrid zone at the front of this invasion is labile in the face of decadal-scale shifts in sea surface temperature (Hilbish et al., 2010). On a smaller geographic scale, *M. trossulus* was more abundant at subtidal, cooler habitats in San Francisco Bay, while *M. galloprovincialis* dominated at nearby intertidal, warmer sites (Schneider and Helmuth, 2007); however, these field patterns may be confounded by the effects of low salinity (Braby and Somero, 2006a). A more thorough understanding of the physiological and behavioral responses of these congeners to episodes of elevated body temperature may aid in the interpretation of these patterns and in the prediction of future species distributions in a warming world.

To address these issues, we first exposed mussels from each of these species to repeated laboratory conditions of elevated body temperature, in both air (emersion, the typical scenario of heat stress in the field) and SW (immersion, to allow comparisons with previous physiological studies). We continuously recorded valve gaping behavior throughout these experiments, including during the post-stress recovery periods. In a subsequent experiment, we tested whether the differences we observed in the first experiment among species and among treatments in the behavioral propensity to seal the valves completely during post-episode recovery periods might have ecological consequences *via* effects on organismal survival. Specifically, we hypothesized that individuals suffering from carryover physiological consequences of thermal stress might require constant access to oxygenated SW to fuel repair of cellular damage accumulated during bouts of high body temperature (or for other benefits of having the valves open such as waste excretion). Therefore, we predicted that forced valve sealing during the recovery period, simulating events such as the presence of a predator, might increase post-episode mortality, and that the consequences of forced valve sealing would be more severe after multiple episodes of elevated body temperature than after a single episode. We further predicted that the more cool-adapted of the blue mussel species, *M. trossulus*, would show the most pronounced effect of forced valve closure on survival during the recovery period. We simulated an anti-predator response during the post-episode recovery period by artificially sealing mussels closed for varying periods and monitoring their subsequent survival relative to that of unsealed individuals and individuals that had not experienced elevated body temperature. Finally, we quantified adductor muscle tissue glycogen content to ascertain whether depletion of fuel for anaerobic metabolism could provide an alternative explanation for the observed lack of a tendency to seal the valves following episodes of elevated body temperature. We discuss how interactions between mussels' simple valve opening/closing behavior and thermal stress physiology, as well as variation in these interactions among closely related or

sympatric species, could have important implications for ecological processes in intertidal communities under both current and projected future environmental scenarios.

MATERIALS AND METHODS

Animal maintenance and species identification

Adult ribbed mussels (*M. californianus*) were collected from a single location in the rocky intertidal zone at Hopkins Marine Station (HMS) in Pacific Grove, CA, USA (36.6216°N, 121.9042°W). The native and invasive blue mussel congeners are extremely difficult to differentiate based on morphology. They are also capable of hybridizing, and HMS sits within the mosaic hybrid zone (Braby and Somero, 2006a; Hilbish et al., 2010). Thus, blue mussels from putative pure populations of *M. trossulus* and *M. galloprovincialis* were collected from floating docks outside the hybrid zone in Newport Harbor, OR, USA (44.6226°N, 124.0520°W), and Santa Barbara Harbor, CA, USA (34.4089°N, 119.6893°W), respectively. Blue mussels were transported to HMS over ice and in air. All three species were subsequently held under constant immersion and natural photoperiod in outdoor, flow-through SW tanks at HMS (salinity 34±1 p.p.t.; temperature 12–15°C) for at least 4 weeks and for no longer than 12 weeks prior to the start of any experiment. All experiments described here were carried out on adult individuals of comparable size (~50–70 mm valve height) among all species. Mussels were fed three times a week with a commercial bivalve diet (Shellfish Diet 1800, Reed Mariculture, Campbell, CA, USA) during this common garden acclimation period.

We genetically confirmed the species identity for the individual blue mussels used in the gaping experiments, using the *Glu-5′* and *ITS-1* nuclear markers, following previously established methods (Heath et al., 1995; Rawson et al., 1996). Only 1 of the 79 individuals that were genotyped was determined to be different from the putative species (one *M. galloprovincialis* collected in Oregon); no hybrids were identified. This misidentified individual was excluded from all analyses. The species identity of the blue mussels used in the survival experiments was not confirmed. These individuals were collected from the same locations as those used in the gaping experiment, and we assumed a negligible rate of species misidentification.

Gaping experiment: behavioral responses to repeated temperature ramps

Temperature ramps were conducted in SW and in air. In all cases, temperature was increased and decreased gradually (~13°C h^{-1}) from control conditions of 13°C. This rate of change is slightly faster than maximum heating and cooling rates observed in *M. californianus* mimics placed into intertidal mussel beds (8.1 and −10.9°C h^{-1}, respectively) (Denny et al., 2011). However, those field rates were obtained on days when the mimics' 'body temperature' reached levels lower than those tested in the current experiments, and we would expect more rapid rates of heating and cooling on the warmest days. In each experiment, the animals (N=6–10 per species and treatment) were held at the peak temperature (SW 33±1°C; air 32±1°C) for 45 min before cooling commenced at the same rate. An additional set of individuals (N=6–8 per species) were also exposed to ramps to 25°C under constant immersion to examine the effect of different maximum temperatures on valve gaping behavior. The upper temperature of 33°C was chosen to mimic some of the most extreme conditions to which these mussels are likely to be exposed in the wild (Harley, 2008; Petes et al., 2007), while the lower temperature (25°C) lies within the range at which these species induce expression of the molecular chaperone Hsp70, an indicator of heat-induced protein damage (Buckley et al., 2001; Lockwood et al., 2010).

In each scenario, repeated heat ramps commenced at the same time of day on three successive days; a ~20 h recovery period at 13°C and under constant immersion separated each temperature ramp. This design approximates body temperature patterns experienced in the wild (Denny et al., 2011). Typically, mussels along the Pacific coast of North America experience a mixed semidiurnal tide with two low tides of differing heights; only when lower low water occurs near midday and coincides with warm air temperatures and clear skies do body temperatures typically rise to high levels (Helmuth, 1999). Thus, these mussels experience at most one episode of elevated body temperature every 24 h in the field. The animals in these experiments experienced a single episode of emersion per day, as they likely would during significant portions of the lunar tidal cycle (WWW Tide/Current Predictor: http://tbone.biol.sc.edu/tide/tideshow.cgi).

SW temperature ramps were conducted in an insulated ice chest. SW temperature was controlled with a programmable laboratory water bath temperature controller (Polystat, Cole-Parmer, London, UK). The outflow circulation from the water bath was passed through one half of a countercurrent heat exchanger, while a pump in the ice chest passed SW through the other half of the heat exchanger and back into the ice chest. The air temperature ramps were run in the same experimental setup. To simulate emersion at low tide, most of the SW was pumped out of the ice chest at the beginning of a temperature ramp, leaving the undisturbed mussels exposed to air on top of plastic racks. Air temperature was controlled by ramping the temperature of the underlying SW (as above), and a small electric fan ensured mixing of the air within the ice chest. We did not measure humidity in these chambers, but we expect it to approximate the typical conditions at the HMS field site (85±5% relative humidity in July 2010). At the conclusion of the temperature ramp (i.e. when air temperature had again reached ~14°C), fresh 13°C SW was pumped back into the chamber to a level above the mussels' valves.

In both SW and air, control experiments were conducted over the same period of 3 days, but temperature was regulated at the control level (13±1°C in SW or air); the control temperature air exposures were of the same duration as the corresponding air temperature ramps. Mussels were not fed during the 3 days of the gaping trials, so that differences in food availability between emersed and immersed mussels would not confound the comparisons.

At the conclusion of each trial (following the last 20 h recovery period), we sampled gill and posterior adductor muscle tissues and flash froze them in liquid nitrogen. Tissue samples were stored at –80°C until analysis.

Valve gaping measurements

Mussel valve gape was monitored with custom-built, differential Hall Effect circuits (modified from Wilson et al., 2005). Each Hall Effect sensor (SS59ET, Honeywell Sensing and Control, Golden Valley, MN, USA) produces a change in voltage as the sensor moves within a magnetic field. The voltage output decreases non-linearly as the distance between the sensor and a magnet increases. The voltage output of a single Hall Effect sensor is sensitive to ambient temperature. Thus, two Hall Effect sensors were sealed back-to-back in a 0.5 ml microcentrifuge tube, and the voltage difference between the two outputs was determined after passing through a differential analog amplifier. This voltage difference was tested and found to be insensitive to changes in temperature (supplementary

material Fig. S1), allowing the use of the apparatus in temperature ramp experiments. For gaping experiments, the sealed Hall Effect apparatus was glued to one valve of the mussel shell, and a small magnet encased in heat-shrink tubing was glued to the opposite valve. The voltage output (proportional to valve gape) was converted to a digital signal *via* a Powerlab A/D converter system and recorded at 10 Hz using the associated LabChart v6.0 software (ADInstruments, Inc., Colorado Springs, CO, USA). Ambient temperature from a calibrated K-type thermocouple was recorded simultaneously (LabChart). In independent pilot experiments we found that body temperature – determined by sealing the tip of a K-type thermocouple within a mussel's body cavity – tracked ambient temperature to within 1°C in both SW and air under the heating/cooling rates used in our experiments (data not shown). Four individuals could be monitored simultaneously using this apparatus.

Gape analyses

For each individual, we determined the percentage of each temperature ramp or emersion episode the mussels spent with their valves completely closed; the voltage corresponding to complete sealing of the valves was determined by pinching the mussel's valves together at the end of each experiment. For each recovery period (each was ~20 h long) we measured the number of adductions (stereotypical valve closure movements), the number of complete closure events in which the valves were sealed shut, and the duration of each of those complete closure events (to the nearest minute). From the last two measurements we calculated the total time spent with the valves sealed during each 20 h recovery period. For the control SW individuals, the totals over all 3 days of the experiment were normalized to 20 h to allow comparisons with the other treatments.

The resulting individual means for the recovery period data were analyzed using a generalized linear mixed effects model approach (GLMM) (Faraway, 2006) using the GLIMMIX procedure in SAS software v9.2 (SAS Institute Inc., Cary, NC, USA). Treatment, species and ramp, as well as each of the three two-way interactions, were each treated as fixed effects in the model. Individual ID was modeled as a random effect to account for the repeated measurements on each mussel following the three temperature ramps. Each response variable (adductions, number of complete closure events, mean duration of complete closure events, and total time closed) was modeled using a Poisson distribution with a log link function. The mean duration of complete closure events and total time closed were both strongly right-skewed and fitted the Poisson distribution better than a normal distribution.

For each species and treatment, we also constructed empirical cumulative distribution function curves (using the Kaplan–Meier method in Matlab v7.12; The Mathworks, Inc., Natick, MA, USA) for the duration of individual complete closure events over the entire 3 days of the experiment. The shapes of these distribution functions were compared in a pairwise fashion using an Anderson–Darling (AD) statistic (Scholz and Stephens, 1987). Because of the unequal sample sizes among treatments, a jackknife procedure was employed in Matlab to estimate the probability of achieving an AD statistic greater than the observed value out of 100,000 resampling iterations for each pairwise comparison. Using the resulting table of pairwise jackknife *P*-values, the empirical cumulative distribution functions were assigned to statistically homogeneous groups using the multcompView package (Piepho, 2004) in R software v2.14.0 (R Development Core Team 2011, R Foundation for Statistical Computing; http://www.R-project.org). Statistical significance was set at $\alpha<0.05$ in all analyses.

Adductor muscle glycogen analysis

Adductor muscle glycogen content was determined for individual mussels in duplicate using a modification of the amyloglucosidase method, followed by enzymatic glucose determination (modified from Passonneau and Lauderdale, 1974). Absorbance at 340 nm (proportional to NADH concentration remaining in the assay cocktail) was measured on a microplate reader for both undigested background glucose aliquots and aliquots in which tissue glycogen had been completely digested to glucose by amyloglucosidase. This analysis was carried out only on samples from the gape experiment.

Survival experiment: effects of context, number of episodes and valve sealing during recovery

For the survival and forced valve sealing experiment, different groups of mussels from a single species were exposed to temperature ramps as above (in air or in SW) using the same ice chest apparatus as for the gape experiment. Valve gape was not recorded in the survival experiment. At the conclusion of a single temperature ramp, individuals were allowed to behave normally at control temperature for ~1 h; most gaped widely after being exposed to high temperature. After that time, the mussels were randomly divided into three groups: unsealed, sealed for 1 h and sealed for 5 h. Individuals in the 'sealed' groups were held closed for the appropriate duration with plastic cable ties. In preliminary trials we used the Hall Effect apparatus to demonstrate that mussels of each species could not maneuver out of these cable ties (i.e. gape was constant and zero; data not shown). The chosen maximum sealed duration of 5 h approximated the overall mean amount of time that individual mussels would spend sealed out of every 20 h during the control, SW-immersed gaping trials. This duration also greatly exceeded the amount of time mussels of all three species voluntarily spent closed following any given high temperature exposure (see Fig. 1 below). Control animals of each species that had not experienced a temperature ramp were also divided into the same three groups.

This process was repeated for additional groups of mussels after they had experienced three consecutive days of high body temperature in air or SW (as in the gaping study). Thus, we could examine whether there are context-specific (i.e. immersed *versus* emersed), cumulative effects of repeated high temperature episodes on survival, both with and without valve sealing during the recovery period. There were no 13°C air or 25°C SW treatments for the survival experiment because there were only minor shifts in behavior following exposure to these conditions in the gaping experiment.

We assessed survival in 5–10 individuals of each species in each group (1 or 3 days of exposure; air or SW exposure; 0, 1 or 5 h sealed). Survival was monitored daily for the first ~15 days after the completion of the temperature ramp and then weekly thereafter to 90 days. Dead mussels can be distinguished by widely opened valves and/or unresponsiveness to manipulation. Mussels were housed and fed throughout this period as described above. The complete set of survival time data was analyzed with a Cox proportional hazard regression model in IBM SPSS Statistics software v19.0.0 (SPSS, Inc., Armonk, NY, USA) (Cox and Oakes, 1984; Sievert and Keith, 1985). This model evaluates the effects of covariate parameters on the hazard of a mortality event. Covariates with positive coefficients ($\beta>0$) decrease survival, and those with $\beta<0$ increase survival. Coefficient magnitude reflects the relative effect of covariates on the survival rate, and the global 'protective effect' (i.e. relative survival benefit) of belonging to a particular covariate group may be evaluated as $1/\exp(\beta)$. The survival times for mussels still alive at the end of 90 days were right censored for this analysis, as they represent incomplete observations (i.e. some

mussels may have died after 90 days, but this cannot be known). Species, treatment (13°C SW controls, 33°C SW once, 33°C SW three times, 33°C air once, 33°C air three times), valve sealing duration, and a species × treatment interaction term were used as explanatory variables. We initially included an experiment × date term (not all mussels of a given species were exposed to given conditions on a single date), but this term was insignificant (likelihood ratio test, $P>0.35$) and was excluded from the final regression model.

RESULTS

Species differences in behavior under control conditions

Although behavior varied among individuals within each species, there were consistent differences among the three mussel species in their baseline behavioral patterns. The two blue mussels were more active behaviorally under control, immersed conditions, exhibiting more frequent valve adductions (one-way ANOVA, $P_{species}<0.001$; supplementary material Fig. S2A). *Mytilus trossulus* closed its valves completely for prolonged periods; on average, individual valve closure events under control conditions were 3.3 times as long as in *M. galloprovincialis* and 2.5 times as long as *M. californianus* (one-way ANOVA, $P_{species}=0.038$; supplementary material Fig. S2B). In one case, an individual *M. trossulus* closed its valves voluntarily for more than 32 h, while the longest voluntary closures in *M. californianus* and *M. galloprovincialis* were 17 and 15 h, respectively. Corresponding values for the total time spent sealed in an average 20 h control period were significantly higher in the two blue mussels than in *M. californianus* (Fig. 1, Table 1).

Acute behavioral responses to emersion and/or elevation of body temperature

The three mussel species exhibited similar acute behavioral responses to emersion and to elevated body temperature. All three species closed their valves quickly when exposed to air, remained completely sealed for 50% or more of the ensuing emersion period (regardless of temperature), and widely reopened the valves shortly after SW was pumped back into the chamber following an emersion episode (supplementary material Fig. S3). The behavioral responses to elevated body temperature ramps under constant immersion in SW suggested a temperature threshold for eliciting valve closure in this context. Mussels of all three species that were exposed to 25°C in SW did not seal the valves for prolonged periods during temperature ramps (supplementary material Fig. S3). In contrast, mussels exposed to 33°C SW closed their valves tightly for prolonged periods. The temperature at which the valves initially closed was much higher during the 33°C SW temperature ramps than during the emersion temperature ramps. However, there was no significant effect of species ($P=0.190$) or ramp number ($P=0.414$) on the temperature of first valve closure in SW (grand mean 26.5°C, range 20.9–32.9°C). Analyzing only those mussels exposed to 13°C in air, 33°C in SW and 33°C in air (i.e. those exposures in which prolonged valve closures were observed), despite slight differences in the temperature at which the valves reopened there were no significant differences among species in the fraction of each exposure spent completely sealed ($P_{species}=0.195$). There was a strong treatment effect ($P_{treatment}<0.001$); individuals of all three species spent significantly less time sealed during the 33°C SW temperature ramps than during those in air (Fig. 2). There was also a significant effect of ramp number ($P_{ramp}<0.001$) and a significant ramp × treatment interaction ($P_{ramp\times treatment}<0.001$). In particular, *M. californianus* exposed to 13°C in air and 33°C in SW responded differently to the second and third exposures than to the first (Fig. 2).

Behavioral responses in the post-episode recovery period

Differences among the mussel species, and among treatments, were far more pronounced during the post-episode recovery period than during the temperature/emersion episodes themselves. Treatment,

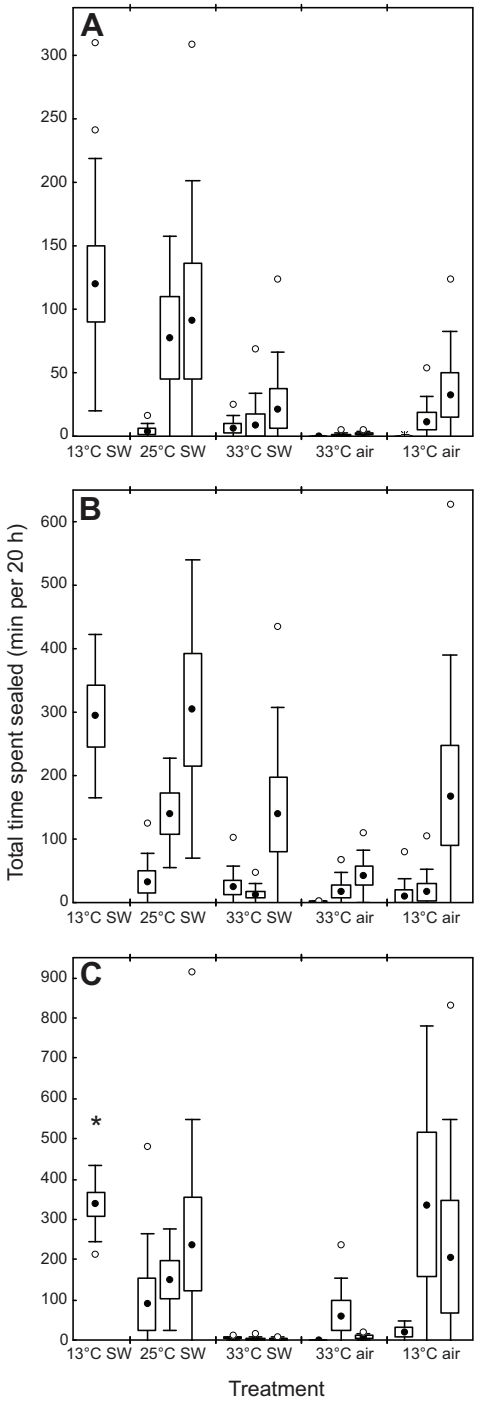

Fig. 1. The amount of time mussels of each species spent with their valves completely sealed in each recovery period in each of the treatments: (A) *Mytilus californianus*, (B) *Mytilus galloprovincialis* and (C) *Mytilus trossulus*. For the seawater control group (13°C SW), the total amount of time spent sealed in 72 h was normalized to the 20 h recovery period between heat ramps. Box plots indicate the mean ± s.e.m. (box limits) and the mean ± s.d. (error bars); N=6–10 individuals per treatment. Outliers and extreme values are indicated by open circles and asterisks, respectively. Note the differences in y-axis scales.

Table 1. Statistical summary of the GLMM analyses for each of the four mussel behavior response variables that were quantified during the recovery periods

		Adductions		Complete closures		Duration of complete closures		Total time spent closed	
Effect	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	4	3.26	0.013	4.84	<0.001	31.95	<0.001	35.33	<0.001
13°C SW		a		a,b		a		a	
25°C SW		a,b		a		b		a	
33°C SW		a,b		a,b		c		b,c	
33°C air		a,b		b		c		c	
13°C air		b		b		b		b	
Species	2	26.72	<0.001	19.65	<0.001	4.88	0.009	13.07	<0.001
M. californianus		a		a		a		a	
M. galloprovincialis		b		b		b		b	
M. trossulus		b		b		b		b	
Ramp	2	82.73	<0.001	9.67	<0.001	308.80	<0.001	633.26	<0.001
1		a		a		a		a	
2		a,b		a,b		b		b	
3		b		b		b		b	
Treatment × species	8	0.97	0.462	1.19	0.305	2.32	0.021	2.22	0.027
Species × ramp	4	31.11	<0.001	1.23	0.301	56.57	<0.001	152.66	<0.001
Treatment × ramp	8	15.48	<0.001	7.69	<0.001	226.21	<0.001	399.52	<0.001

GLMM, generalized linear mixed model.
In each case the denominator degrees of freedom (d.f.) was 212. Under each main effect (treatment, species, ramp), shared lowercase letters indicate statistically homogeneous groups in the GLMM within each response variable.

species and ramp number all had significant effects on each of the four behavioral response variables in the GLMM (Table 1). Across experimental contexts, *M. galloprovincialis* and *M. trossulus* performed more valve adductions ($P<0.001$) and more complete valve closures ($P<0.001$) of longer duration ($P=0.009$) than *M. californianus*, yielding a significantly greater total time spent sealed ($P<0.001$).

The most pronounced effect on behavior was the dramatic reduction of the total time spent with the valves completely sealed that was observed in some contexts. Although mussels of the three species tended to be equally – or, in some cases, more – active following temperature/emersion episodes relative to their respective behavior under control conditions (quantified by the number of valve adductions; supplementary material Fig. S2A), emersion and/or elevated body temperature elicited substantial decreases in the total amount of time during which the valves were completely closed. Species, treatment, ramp number and each of the three two-way interactions all had significant effects on the total amount of time

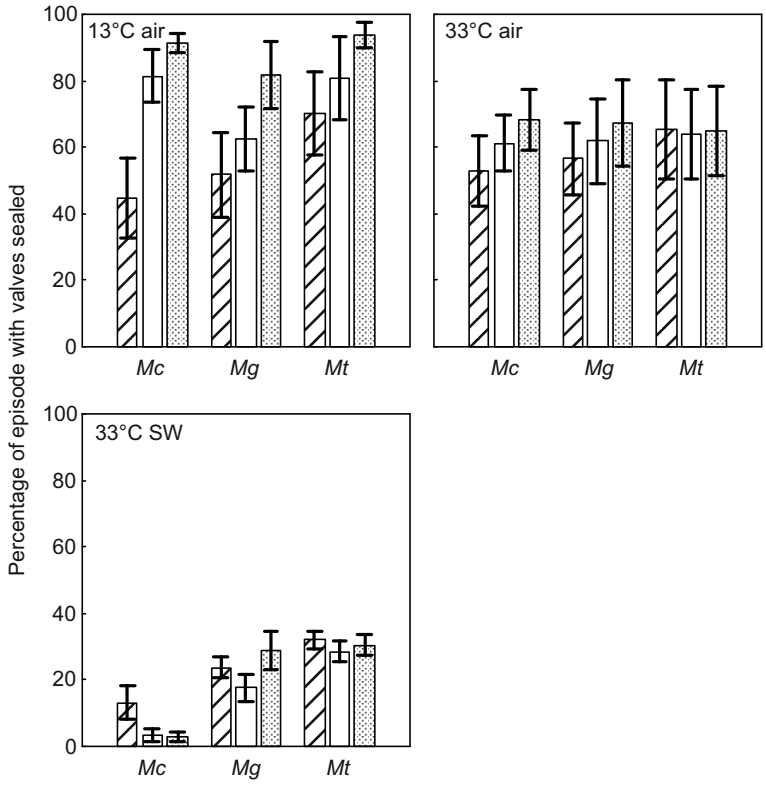

Fig. 2. Differences among treatments in the percentage of emersion/elevated body temperature episodes spent with the valves completely sealed, separated by the treatments in which prolonged valve closure was consistently observed in each mussel species. Different shading represents the first, second and third exposure. *Mc*, *M. californianus*; *Mg*, *M. galloprovincialis*; *Mt*, *M. trossulus*. Error bars represent ±1 s.e.m.

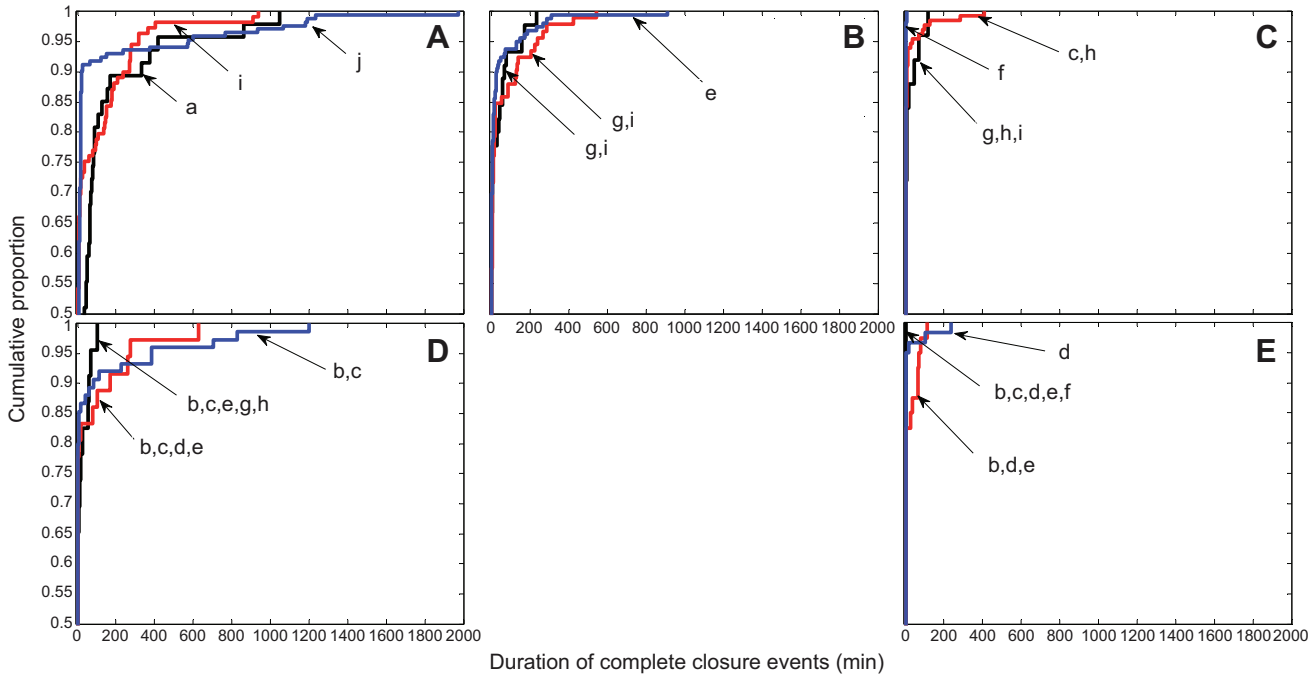

Fig. 3. Empirical cumulative distribution function curves for the duration of complete valve closure events during recovery from emersion and/or elevated body temperature episodes in each mussel species, separated by treatment: (A) 13°C SW, (B) 25°C SW, (C) 33°C SW, (D) 13°C air and (E) 33°C air. Within each species, all events from all individuals over the 3 day experiment were pooled to create each curve. Curves sharing common letters indicate statistically homogeneous groups (at $P>0.05$) across all three species and five treatments, as determined by a jackknife distribution of the Anderson–Darling test statistic using 100,000 iterations for each pairwise comparison. See Materials and methods for further details. Black, *M. californianus*; red, *M. galloprovincialis*; blue, *M. trossulus*.

spent closed during a recovery period in the GLMM (Table 1). There were particularly pronounced and persistent decreases in the total time spent sealed during recovery for *M. californianus* after exposure to 33°C in air and for *M. trossulus* after exposure to 33°C in either air or SW (Fig. 1). These decreases were driven largely by significant reductions in the durations of individual closure events ($P_{\text{treatment}}<0.001$; supplementary material Fig. S2B), rather than by dramatic decreases in their quantity (already few under control conditions; supplementary material Fig. S2C).

In all species and treatments, the amount of time spent sealed during the first recovery period was considerably less than control values (Fig. 1). Only in the second or third recovery period, if at all, was behavior restored to control patterns (e.g. *M. trossulus* in 13°C air, all species in 25°C SW; Fig. 1). There was considerable variability among individuals within each species in the degree of restoration of control-like behavior, indicated by the large standard deviations in Fig. 1 and supplementary material Fig. S2.

Similar to the acute behavioral responses described above, the behavior of all three species in the recovery period suggested a distinction between the responses to 25°C and 33°C in SW. For the total time spent sealed and the duration of individual closure events, the behavior following exposure to 25°C SW was statistically indistinguishable from that in 13°C SW controls while being significantly different from that in 33°C SW (Table 1).

The patterns in total valve closure time were reflected in species- and treatment-specific empirical cumulative distribution functions of the durations of valve sealing events (Fig. 3). In all three species, the very long valve closure events observed under control conditions (Fig. 3A) were far less common after the animals were exposed to 33°C body temperature in either air or SW (Fig. 3C,E). As for the total time sealed, this effect was particularly evident for *M. californianus* in 33°C air and for *M. trossulus* in 33°C SW (maximum valve closure durations of 5 and 4 min, respectively). Notably, for both *M. galloprovincialis* and *M. californianus*, valve closure durations were shortened similarly by exposure to 13°C air as by exposure to 33°C air (Fig. 3D,E), suggesting that emersion alone may drive a behavioral shift in these species. In contrast, valve closure durations of *M. trossulus* were further reduced in 33°C air. Emersion yielded statistically indistinguishable patterns of valve closure durations among the three mussel species, regardless of temperature (Fig. 3D,E).

In SW, a temperature threshold effect on behavior was particularly evident in *M. trossulus*. Specifically, there was a small difference in the shape of the curves between 13°C and 25°C for both blue mussels (insignificant in *M. galloprovincialis*), followed by a substantial decrease in the proportion of longer valve sealing events between 25°C and 33°C for both species (Fig. 3A–C). In contrast, valve closure durations of *M. californianus* were similarly affected by exposure to 25°C and 33°C SW. As for the analysis of total time spent sealed, *M. galloprovincialis* appeared to be the least impacted by elevated body temperature in SW.

Muscle glycogen

Although the glycogen content of adductor muscle did not vary among the mussel species under control conditions, the three species exhibited divergent patterns in the cumulative response to repeated episodes of different combinations of emersion and elevated body temperature. The 25°C SW group was excluded from these analyses because of a lack of pronounced behavioral changes under this treatment. Glycogen content was significantly affected in a two-way ANOVA by species ($F_{2,54}=9.05$; $P<0.001$) and treatment ($F_{3,54}=4.40$; $P=0.008$), as well as by a significant species × treatment interaction ($F_{6,54}=6.70$; $P<0.001$). For clarity, we have indicated statistically homogeneous groups based on the results of a species-

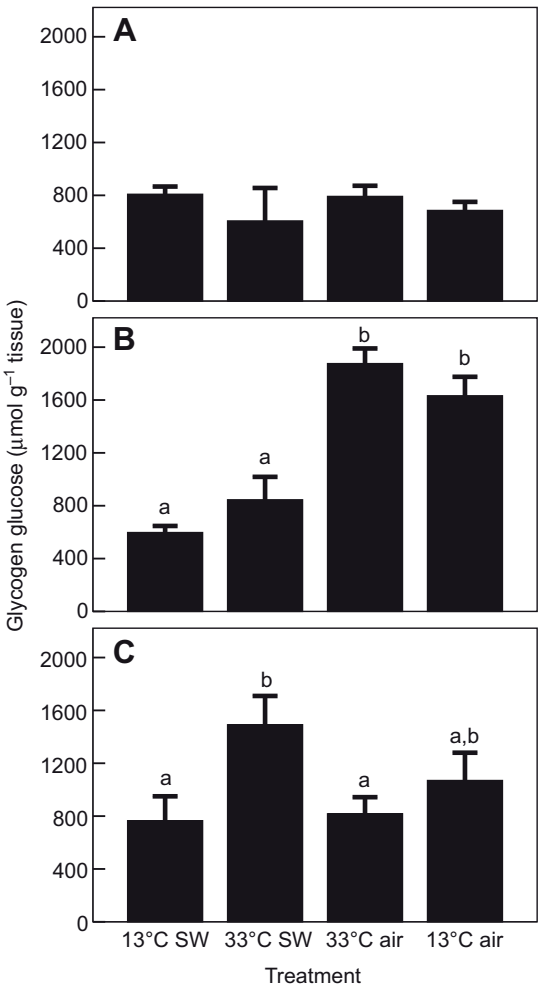

Fig. 4. Changes in adductor muscle tissue glycogen content following three consecutive days with episodes of exposure to elevated body temperature and/or to emersion for the three mussel species: (A) *M. californianus*, (B) *M. galloprovincialis* and (C) *M. trossulus*. Bars sharing common letters indicate statistically homogeneous groups (at $P>0.05$) within each species; there were no differences among treatments for *M. californianus*. Error bars represent ±1 s.e.m.

specific, one-way ANOVA within each panel of Fig. 4. Adductor muscle glycogen of *M. californianus* did not vary among treatments (Fig. 4A). In contrast, *M. galloprovincialis* increased its muscle glycogen content significantly following three exposures in air, regardless of the temperature (Fig. 4B). *Mytilus trossulus* also increased tissue glycogen content, but only after three episodes of elevated body temperature in SW and to a lesser extent at control temperature in air (Fig. 4C).

Survival following thermal stress

All individuals of *M. californianus* and *M. galloprovincialis* survived the 3 day protocol during the gape experiment. In contrast, 5 *M. trossulus* died (diagnosed based on lack of activity in the gape signal and confirmed at the conclusion of the experiment): 2 died in 33°C SW after the second temperature ramp, 1 died in 33°C air after the second temperature ramp, and 2 died in 13°C air after the third emersion period. These individuals were excluded from the behavioral analyses and from biochemical assays.

We monitored mussels for a 90 day period in the survival experiment (Fig. 5), during which we observed ongoing mortality and significant species-specific differences. All three mussel species were severely challenged by exposure to one or three episodes of elevated body temperature, with 144 mortality events out of 295 total individuals tested (48.8% mortality). Survival varied dramatically among the three mussel congeners ($P_{species}<0.001$) as well as among temperature/emersion treatments ($P_{treatment}<0.001$). Overall, *M. californianus* exhibited the lowest hazard rate of mortality (proportional to β in Table 2) across all treatments, while mortality rates in *M. trossulus* were more than 80% (Fig. 5). The global 'protective effect' of belonging to *M. californianus*, evaluated as 1/exp(β), was a 12.5-fold decrease (95% confidence interval CI 5.4–29.4; $P<0.001$) in the odds of a mortality event relative to the extremely sensitive *M. trossulus*. The analogous protective effect for *M. galloprovincialis* relative to *M. trossulus* was a 4.1-fold decrease (95% CI 2.2–7.5; $P<0.001$). Based on the overlap in these two confidence intervals, *M. californianus* and *M. galloprovincialis* enjoyed a similar survival advantage over *M. trossulus*.

Mortality rates were low in control SW conditions in all three species, and they increased with exposure to elevated body temperature. The highest mortality rates occurred in mussels exposed to one or three episodes of 33°C body temperature in SW, while mortality was much lower after exposure to high body temperature in air (Table 2, Fig. 5). In particular, *M. trossulus* suffered extensive mortality much earlier following exposure in SW relative to exposure in air. Controlling for the other explanatory variables, the protective effect of exposure to control conditions relative to three episodes of 33°C in SW was a 28.6-fold decrease in the odds of a mortality event (95% CI 3.8–200.0; $P=0.001$). Analogous protective effects for exposure to 33°C in air for one or three episodes relative to three episodes of 33°C in SW were 2.5 (95% CI 1.2–5.3; $P=0.019$) and 3.4 (95% CI 1.5–7.9; $P=0.004$), respectively. Based on the overlap in these last two confidence intervals and the lack of a significant difference in survival between one and three episodes of elevated body temperature in SW ($P=0.447$, Table 2), there was no evidence to support the predicted additive impact on survival of exposure to repeated episodes of elevated body temperature. There was also no evidence in the model for a significant species × treatment interaction ($P=0.389$). At nearly every point under all treatment conditions, survival was lowest in *M. trossulus*, intermediate in *M. galloprovincialis* and highest in *M. californianus* (Fig. 5).

Also contrary to our prediction, there was no evidence for an effect of imposed valve sealing during the recovery period on subsequent survival for any species under any treatment scenario (Table 2, $P_{sealing}=0.880$).

DISCUSSION

We have shown that behavior and survival both vary significantly among three congeners of *Mytilus* and are dependent on the specific recent exposure regime. The distinct, context-specific responses we observed suggest that behavioral modifications may be diagnostic of the recent thermal/emersion history of individual mussels. If so, behavior shortly following episodes of emersion and/or elevated body temperature might be used to predict long-term outcomes. Because we did not follow survival of the individuals used in the gape experiment over longer periods, we unfortunately cannot use specific behavioral patterns to predict individual survival probability here. Nor can we correlate biochemical measures such as tissue glycogen content with subsequent survival probability. However, our results do suggest promising avenues that warrant further study.

Overall, *M. californianus* and *M. galloprovincialis* exhibited less severe behavioral consequences (i.e. smaller effect sizes) and

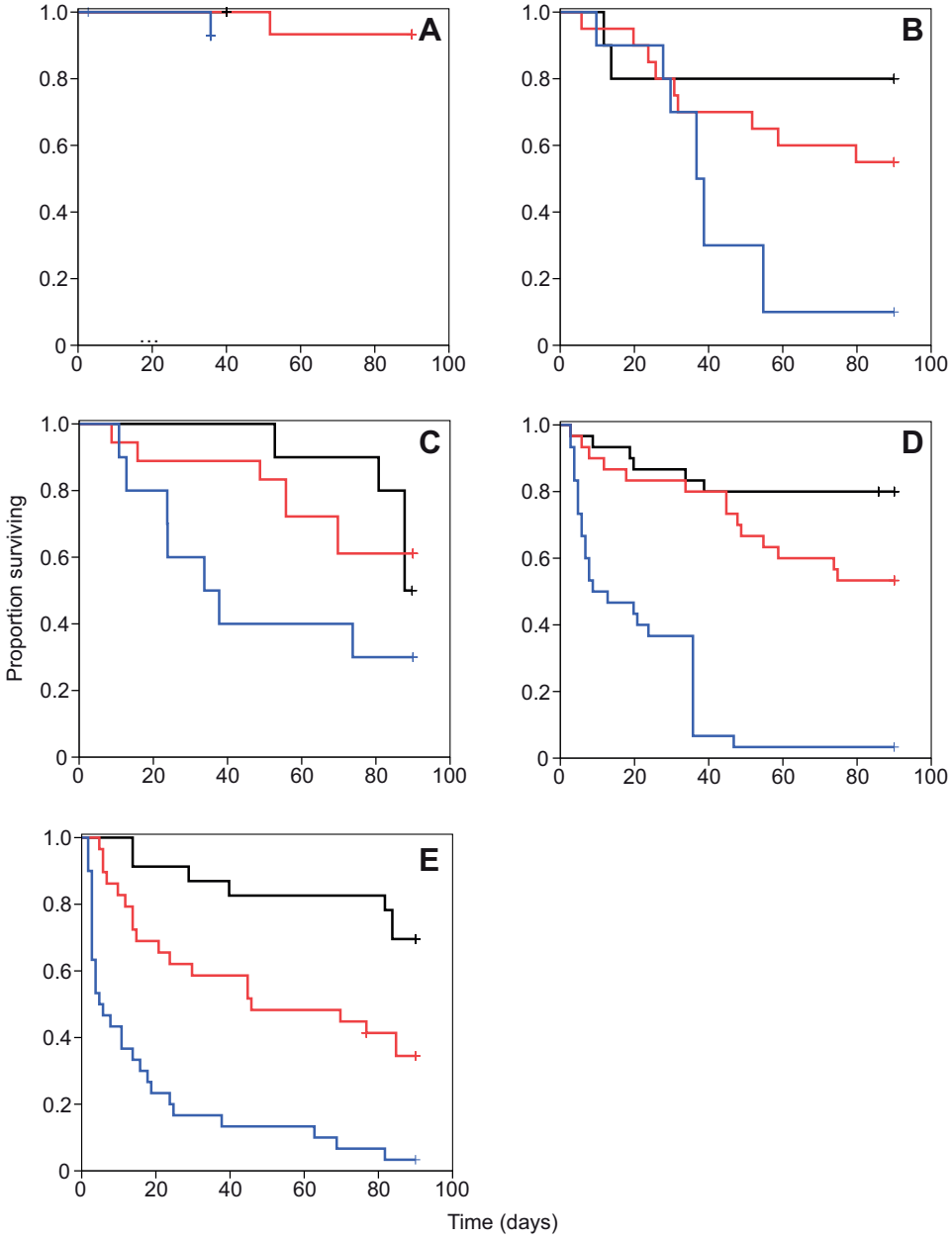

Fig. 5. Kaplan–Meier cumulative survival curves for each of the three mussel species, separated by treatment: (A) 13°C SW, (B) 33°C air ×1, (C) 33°C air ×3, (D) 33°C SW ×1 and (E) 33°C SW ×3. Censored values are indicated by plus symbols. See Materials and methods for details of statistical analyses. Black, *M. californianus*; red, *M. galloprovincialis*; blue, *M. trossulus*.

enjoyed higher survival following elevated body temperature than did *M. trossulus*, in accord with our expectations based on a number of other studies (Braby and Somero, 2006b; Lockwood et al., 2010; Lockwood and Somero, 2011; Schneider, 2008; Schneider and Helmuth, 2007; Tomanek and Zuzow, 2010). These data add to the growing literature demonstrating that, among mussel species, disparate combinations of physiology and behavior play a crucial role in ecological success of species, notably in the context of biological invasions (de Zwaan et al., 1991; Lockwood and Somero, 2011; Nicastro et al., 2010; Schneider et al., 2005; Shinen and Morgan, 2009).

Our data also provide evidence that changes in behavior may be sensitive indicators of underlying physiological perturbations elicited by environmental stress. For example, the differences observed in behavior between exposure to 25 and 33°C correlate with differences noted in the expression of stress-related genes in *M. trossulus* and, to a lesser extent, in *M. galloprovincialis* between heat-stress temperatures of 24 and 32°C (Lockwood et al., 2010).

The evidence we present for the existence of complex interactions between behavior and thermal stress physiology in these three species shows that such interactions must be taken into account in the design of future studies, in order to gain a more comprehensive and realistic understanding of the role of environmental stress in the ecology of intertidal and sublittoral communities. Specifically, our data offer important insights into several questions regarding the context-specific interplay between physiology and behavior in these three mussel species.

Can data from thermal challenge in SW be used to predict the ecological consequences of thermal stress in the intertidal zone?

Although intertidal mussels are most likely to experience episodes of elevated body temperature when emersed in air at low tide

Table 2. Statistical summary of the Cox proportional hazard model results for 90 days survival of mussels after exposure to emersion and/or elevated body temperature

Model term	β	s.e.m.	Wald statistic	d.f.	*P*	exp(β)	95.0% CI for exp(β)	
							Lower	Upper
Species			**44.000**	**2**	**<0.001**			
M. californianus	–2.532	0.427	35.169	1	<0.001	0.080	0.034	0.184
M. galloprovincialis	–1.410	0.309	20.858	1	<0.001	0.244	0.133	0.447
M. trossulus								
Treatment			**20.822**	**4**	**<0.001**			
13°C SW (control)	–3.338	1.020	10.713	1	0.001	0.035	0.005	0.262
33°C air ×1	–0.904	0.386	5.482	1	0.019	0.405	0.190	0.863
33°C air ×3	–1.230	0.428	8.278	1	0.004	0.292	0.126	0.676
33°C SW ×1	–0.203	0.267	0.578	1	0.447	0.817	0.484	1.377
33°C SW ×3								
Sealed			**0.255**	**2**	**0.880**			
0 h	0.065	0.192	0.114	1	0.736	1.067	0.732	1.554
1 h	0.113	0.235	0.232	1	0.630	1.120	0.706	1.776
5 h								
Species × treatment			**8.472**	**8**	**0.389**			

Significance is indicated for each model term in bold type. Underlying, indented rows indicate statistical comparisons of the hazard of a mortality event in the indicated subgroup relative to the last subgroup in the list. Subgroups have been removed from the insignificant 'Species × treatment' term to save space.
d.f., degrees of freedom; CI, confidence interval.

(Helmuth, 1998), some studies of gene and protein expression in these species have exposed individuals to elevated body temperature only in SW (Lockwood et al., 2010; Tomanek and Zuzow, 2010). Our behavioral and survival data demonstrate at times profound differences between these two contexts, suggesting a limited ability to extrapolate between them. In proteomics studies of these three mussels, we have observed similarly distinct patterns of protein expression between exposure to elevated body temperature in air and SW (Dowd, 2012) (W.W.D. and G.N.S., manuscript in preparation). Although stress imposed in SW can lead to a valid differentiation of physiological processes among congeners, the inter-congener differences we have documented may ultimately provide mechanistic explanations for the context-specific distribution patterns of blue mussels observed in the field (Braby and Somero, 2006a; Schneider and Helmuth, 2007).

Context-specific differences in our dataset included acute behavioral responses, behavior (particularly the total time spend with the shell valves sealed) in the post-episode recovery period, and long-term post-episode survival. Mussels exposed to air sealed their valves and kept them sealed for a large portion of the exposure episodes. In contrast, mussels in SW often closed and then reopened their valves, even near the peak of the high temperature episodes. This disparity likely reflects the desiccation tradeoff in air that does not exist for gaping mussels when immersed in SW. Although some mussel species are known to gape extensively at low tide (Nicastro et al., 2010), there appears to be little variation among the three *Mytilus* congeners examined in this study in their acute response to air exposure. This observation implies shared susceptibility to desiccation, as would be expected from the similar morphologies of the three congeners. However, we did not confirm this by measuring body water content before and after episodes of emersion. Notably, individual *M. californianus* and *M. galloprovincialis* whose valves were forcibly held open during emersion, even at control temperatures, did not survive (W.D., unpublished; *M. trossulus* was not examined).

Despite the fact that by some measures behavioral modifications were similarly pronounced in the recovery periods following exposure to elevated body temperature in air and in SW, subsequent survival was significantly lower in mussels after exposure to elevated body temperature in SW. This effect was most apparent in *M. trossulus*. Similarly, median lethal temperature (LT_{50}) of *M. edulis* was up to 5°C lower in water than in air (Jones et al., 2009). Although the mechanistic causes of this disparity remain elusive, this observation again argues for careful approximation of field conditions when designing laboratory experiments and attempting to extrapolate to ecological consequences. Interestingly, Schneider and Helmuth observed the opposite pattern (i.e. lower survival in air) or no difference between shaded intertidal and subtidal locations when *M. galloprovincialis* and *M. trossulus* were outplanted to field plots in San Francisco Bay (Schneider and Helmuth, 2007). However, temperatures in this field study never reached those used in the present laboratory experiments, limiting our ability to directly compare these results. Of course, other factors such as species interactions and food availability could play important roles in the field, and further work is needed to incorporate these additional aspects into ecophysiological studies.

Are the consequences of thermal stress additive over multiple episodes?

Although we expected that exposure to multiple episodes of elevated body temperature might dramatically impact behavior and decrease survival relative to exposure to a single episode, we found no evidence to support these predictions. There were no pronounced changes in acute behavioral responses to emersion/elevated body temperature between the first and subsequent episodes (with the possible exception of *M. californianus* in 13°C air; Fig. 2). Thus, although the acute behavioral response varied across contexts (described above), it did not depend upon recent thermal history. Similarly, we found no evidence for additive or cumulative effects on behavior during the recovery period following episodes of emersion or elevated body temperature. Rather, behavior during these recovery periods fell into one of two other patterns. In the first, the behavior was altered dramatically following the first episode and remained at the new level throughout the 3 day protocol (e.g. total time spent sealed for *M. trossulus* in 33°C SW remained extremely low during all three recovery periods; Fig. 1C). In the second pattern, after an initial adjustment following the first episode, behavior tended to recover to control levels by the third episode

(e.g. total time spent sealed for *M. galloprovincialis* in 33°C SW; Fig. 1B). In emersion scenarios only, this second pattern may have been partly a function of prior acclimation to conditions of constant immersion, yet the added impacts of elevated body temperature are clearly demonstrated by comparison of the 33°C air treatment with the 13°C air control. Notably, the *M. trossulus* that died during the 3 day gape experiment – and that were excluded from our behavioral analyses – demonstrated a gradual loss of valve movements that is diagnostic of moribundity (supplementary material Fig. S3P).

We initially interpreted the re-establishment of control-like behavior over the course of the gape experiment as evidence that the organisms had successfully acclimated to cope with the new exposure regime. However, one of the more striking results of the survival experiment was the delayed mortality observed in all three species after episodes of elevated body temperature. Even in the relatively thermally sensitive *M. trossulus*, long thought to be more susceptible than its close relative (and successful invader) *M. galloprovincialis*, the vast majority of individuals survived 1 or 3 days of episodic exposure to 33°C in either air or SW (only 3 mortalities out of more than 100 combined individuals in the gape and survival experiments). Taken alone, this less than 3% acute mortality rate would dramatically underestimate the true mortality rate, which reached more than 80% by the end of 90 days. In the present study, in some cases individuals may have succumbed to factors not related to the consequences of thermal stress. We cannot specify a physiological cause of this delayed mortality without further data, but others have correlated delayed mortality with acute, stress-induced physiological perturbations, for example when examining post-release survival in fisheries (e.g. Moyes et al., 2006). In contrast to a previous study that observed significantly higher mortality in the native (*M. trossulus*) than in the invasive blue mussel (*M. galloprovincialis*) under common garden conditions (Braby and Somero, 2006b), there were no species differences in survival under control conditions in the present study. However, differences in experimental protocols between these two studies (e.g. flow-through *versus* recirculating aquaria, acclimation time before survival monitoring began) confound direct comparisons. We are also unable to correlate behavior during the exposure protocol with subsequent survival. Nevertheless, these data emphasize the need to conduct future experiments over similarly long periods (e.g. Schneider and Helmuth, 2007) to fully evaluate the consequences of exposure to environmental stress.

Surprisingly, we also found no cumulative effect on survival of multiple days of exposure to elevated body temperature. Mussels exposed to three episodes of elevated body temperature were no more likely to perish than individuals exposed to only a single episode. Whatever the macromolecular damage or other physiological disruption that leads to mortality in some individuals of these mussel species, it appears to be inflicted by a single episode of exposure to 33°C body temperature. This finding questions the importance of mimicking 'heat waves' observed in the field (Denny et al., 2011; Petes et al., 2007) in the design of laboratory experiments. Two previous studies suggested that the LT_{50} of *M. edulis* decreases with increasing number of exposures (Jones et al., 2009; Sorte et al., 2011). However, in each case survival was only monitored for 18 h following individual thermal challenges; our results show that mortality should be monitored over much longer periods to adequately assess the effects of stress on long-term survival. When appropriate post-stress survival times are used, it may be possible to extrapolate from the physiological consequences of single high temperature episodes to ecological consequences for thermally stressed populations.

Although many mussels did not survive following episodes of elevated body temperature, other individuals appeared healthy at the end of 90 days. It will be very informative in future studies to examine the underlying genetic variation that could explain the survival differences among individual mussels, as seen in other organisms (e.g. Krebs and Feder, 1997; Rank and Dahlhoff, 2002).

Why do mussels maintain their shell valves in an open position following episodes of elevated body temperature?

Overall, the behavioral results suggest that mussels of all three species exhibit a reduced propensity for maintaining their valves in a closed position for prolonged periods following specific exposures, particularly following episodes of elevated body temperature in air. There are several potential explanations for this pattern. We discuss four here, the first three of which we can largely refute based on our data and information in the literature:

(1) Sustained valve gaping during recovery might be an absolute requirement for survival, perhaps because of the need to repay an oxygen debt or to excrete wastes accumulated during exposure episodes when the valves are sealed and metabolism shifts to less-efficient anaerobic pathways (Bayne et al., 1976). However, in our survival experiment, forced valve sealing for up to 5 h during the recovery period had no detectable effect on long-term survival. Thus, the observed decreases in total valve closure times in all three mussel species following episodes of elevated body temperature may serve other physiological purposes or may indicate other effects of thermal stress (e.g. thermal damage to muscle proteins), but they do not appear to represent a behavioral strategy necessary for survival. It is possible that longer periods of valve closure might elevate the likelihood of mortality, but we chose a maximum duration of valve sealing comparable to voluntary behavioral patterns under control conditions.

(2) Sustained valve gaping may indicate depletion of fuel stores needed for contraction of the adductor muscles. In our experiments, in no scenario was there any indication of a cumulative decrease in adductor muscle glycogen. In some cases tissue glycogen in the blue mussels actually increased over control levels, despite the fact that the animals were not fed during the 3 day protocol. Transfer of glyconeogenic products from another tissue, e.g. the hepatopancreas, might underlie these increases. However, we examined glycogen concentrations exclusively in adductor muscle, so trans-tissue movement of substrates cannot be evaluated here. Furthermore, we could not distinguish any systematic correlation between behavioral shifts and changes in muscle glycogen content. Thus, we conclude that a lack of metabolic substrate for anaerobic metabolism is not the driving factor in determining whether mussels seal their valves during recovery from exposure to emersion and/or elevated body temperature. It is possible that substrates other than glycogen-derived glucose fuel the adductor muscle contraction, or that glycogen is transiently depleted by the end of episodes of elevated body temperature and is then rapidly restored. Our current data and experimental design cannot address these possibilities. However, the biochemical underpinnings of sustained muscle contraction in sea mussels further argue against substrate limitation as an explanation for the observed behavioral patterns. Specifically, *Mytilus* adductor muscles exhibit a 'catch' mechanism, likely enabled by the protein twitchin (Funabara et al., 2007), which dramatically decreases the energetic cost of sustained contraction (Twarog, 1976). Thus, only a relatively small initial pulse of energy is required to keep the valves in a sealed position.

(3) The biochemical constituents of adductor muscle may themselves be damaged by episodes of elevated body temperature,

preventing individuals from closing. Data from the gaping experiment clearly refute this hypothesis. Individuals of all three species continued to exhibit as many or more adduction movements following episodes of elevated body temperature as under control conditions. These mussels also were able to close and seal their valves during subsequent episodes, especially in air. These results suggest that the muscular apparatus maintains its contractile ability. It would be interesting to evaluate whether the adductor muscles retain their ability to 'catch', a performance with clear ecological consequences for the ability to avoid predation.

(4) The observed lack of prolonged valve closure in the periods following thermal challenge represents avoidance of hypoxia–reoxygenation cycles. Gene and protein expression data implicate oxidative stress as a prominent consequence of exposure to thermal extremes in *Mytilus* mussels (Lockwood et al., 2010; Tomanek and Zuzow, 2010). Given these results, and knowledge from ischemia and stroke models that cycles of hypoxia and reoxygenation elevate the production of reactive oxygen species (Li and Jackson, 2002), we hypothesize that sustained valve gaping minimizes oxidative stress. Specifically, sustained valve gaping in the recovery period would avoid compounding macromolecular damage suffered during episodes of elevated body temperature with the subsequent effects of oxidative stress. Further data are needed to fully test this hypothesis. Such a strategy would help to mitigate the consequences of thermal challenge in at least one other way. Specifically, valve gaping during recovery would permit more efficient aerobic ATP generation to fuel macromolecular repair. This prediction corresponds well with gene and protein expression data indicative of damage to proteins (induction of heat shock proteins and proteasome constituents) (Buckley et al., 2001; Gracey et al., 2008; Lockwood et al., 2010; Tomanek and Zuzow, 2010), and it is further supported by the discovery that thermal stress leads to significant increases in single- and double-stranded DNA breakage (Yao and Somero, 2012). Other gene expression studies have indicated alternating phases of oxidative and reductive metabolism in *M. californianus* (Connor and Gracey, 2011; Gracey et al., 2008). Notably, an episode of elevated body temperature in air disrupted these rhythms (Connor and Gracey, 2011). In light of these findings, our behavioral data support the hypothesis that episodes of thermal stress canalize physiological processes in intertidal mussels, and that oxidative stress may be the predominant underlying mechanism.

Whatever the mechanisms are that dictate the observed lack of prolonged valve closure events during the recovery phase, the long-term survival correlates of this behavioral shift clearly varied among mussel species. For example, despite similarly pronounced shifts in the total time spent with the valves sealed between *M. galloprovincialis* and *M. trossulus* in 33°C air, the former species always enjoyed a substantially higher survival rate. Ultimately, the combined behavioral and physiological repertoires of the invasive *M. galloprovincialis* confer a strong advantage at elevated temperatures.

Conclusion

Adult mussels of the Genus *Mytilus* possess a simple, physiologically and ecologically relevant behavioral repertoire that also can be easily manipulated. It will be valuable to extend these analyses to field-acclimatized individuals, rather than the common garden approach used in the present experiments. These animals were not fed during the 3 day experiments, and the results might be different if food were presented during the 3 day experimental protocol or if the feeding regime during acclimation were changed (Norkko et al., 2005; Schneider et al., 2010). In addition, future research might well evaluate other potential, sublethal consequences of prolonged valve gaping during recovery from episodes of elevated body temperature. For example, mussels and their sea star predators may vary in the consequences of exposure to environmental stress (Petes et al., 2008). Thus, ecological consequences of prolonged valve gaping might include altered tradeoffs between opening to access food and closing to minimize predation risk (Robson et al., 2010), or increased vulnerability to predation *per se*.

Clearly, the degree of interaction between physiology and behavior in response to environmental stress will depend upon both the complexity of the behavioral repertoire of the species in question and the degree and type(s) of stress encountered. Unanticipated behaviors could have profound effects on our predictions of the consequences of environmental stress. For example, sea stars (*Pisaster ochraceus*) were recently found to trap increasing quantities of cool SW in their coelomic cavity following exposure to episodes of elevated body temperature (Pincebourde et al., 2009). If, as such observations and our data suggest, the behavioral and physiological capacities to cope with environmental stress vary significantly among members of intertidal communities, this could lead to complex and unexpected ecological responses to environmental variability and directional environmental change.

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