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Abstract. In the rocky intertidal, organisms frequently experience a wide range of daily body temperatures depending on the stage of the tide and the time of day. In the intertidal, the thermal adaption of a species and its ability to invade a new region may be closely linked. In this research, the physiological effects of thermal stress in both low tide and high tide conditions are compared between *Mytilus galloprovincialis*, a worldwide mussel invader, and *M. trossulus*, a sibling species. In a seawater tank, mussels were exposed to one of three aerial temperature treatments (20, 25, 30 °C) in a cycle with one of two water temperatures (18, 12 °C). In 18 °C water, there was no effect of the aerial treatments on growth or survival in either species. In contrast, in 12 °C water, aerial exposure affected the survival and growth of both species. Growth and survival rates of *M. galloprovincialis* were higher in all conditions than the rates of *M. trossulus*, especially in the 18 °C water experiments and in the aerial exposure treatments of the winter 12 °C water experiment. *M. galloprovincialis* appears to be warm-adapted with regard to both low tide and high tide thermal stress. These results when paired with previous research suggest that as climates shift due to global warming, the temperatures favorable to *M. galloprovincialis* will become more common.

Introduction

Non-native species can cause a devastating ecological impact on invaded communities (Hoffman *et al.*, 1999; Sax *et al.*, 2005). For instance, endangered species frequently risk extinction due to competition or predation from intro-duced species (Wilcove *et al.*, 1998). As barriers to organism dispersal continue to break down as a result of anthropogenic activities (*e.g.,* release of ballast water), the threat of invasion continues to rise in both terrestrial and marine communities (Carlton and Geller, 1993; Cohen and Carlton, 1998; Mack *et al.*, 2000; Wonham *et al.*, 2001). Therefore, quantifying the attributes of organisms and ecosystems that facilitate or interfere with successful invasion is crucial to forecasting future invasions (*e.g.,* Mack *et al.*, 2000; Sax *et al.*, 2005; Miller *et al.*, 2007). However, such quantification remains a challenge because of the many factors that may influence invasion success (Mack *et al.*, 2000).

Some progress has been made in identifying the general characteristics of successful invaders, such as their life-history strategies (*e.g.,* reproductive potential, body size), adaptations to certain habitats, and release from predators and diseases in invaded communities (Rejmanek, 1996; Calvo-Ugarteburu and McQuaid, 1998; Radford and Cousens, 2000; Blackburn and Duncan, 2001). Other mechanisms that also may contribute significantly to the successful invasion of a species include its thermal adaptation (*e.g.,* Stachowicz *et al.*, 2002), which is the focus of the present research. The rocky intertidal is an ecosystem where thermal adaptation and invasability may be intimately intertwined, considering that marine intertidal organisms frequently contend with a wide range of daily body temperatures due to alternating exposure to the aquatic and aerial environments (*i.e.,* high and low tides, respectively). For instance, during low tide the body temperature of a mussel can be 20 °C warmer than during high tide when body temperature tracks water temperature (Helmuth, 2002). Furthermore, recent studies that have compared the thermal tolerance of native and invasive marine species suggest that successful invaders have higher fitness in warmer climates compared to native species that occupy

similar niches (*e.g.,* Stachowicz *et al.*, 2002; Nehls *et al.*, 2006; Agius, 2007; Saunders and Metaxas, 2007; Schneider and Helmuth, 2007). An organism's physiological tolerance to thermal stress likely plays a significant role in its invasion potential.

The *Mytilus edulis* complex (blue mussels: *M. edulis*, *M. trossulus*, and *M. galloprovincialis*) represents an excellent system in which to study the role of thermal stress in intertidal species invasion. These species occur in subtidal and intertidal coastal habitats. Additionally, *M. galloprovincialis* is considered one of the 100 most successful invasive species in the world (Lowe *et al.*, 2000). Over the past century, this species has established populations in the United States, South Africa, Japan, and Hong Kong (Wilkens *et al.*, 1983; Lee and Morton, 1985; Hockey and Schurink, 1992; Geller, 1999; Wonham, 2004). The United States and Japanese invasions have occurred in areas where *M. trossulus* occurs naturally; thus, *M. galloprovincialis* has competed with and often replaced a sibling species (Wilkens *et al.*, 1983; Geller, 1999). In the United States, *M. galloprovincialis* is suggested to have been introduced into southern California, where *M. trossulus* was endemic, in the early 1900s (Geller, 1999). The two mussels are morphologically similar species that hybridize when they co-occur (Seed, 1992). However, since the invasion, pure populations of *M. galloprovincialis* have expanded northward. Currently, mussels in southern California are almost exclusively *M. galloprovincialis*; mussels in northern California and Oregon are predominately *M. trossulus*; and both species as well as their hybrids occur in central California (Rawson *et al.*, 1999).

To date, most studies of the population distribution in *Mytilus* spp. have focused on the limiting effects of water temperature on dispersal (*e.g.,* Suchanek *et al.*, 1997). In central California, Sagarin *et al.* (1999) and Barry *et al.* (1995) correlated intertidal faunal shifts between the early 1930s and the early 1990s with increased water temperatures. Those authors did not look at *Mytilus* spp., but a range expansion of *M. galloprovincialis* is thought to have occurred during the same time (see Geller, 1999). Observational studies suggest that *M. trossulus* prefers cooler water and *M. galloprovincialis* prefers warmer water (Sarver and Foltz, 1993; Suchanek *et al.*, 1997). This difference in thermal preference has been further supported by research examining the degree of protein damage these species suffered under different regimes of water temperature (Hofmann and Somero, 1996a; Braby and Somero, 2006a). Thus, superior adaptation to warmer water may be one reason why *M. galloprovincialis* has been a successful invader.

Moreover, recent evidence suggests that exposure to aerial temperatures also affects the fitness of intertidal organisms (Hofmann and Somero, 1996b; Przeslawski, 2005; Schneider and Helmuth, 2007). For example, immunity,

reproduction, and the acid-base balance of an intertidal organism can be affected by low tide temperature (*e.g.,* Petes *et al.*, 2007; Allen and Burnett, 2008). Hofmann and Somero (1996b) provide evidence that *M. trossulus* is thermally sensitive to aerial temperatures. A key finding of this study is that exposure to damaging temperatures occurs exclusively during aerial exposure at low tide. For intertidal organisms, temperature patterns in air can differ substantially from patterns in water (Helmuth *et al.*, 2006). For example, along the west coast of the United States, although water temperatures generally decrease as one moves from south to north, maximum aerial mussel (*M. californianus*) body temperatures exhibit a mosaic pattern of alternating "hot" and "cold" spots (Helmuth *et al.*, 2002, 2006). Patterns of heat-shock expression in *M. californianus* also appear to display a complex mosaic along this latitudinal gradient (Sagarin and Somero, 2006; Place *et al.*, 2008). The relative physiological effects of emersed (*i.e.,* aerial exposure) and immersed (*i.e.,* water) thermal stress on mussels within the *M. edulis* complex remains relatively unexplored.

In the present study, I investigated the physiological effects of thermal stress on *M. galloprovincialis* (the invader) and *M. trossulus* (the native congener). To this end I examined the effects of both aerial exposure and water temperature on the growth and survival of both species under laboratory conditions. I hypothesized that *M. galloprovincialis* would show higher levels of growth and survival in both warmer water and aerial environments than would *M. trossulus* (Hofmann and Somero, 1996a; Braby and Somero, 2006a) and that aerial exposure to thermal stress would be an important factor in the growth and survival of the two species.

Materials and Methods

Temperature regime

In order to simulate the natural range of tidal thermal stress, mussel populations of *Mytilus galloprovincialis* and *M. trossulus* were established in seawater tanks that mimicked a daily tidal cycle by filling and draining at different times. During high tide, mussels were completely immersed in water. However, during simulated low tide, the mussels were exposed to one of three aerial temperature treatments. These treatments included body temperatures of 20 °C (cool), 25 °C (warm), and 30 °C (hot). A subtidal control group also was established that remained underwater during the entire tidal cycle. Separate experiments were conducted in two different water temperatures: 18 °C and 12 °C. Although in nature a variety of air and water temperatures occur within the distribution of *M. galloprovincialis* and *M. trossulus*, the above temperature regimes in this research represented those that (1) exist where the species co-occur in California (Braby and Somero, 2006b) and (2) are found

during the summer along the west coast of the United States (see Helmuth *et al.*, 2006; Braby and Somero, 2006b; Schneider and Helmuth, 2007).

Each experimental combination of each temperature combination was run for 9 weeks. Each water temperature was run twice. The experiments using the 18 °C water temperature were conducted from 24 June to 24 August in 2004 and from 4 July to 5 September in 2005. Experiments using the 12 °C water temperature ran from 1 February through 1 April and from 4 July to 5 September in 2005.

Experimental tanks

Experiments were run in an indoor seawater hall at the University of South Carolina, Columbia, South Carolina. A re-circulating tank (2.1 m × 0.65 m × 0.6 m) was used with a chiller attached to the system to maintain a constant water temperature of either 12 °C or 18 °C. During the summer of 2005, two separate tanks were used for the 18 °C and 12 °C trials that were run simultaneously. A pump circulated the water continuously 24 h a day within the tank. To simulate a single daily low tide, a second pump released water from the system for 6 h a day. Two separated halogen lamps (Regent-WQ; no emission of ultraviolet light) were located over the tanks to regulate the body temperatures in the hot (≈30 °C; 500 W) and warm (≈25 °C, 300 W) aerial exposure treatments. The lamps were on timers to coincide with the low tide cycle. Additionally, dividers were placed between the four treatments within each tank to ensure that the halogen lamps affected only the hot and warm aerial exposure treatments and not the cool and subtidal treatments. Practical salinity was maintained at 28.

In each of the thermal treatment conditions, the mussels were placed on four tiles (14 cm × 14 cm) located under the surface of the water during the simulated high tide. In the immersed treatment (*i.e.,* subtidal control), the mussels were placed on tiles closer to the bottom so that they remained submerged during the simulated low tide; subtidal mussels were 25 cm above the bottom of the tank. Individuals of *M. trossulus* and *M. galloprovincialis* were labeled, mixed together, and assigned to one of the four tiles within each treatment. Individual mussels were not disturbed during the experiment unless clumps formed due to mussel movement (*i.e..* mussel(s) attached on top of another mussel potentially shading bottom layer; see Schneider *et al.,* 2005). When clumping did occur, mussels on the top were carefully removed and placed in an open space on their tile. Tiles were rotated biweekly to ensure that all mussels experienced similar overall thermal regimes. Furthermore, the locations of the four treatments within the tank were randomly changed on a weekly basis. Partial water changes were also carried out weekly.

Organismal maintenance

All mussels were obtained from Penn Cove Shellfish in Coupeville, Washington. This aquaculture facility raises *M. trossulus* and *M. galloprovincialis* under similar conditions. Both species were originally collected from Washington State populations; *M. galloprovincialis* is invasive in this area and the source population is unclear (pers. comm., I. Jefferds, Penn Cove Shellfish) but has likely been introduced multiple times (see Wonham, 2004). Mussels remain underwater in Puget Sound, Washington, after the larvae settle on submerged ropes in the aquaculture facility. Therefore, their thermal history includes no aerial exposure. The mussels were shipped overnight to Columbia, South Carolina. About 10 mussels of each species were genetically identified with a diagnostic genetic marker, *Glu-5′*, after each shipment to confirm correct species assignment (KRS, unpubl. data). DNA was extracted with phenol chloroform and amplified by polymerase chain reaction (PCR) according to Rawson *et al.* (1996).

Upon arrival, mussels were put into seawater that had a temperature that corresponded with the water temperature at the aquaculture facility when they were collected. Mussels were collected in January and June when the water temperatures were ≈8–10 °C and ≈11–12 °C, respectively. If the required experimental water temperature was higher than the temperature at the aquaculture facility, it was increased slowly, 1 °C every 1.5 days. The maximum increase was 6 °C. All mussels were held in the tank for 10 days before being exposed for an additional week to periodic air exposure at room temperature (≈20 °C; 1 h per day to a maximum of 6 h). During the first week of each experiment, the mussels in the hot (30 °C) and warm (25 °C) aerial treatments were slowly exposed to the additional thermal exposure during low tide. Lamps were turned on for 0.5 h the first day, followed by an additional hour each day to a maximum of 6 h per day, which was maintained for the subsequent 8 weeks.

Mussels were fed daily an algal paste (*Chaetoceros-B*, *Phaeodactylum tricornutum*, *Nannochloropsis oculata*), obtained from Innovative Aquaculture (SPAT formula), upon arrival and throughout the experiment. The algal paste was mixed with seawater to form a 10% paste solution that was frozen in ice cube trays for easy and equal feedings. The number of ice cubes provided in a feeding depended on the number of live mussels at the time (about one ice cube to each 50 mussels). Mussels were fed 1 h after the tidal cycle to ensure equal initial feeding between all treatments.

All mussels used in the experiment were 20–30 mm in length. Prior to the start of the experiment, individuals were weighed (wet weight, after external drying; Ohaus Explorer, accuracy 0.00 g) and measured for length. Small tags containing identification numbers were glued to the mussels' shells. At the end of each experiment, all surviving mussels

were measured for length and weight to calculate their growth. Mussel mortality was checked daily at feeding time. A mussel was considered dead if it was floating or if its valve was open and it also did not respond to physical stimulation. In the first experiment (*i.e.,* 18 °C water temperature, 2004), 30 mussels of each species were used in each treatment. In the other three experiments (18 °C, 2005; 12 °C winter 2005 and summer 2005), 50–70 mussels were used per species per treatment.

Temperature monitoring

Mussel body temperatures were monitored throughout the experiments by biomimetic temperature loggers that thermally mimic the body temperatures of living mussels (Helmuth *et al.*, 2002; Fitzhenry *et al.*, 2004). To monitor temperature, additional mussel shells were emptied and filled with silicone; then iButton temperature loggers were placed in the centers of the shells (iButton data loggers, 1-cm diameter, sample interval of 5 min, accuracy of 0.5 °C). One biomimetic temperature logger was deployed in the middle of each treatment (*i.e.,* four loggers per tank) and exchanged weekly. The use of loggers was especially important because the body temperature of an intertidal organism during low tide can be very different than the air and water temperature (Helmuth, 1998, 2002). Thus, monitoring air temperature alone would not have provided an accurate estimate of the body temperature mussels experienced during the simulated low tide. During immersion, however, body temperature tracks water temperature.

Statistical analyses

A four-factor nested mixed-effect model analysis of variance (ANOVA) was run to look at the effects of aerial exposure, water temperature, season of trial, and species on growth (separate analyses for each growth variable—*i.e.,* change in length and weight; Table 1). Several significant interactions were found between water temperature, aerial exposure, and species (Table 1). Thus, to compare the effects of aerial exposure on the growth, a two-way ANOVA were performed looking at species and aerial exposure for each trial. All statistics were performed separately for each of the four individual experiments (*i.e.,* 18 °C 2004, 18 °C 2005, summer and winter 2005 at 12 °C). To further elucidate the aerial treatment effects, pairwise comparisons between treatments within species were made using a Student's *t*-test; sequential Bonferroni corrections were conducted to correct for multiple comparisons (Rice, 1989). For all growth analyses data, only surviving mussels were included. ANOVAs were done in SAS 9.1 and JMP 5.1.

To examine the effects of aerial exposure on survivorship (*i.e.,* days until death), a Kaplan-Meier survival analysis (Kaplan and Meier, 1958) was performed. Differences between species survivorship within an individual treatment were also examined using a survival analysis. Survival (*e.g.,* dead or alive after completion of experiment) was examined with an original logistic model to look for interactions between species and aerial exposure. All statistics were performed separately for each of the four individual exper-

Table 1

Four-way nested mixed-effect model ANOVA of growth (both length and weight) where Season of Trial (i.e., summer 2004) is a random factor and all others are fixed

Source[1]	Change in Length					Change in Weight				
	DF	MS	F	P	Significance[2]	DF	MS	F	P	Significance[2]
Water	1	6.69	13.60	0.0002	*	1	1.46	48.13	<0.0001	*
Trial (Water)	2	2.93	5.96	0.0027	*	2	2.47	80.93	<0.0001	*
Aerial	3	31.11	73.72	<0.0001	*	3	1.00	12.00	0.0061	*
Species	1	221.77	46.20	0.021	*	1	11.49	10.07	0.0866	ns
Water × Aerial	3	1.70	4.03	0.0641	ns	3	0.040	0.047	0.9852	ns
Water × Species	1	22.30	4.60	0.1652	ns	1	0.97	0.91	0.4418	ns
Trial (Water) × Aerial	6	0.42	2.53	0.523	ns	6	0.082	2.71	0.0131	*
Trial (Water) × Species	2	4.80	9.74	<0.0001	*	2	1.07	35.22	<0.0001	*
Aerial × Species	3	13.83	153.70	<0.0001	*	3	0.47	25.56	0.0008	*
Water × Aerial × Species	3	0.74	8.20	0.0152	*	3	0.012	3.54	0.0878	ns
Temperature (Water) × Aerial × Species	6	0.09	0.19	0.9809	ns	6	0.0031	0.10	0.9959	ns
Error	10,80	0.49				10,30	0.030			

[1] Water = water temperature of 12 °C or 18 °C; Trial = time period when the experiments were run; Aerial = exposure treatment during low tide; and Species = *M. galloprovincialis* and *M. trossulus*.

[2] Asterisk (*) indicates that *P* is significant at the 0.05 level; ns indicates that *P* is nonsignifiant.

iments (*i.e.,* 18 °C 2004, 18 °C 2005, summer and winter 2005 at 12 °C). Survival analyses were done with JMP 5.1.

Results

Temperature monitoring

Although lamp height was adjusted periodically, the hot- and warm-treatment lamps remained, on average, at 60 cm and 70 cm, respectively, above the mussels. The body temperatures of the mussels in the cool treatment remained at ambient air temperature ($\approx$20 °C). Daily body temperature highs during intertidal exposures were very close to expected values during the experiments; however, there were some variations that were partially due to fluctuations in the room conditions (Table 2). Water temperatures varied slightly more than anticipated. The mean temperature in the 18 °C water treatment was 16.9 °C with a standard deviation of $\pm$ 0.71 °C in 2004 and 18.42 $\pm$ 0.86 °C in 2005 (Table 2). For the 12 °C water treatment, the means were 12.46 $\pm$ 0.84 °C in the winter and 11.90 $\pm$ 0.65 °C in the summer of 2005 (Table 2).

Growth

18 °C water experiments. Aerial exposure treatments affected shell length growth differently for each species in both 2004 and 2005 (interactions between species and treatment: $P < 0.0001$; Table 1) but not for weight gain (interaction: 2004, $P = 0.29$; 2005, $P = 0.07$). There was a significant effect of aerial exposure for *M. galloprovincialis* on shell length growth (2004: $F_{3, 86} = 13.09$, $P < 0.0001$; 2005: $F_{3, 175} = 26.34$, $P < 0.0001$) and weight gain (2004: $F_{3, 87} = 4.5$, $P = 0.0055$; 2005: $F_{3, 175} = 15.6$, $P < 0.0001$), but no significant effects were observed with *M. trossulus* (Table 3 and Fig. 1a). Growth was significantly higher only in the immersed mussels compared to all three periodically emersed treatments for *M. galloprovincialis* (all comparisons, $P < 0.007$; Table 3 and Fig. 1a). Therefore, no significant effect of different aerial body temperatures (*e.g.,* 20° C *vs.* 25 ° C) on growth was observed for either species

when animals were exposed to 18 °C water. *M. galloprovincialis* grew more than *M. trossulus* when comparisons were made between all treatments for shell length and weight gain (all comparisons, $P < 0.01$); growth was very limited in *M. trossulus* (Table 3 and Fig. 1a).

12° C water experiments. Under 12 °C conditions, aerial exposure treatments affected the species' shell length growth and weight gain differently in both winter and summer 2005 experiments compared to the 18 °C experiments (interactions: $P < 0.0001$; Table 1). There was a significant effect of aerial body temperature on *M. galloprovincialis* shell length growth (winter: $F_{3,211} = 60.8$, $P < 0.0001$; summer: $F_{3,176} = 24.25$, $P < 0.0001$; Table 3 and Fig. 1b) and weight gain (winter: $F_{3,209} = 32.23$, $P < 0.0001$; summer: $F_{3,176} = 24.2$, $P < 0.0001$). Similarly, there was a significant effect of aerial body temperature on shell length growth in *M. trossulus* (winter: $F_{3,133} = 3.8$, $P < 0.01$; summer: $F_{3,175} = 26.34$, $P < 0.0001$). The effect of treatment on weight gain in *M. trossulus* differed between the summer and winter experiments, with a significant effect of treatment observed in the summer experiment but not in the winter (summer: $F_{3,152} = 19.76$, $P < 0.0001$). When pairwise comparisons were made, shell length and weight gain growth was significantly affected by the aerial exposure treatments, including the simulated subtidal mussels, for *M. galloprovincialis* but only between the simulated subtidal treatment and all of the aerial exposure treatments for *M. trossulus* (see Table 3 and Fig. 1b). However, for both species, growth decreased going from the least thermal stressful condition—simulated subtidal—to the most thermally stressful condition—hot aerial exposure treatment. *M. galloprovincialis* grew more than *M. trossulus* when comparisons were made in the winter and summer between all treatments for shell length and weight gain (all comparisons, $P \leq 0.01$) except the hot treatment of the winter trial.

Survival

18 °C water experiments. There were no significant effects of aerial exposure treatment on survival for either *M.*

Table 2

Average daily high mussel body temperature experienced by mussel thermal mimics in a variety of thermal habitats throughout the laboratory experiments ($\pm$ std dev) in 18 °C and 12 °C water

Water temperature	Water temperature[1]		Body temperature[2]		
	Trial	Subtidal	Cool (20 °C)	Warm (25 °C)	Hot (30 °C)
18 °C	Summer 2004	17.53 $\pm$ 2.13 °C	19.87 $\pm$ 2.26 °C	26.73 $\pm$ 2.39 °C	31.68 $\pm$ 3.60 °C
	Summer 2005	19.11 $\pm$ 1.36 °C	20.89 $\pm$ 1.22 °C	26.58 $\pm$ 2.05 °C	30.18 $\pm$ 2.71 °C
12 °C	Winter 2005	13.44 $\pm$ 1.62 °C	19.16 $\pm$ 2.40 °C	25.74 $\pm$ 2.07 °C	31.02 $\pm$ 2.99 °C
	Summer 2005	13.35 $\pm$ 1.60 °C	19.20 $\pm$ 2.17 °C	24.05 $\pm$ 2.93 °C	29.65 $\pm$ 3.46 °C

[1] Subtidal mussels remained underwater during the entire experiment, thus the body temperature mimics the water temperature.
[2] Body temperatures during a simulated low tide intertidal exposure.

Table 3

Growth and survival of Mytilus galloprovincialis *and* M. trossulus *under a variety of simulated intertidal thermal conditions*

| Trial	Species	Intertidal treatment	n^1	Change in length[2] (mm ± std err)	Change in weight[2] (g ± std err)	Percent survival after 9 weeks
a) Experiments run in 18 °C water						
Summer 2004	*M. galloprovincialis*	Hot (30 °C)	26 (30)	1.60 ± 0.15 a	0.35 ± 0.05 a	87
		Warm (25 °C)	17 (24)	1.54 ± 0.19 a	0.33 ± 0.06 a	79
		Cool (20 °C)	21 (27)	1.30 ± 0.17 a	0.33 ± 0.05 a	90
		Subtidal	26 (32)	2.60 ± 0.15 b	0.54 ± 0.05 b	81
	M. trossulus	Hot (30 °C)	11 (29)	0.18 ± 0.09 a	0.15 ± 0.03 a	41
		Warm (25 °C)	13 (26)	0.27 ± 0.08 a	0.11 ± 0.03 a	50
		Cool (20 °C)	8 (27)	0.10 ± 0.10 a	0.10 ± 0.04 a	44
		Subtidal	11 (25)	0.37 ± 0.09 a	0.16 ± 0.03 a	37
Summer 2005	*M. galloprovincialis*	Hot (30 °C)	42 (51)	1.39 ± 0.14 a	0.43 ± 0.04 a	94
		Warm (25 °C)	45 (51)	1.44 ± 0.14 a	0.48 ± 0.03 a	92
		Cool (20 °C)	47 (54)	1.36 ± 0.13 a	0.45 ± 0.03 a	88
		Subtidal	45 (52)	2.78 ± 0.14 b	0.73 ± 0.03 b	94
	M. trossulus	Hot (30 °C)	7 (52)	0.30 ± 0.21 a	0.07 ± 0.02 a	13
		Warm (25 °C)	10 (48)	0.45 ± 0.18 a	0.11 ± 0.02 a	25
		Cool (20 °C)	8 (51)	0.43 ± 0.20 a	0.11 ± 0.02 a	32
		Subtidal	13 (52)	0.59 ± 0.15 a	0.15 ± 0.01 a	19
b) Experiments run in 12 °C water						
Winter 2005	*M. galloprovincialis*	Hot (30 °C)	32 (68)	0.49 ± 0.09 a	0.03 ± 0.02 a	50
		Warm (25 °C)	61 (68)	0.69 ± 0.07 b	0.12 ± 0.02 b	94
		Cool (20 °C)	61 (64)	0.98 ± 0.07 b	0.15 ± 0.02 b	98
		Subtidal	61 (63)	1.76 ± 0.07 c	0.29 ± 0.02 c	98
	M. trossulus	Hot (30 °C)	17 (58)	0.37 ± 0.10 a	0.05 ± 0.02 a	29
		Warm (25 °C)	34 (63)	0.44 ± 0.07 a	0.06 ± 0.01 a	55
		Cool (20 °C)	36 (55)	0.54 ± 0.07ab	0.10 ± 0.01 a	69
		Subtidal	50 (65)	0.69 ± 0.06 b	0.06 ± 0.01 a	86
Summer 2005	*M. galloprovincialis*	Hot (30 °C)	45 (51)	0.72 ± 0.14 a	0.29 ± 0.03 a	90
		Warm (25 °C)	49 (50)	0.97 ± 0.14ab	0.36 ± 0.03ab	98
		Cool (20 °C)	41 (51)	1.24 ± 0.15 b	0.40 ± 0.03 b	94
		Subtidal	45 (51)	2.30 ± 0.14 c	0.66 ± 0.03 c	84
	M. trossulus	Hot (30 °C)	40 (54)	0.19 ± 0.07 a	0.10 ± 0.01 a	76
		Warm (25 °C)	43 (51)	0.18 ± 0.07 a	0.11 ± 0.01 a	86
		Cool (20 °C)	36 (55)	0.28 ± 0.07 a	0.16 ± 0.01 b	76
		Subtidal	37 (50)	0.63 ± 0.07 b	0.21 ± 0.01 c	71

[1] *n* refers to the number alive at the end of the 9-week experiment where the length and growth measurements could be taken; the number in parentheses refers to starting sample size.

[2] Letters next to the length and weight growth measurements refer to the pairwise comparisons between the treatments; length and weight measurements with the same letter are not significantly different from one another.

galloprovincialis or *M. trossulus* in either the 2004 or 2005 experiments when animals were exposed to 18 °C water (Fig. 2a, b). However, there were significant differences between species survivorship within each treatment in 2004 and 2005 (all comparisons, $P < 0.0001$; no interaction between species and aerial exposure treatment: summer 2004, $P = 0.64$; summer 2005, $P = 0.14$). Survival was relatively high for *M. galloprovincialis*; the survival rates after 9 weeks were 84% and 92% in 2004 and 2005, respectively (Table 3). Alternatively, survivorship was low for *M. trossulus* during the same period: 42% in 2004 and 22% in 2005 (Table 3).

12 °C water experiments. Under 12 °C water conditions, in the winter 2005 trial, significant treatment differences in

survival rates were observed in both *M. galloprovincialis* and *M. trossulus* ($P < 0.0001$; Fig. 2c), and there was a significant survival interaction between species and aerial exposure (interaction: $P = 0.02$). For *M. galloprovincialis,* survival rate was reduced in the hot treatment (50%), but it was relatively high for the other three treatments (94%–98%; Table 3). *M. trossulus* also had a reduced survival rate in the hot treatment (29%), but unlike *M. galloprovincialis*, survival slowly increased as severity of simulated intertidal exposure decreased: warm (55%), cool (69%), and subtidal (86%). In all treatments the survival rates of *M. galloprovincialis* were higher than those of *M. trossulus* ($P < 0.05$). When the experiment was repeated in summer 2005 with a similar set of environmental conditions, no significant ef-
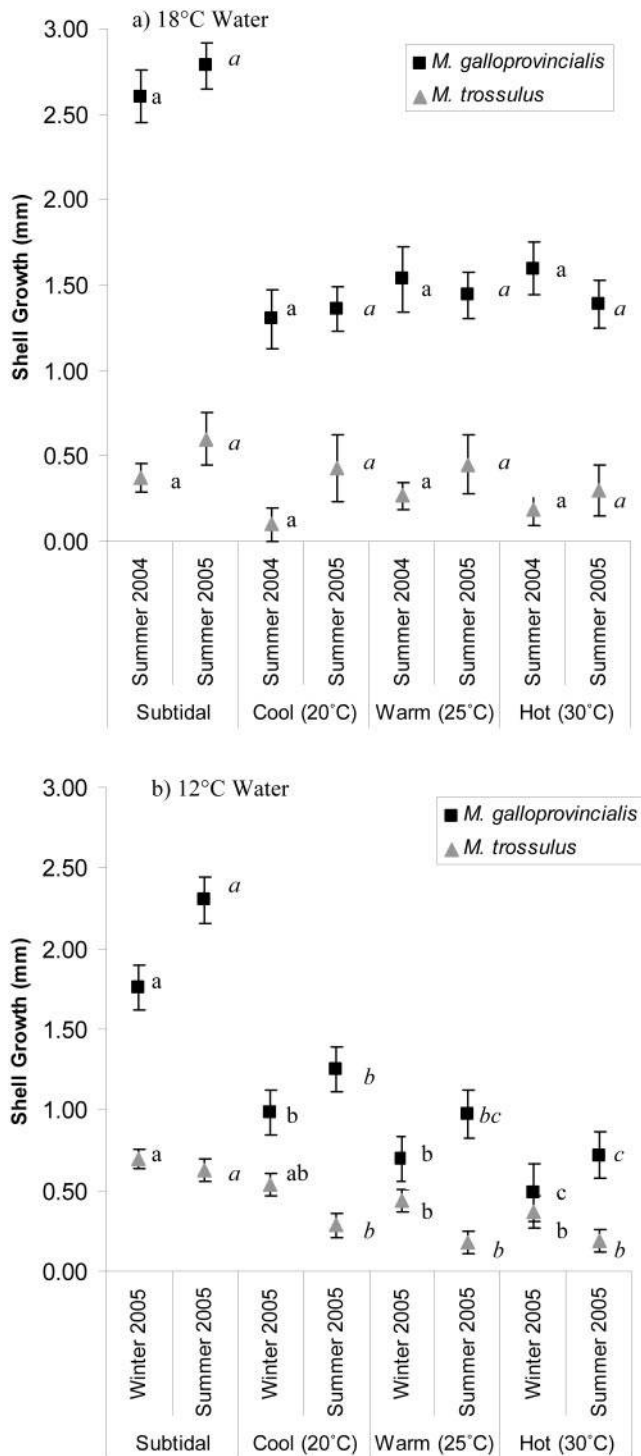
Figure 1. Growth in length of *Mytilus galloprovincialis* and *M. trossulus* under a variety of simulated intertidal thermal conditions in (a) 18 °C or (b) 12 °C water. Length measurements refer to the average change in length at the end of the 9-week experiment; bars around individual points represent the standard error. Letters refer to the pairwise comparisons between the treatments for each species; length measurements with the same letter are not significantly different from one and another (note: letters for the second run of each water temperature are italicized).

fects of aerial treatment were observed for either *M. trossulus* or *M. galloprovincialis*; survival was relatively high in all treatments for both species (Fig. 2d), and there was not a significant survival interaction between species and aerial exposure treatment (interaction: $P = 0.64$). Differences between *M. galloprovincialis* and *M. trossulus* were only marginally significant or were nonsignificant in summer 2005 (hot, $P = 0.06$; warm, $P = 0.03$; cool, $P = 0.1$; subtidal, $P = 0.02$), with *M. galloprovincialis* having slightly higher survival than *M. trossulus* (Fig. 2d).

Discussion

In this research I examined the role that intertidal and subtidal thermal stress plays with regard to the growth and survival of an invasive (*Mytilus galloprovincialis*) and native (*M. trossulus*) marine mussel. Previous work has investigated thermal tolerance differences between these two species with regard to water temperature (see Hofmann and Somero, 1996a; Braby and Somero, 2006a, b) but not aerial exposure (however, see Schneider and Helmuth, 2007, who examined patterns of species distribution in the field). The present study provides data that generally confirm the findings of previous work on the physiological differences in water temperature adaptation between the two species. This study also contributes the first experimental evidence concerning the role that aerial exposure during low tide plays with respect to growth and survival of the two species. Additionally, data show that *M. galloprovincialis* has higher growth and survival rates than *M. trossulus*, regardless of the temperature regimes examined, which supports the hypothesis presented in the introduction that *M. galloprovincialis* would show higher levels of growth and survival in both warmer water and aerial environments than would *M. trossulus*. However the nature of the differences between the species varied across and within the different experiments and treatments.

M. galloprovincialis demonstrated a higher survival than *M. trossulus* in both 18 °C and 12 °C water temperatures. In 12 °C water the differences between the species were small, whereas the differences were much larger in 18 °C water. This pattern of results suggests that 18 °C was detrimentally warm for *M. trossulus* and more suitable for *M. galloprovincialis*. *M. trossulus* mortality began during the first weeks of the study and continued throughout both 18 °C experiments. A 20% decrease in *M. trossulus* survival was observed in 2005 compared to 2004, which may be due to higher water temperature in 2005 (18.4 °C) compared to the 2004 (16.9 °C) experiment (see results).

Heat-shock analyses (Hofmann and Somero, 1996a; Braby and Somero, 2006a) and observational studies (Sarver and Foltz, 1993; Suchanek *et al.*, 1997) are in accord with the present laboratory results suggesting that *M. trossulus* is more heat sensitive than *M. galloprovincialis*.
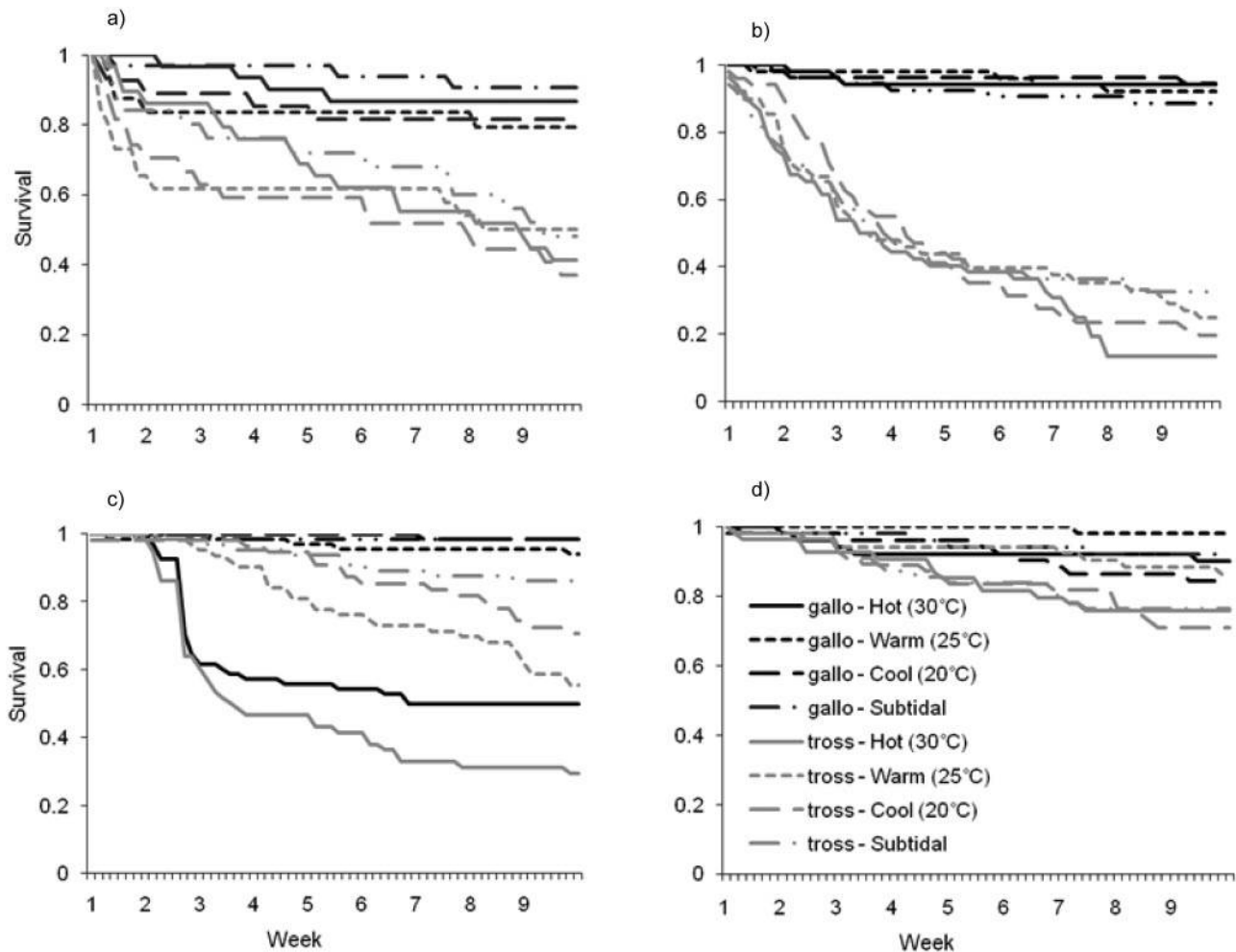
Figure 2. Survival of *Mytilus galloprovincialis* and *M. trossulus* under a variety of aerial exposure treatments throughout the 9-week experiment. Hot (30 °C), warm (25° C), and cool (20 °C) treatments refer to mussel body temperature during simulated low tide (see Table 2). Subtidal mussels remained underwater during the entire experiment, thus the body temperature is the same as the water temperature. A key is located in the bottom right-hand graph; gallo refers to *M. galloprovincialis*, tross refers to *M. trossulus*. (a) 18 °C water temperature—Summer 2004, (b) 18 °C water temperature—Summer 2005, (c) 12 °C water temperature—Winter 2005, (d) 12 °C water temperature—Summer 2005.

However, a recent field observational study in central California showed that *M. trossulus* was more abundant than *M. galloprovincialis* in areas with warm water temperatures, including several sites exceeding 25° C in maximum temperature (Braby and Somero, 2006b). The results of that study highlight the fact that factors other than temperature (*e.g.,* salinity, larvae dispersal) can influence the distribution of organisms.

Notwithstanding the findings of Braby and Somero (2006b), the thermal preference with respect to water temperature of *M. galloprovincialis* and *M. trossulus*, as found in this and other research, is consistent with their current distribution patterns along the west coast of the United States: *M. trossulus* is predominantly found in cooler water from central California through Alaska, and *M. gallopro-*

vincialis is largely found from southern California through northern California (Sarver and Foltz, 1993; Suchanek *et al.*, 1997). If temperatures continue to increase as a result of influences such as global warming, the thermal optimal of *M. trossulus* (*i.e.,* cooler conditions) may occur less frequently, thus opening habitat for invasive warmth-tolerant species such as *M. galloprovincialis*.

In addition to water temperature, aerial exposure during low tide likely influences the distribution patterns of intertidal organisms. This study suggests that the role played by low tide heat stress is probably complicated by multiple factors but remains an important variable in understanding intertidal ecology. Intertidal exposure can have negative effects on the growth and survival of organisms.

In both 12 °C experiments (*i.e.,* winter and summer),

growth was affected by aerial exposure, although this trend was much stronger with *M. galloprovincialis* than with *M. trossulus,* likely due to the profound growth differences between the two species (*i.e., M. galloprovincialis* grew more). The higher intertidal temperatures were associated with lower growth, particularly in *M. galloprovincialis*. In the 12 °C water there was also a negative effect of aerial exposure on the survival of both species, although the effect was greater for *M. trossulus*. This negative effect on survival was observed only in the winter trial. There are several possible reasons for this finding. Differences could be due to a seasonal effect on intertidal mortality such as shifts in water temperature (*i.e.,* thermal acclimation) and food availability. Warmer water temperatures in Puget Sound (≈ 8–10 °C in January, 11–12 °C in June, see Methods), where the mussels were raised, may have reduced the effect of low tide exposure in the laboratory in the summer trial (*e.g.,* Tomanek and Somero, 1999; Stillman and Somero, 2000; Buckley *et al.*, 2001). Food availability also often follows seasonal cycles. In Puget Sound, phytoplankton abundance is very limited in the winter compared to spring and early summer (Newton and Van Voorhis, 2002). The limited food supply during the winter may have had a lasting effect on the mussels' ability to handle thermal stress (see Braid *et al.*, 2005). The prior opportunity to feed in the summer trials may have left the mussels with more energy to combat thermal stress, thus no lethal effects of aerial exposure were seen, although sublethal effects (*i.e.,* growth) were still apparent. This hypothesis requires further investigation. If it is correct, low tide thermal stress events may have a greater effect on survival when they occur after periods of low food availability than they would when food was abundant.

Contrary to the 12 °C experiments, there was not a significant effect of intertidal exposure temperatures on growth or survival in the 18 °C water experiments. However, these trials occurred only during the summer, and as was observed with the 12 °C water experiments, there may have been a seasonal effect in ability to handle low tide stress with regard to survival. Still, in 18 °C, neither species showed the kind of influence on growth that was observed in 12 °C water. Thermal acclimation to aerial exposure due to warmer water conditions has been documented in other species (see Tomanek and Somero, 1999; Stillman and Somero, 2000), as well as in *M. trossulus* (Buckley *et al.,* 2001). Thus, the mussels held in 18 °C water were acclimated to the warmer conditions and likely had less difficulty adjusting to higher body temperatures during low tide.

Although water temperatures tend to follow a latitudinal cline, the body temperatures of intertidal species when measured at low tide do not always follow a similar pattern. Thermal stress during low tide is determined by a complex interaction involving factors such as timing of low tide, terrestrial climate, and wave splash, all of which fluctuate along a coastline (Helmuth *et al.*, 2002, 2006; Gilman *et al.*,

2006). Thus, some northern sites with cool water temperatures may experience warmer low tide body temperatures than southern intertidal sites. Along the west coast of North America, intertidal low tide "hot spots" occur within the range of *M. trossulus* (Helmuth *et al.*, 2002). If *M. trossulus* is more sensitive to warm low tide aerial exposure, than one may expect the abundance or fitness of *M. trossulus* to be reduced in the hot spots because of thermal stress. Additionally, if the distribution of *M. galloprovincialis* expands north, then this species may perform better than *M. trossulus* in these hot spots.

Schneider and Helmuth (2007) have explored aerial exposure thermal tolerance in central California (*i.e.,* intertidal thermal stress). *M. trossulus* genotypes were found in higher frequency in (1) shaded compared to exposed intertidal sites and (2) subtidal compared to intertidal sites (Schneider and Helmuth, 2007). These frequency differences between habitats may be a signature of selection against *M. trossulus* in aerial exposed thermal environments. The laboratory results reported in the present study showed that *M. trossulus* did have increased mortality compared to *M. galloprovincialis* when exposed to warmer thermal environments, but only in the winter 12 °C water experiment.

M. galloprovincialis had a higher growth rate than *M. trossulus* in each of the experiments. In natural populations, *M. galloprovincialis* appears to have a larger maximal size than *M. trossulus* (Braby and Somero, 2006b; Schneider, unpubl. data). If *M. trossulus* is genetically smaller, this may explain its reduced growth rate in the present research; older mussels grow less (*e.g.,* Sukhotin *et al.,* 2002). The size of the two species (*i.e.,* 20–30 mm in length) was controlled in the experiment but age was not. Mussels in the *M. edulis* complex that are 20–30 mm in length can be between from less than 1 year to 7 years in age depending on the habitat and environmental conditions in which they were raised (reviewed in Seed, 1976). Additionally, mussels within the *M. edulis* complex can mature within the first year of life (Seed and Suchanek, 1992). However, no direct reproductive comparisons have been made between *M. trossulus* and *M. galloprovincialis* (for more information about *Mytilus* spp. reproductive timing, see Gosling, 2003; Curiel-Ramirez and Caceres-Martinez, 2004). If one of the species in the laboratory experiments was directing more resources toward reproductive efforts at the time of the study (see Secor *et al.,* 2001), this could have affected the results. The above considerations suggest the importance of controlling for age and reproductive maturity as well as for body size in future comparative investigations of growth and survival.

Other factors can also account for growth and survival differences between *M. trossulus* and *M. galloprovincialis*. For example, neoplasia ("mussel leukemia") is common in populations of *M. trossulus*, but is rarely seen in those of *M. galloprovincialis* (Moore *et al.*, 1991; Elston *et al.*, 1992; Ciocan and Sunila, 2005). The lethality of neoplasia may

increase at higher temperatures: elevated mortality with temperature increases has been observed with other bivalve diseases (reviewed in Gosling, 2003). If any of the study organisms were infected with this disease, it could explain the differences in survival observed in this study. In addition, *M. trossulus* may be more sensitive than *M. galloprovincialis* to captivity (*e.g.,* Braby and Somero, 2006a). Lastly, many other aspects of bivalve physiology are affected during low tide aerial exposure, such as immunity, reproductive stress, and acid-base balance. Clearly, rigorous field studies and expanded laboratory studies are necessary to determine how aerial exposure and water temperature (and the interaction of the two factors) affect intertidal distribution patterns.

Global temperatures are predicted to rise (Solomon *et al.*, 2007). Empirical and theoretical works suggest that the frequency of non-native invasions will increase, and the range of existing invaders will spread (Beerling *et al.*, 1995; Kremer *et al.*, 1996; Dukes and Mooney, 1999; Stachowicz *et al.*, 2002; Kriticos *et al.*, 2003). The differences found between *M. galloprovincialis* and *M. trossulus* in thermal physiology support the thesis that climate change facilitates species invasions. *M. galloprovincialis* outperforms its sibling species, *M. trossulus*, in warm thermal conditions, and the current results suggest that *M. galloprovincialis* will spread, at least *vis-à-vis M. trossulus*, as water and air temperatures increase. On the other hand, *M. galloprovincialis*'s sibling species, *M. trossulus*, is likely to remain a non-invader, and current southern distributions may retract due to low survivorship in warmer conditions. Considering the large negative effects that invasive species frequently have on ecosystems (*e.g.,* Pimentel *et al.*, 2000), understanding how invasive species interact with their thermal environments increases our insight into their current and future distributions. Future studies should continue to explore how subtidal and intertidal temperatures influence the adaptation of invasive and native species to both laboratory and field environments. Field studies that complement the laboratory experiments presented here will increase our understanding of the interaction between abiotic stressors and the success of invasive species in intertidal communities. Work that examines intertidal thermal stresses will facilitate our knowledge of current distribution patterns while helping to predict future distributions under a variety of climate scenarios.

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