RESEARCH ARTICLE

Synchronization of seasonal acclimatization and short-term heat hardening improves physiological resilience in a changing climate

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Abstract

1. Animal survival and species distribution in the face of global warming and increasing occurrences of heatwave largely depend on how heat tolerance shifts with plastic responses at different spatiotemporal scales, including long-term acclimation/acclimatization and short-term heat hardening. However, knowledge about the interaction of these plastic responses is still unclear.

2. To understand how plastic responses at different time-scales work together to adjust heat tolerance of organisms, we examined the effect of heat hardening on the upper thermal limits of an intertidal mudflat bivalve, the razor clam *Sinonovacula constricta*, for different seasons using heart rate as a proxy.

3. We observed a stronger heat hardening response of *S. constricta* in warm seasons, implying that heat hardening worked synchronously with seasonal acclimatization to increase resistance of the clams to high temperatures in warm seasons. In warm seasons, heat hardening increased heat tolerance by 2–4°C and showed a 24-hr temporal dependence, suggesting an adaptation to the diel fluctuation of thermal regimes in summer.

4. Furthermore, thermal stress resembling seasonal maximum environmental temperature induced stronger heat hardening effects, indicating that heat hardening is an essential plastic response to extreme hot weather, complementing seasonal acclimatization.

5. Our results suggest that high temperature risk can be alleviated jointly by seasonal acclimatization and heat hardening, and emphasize the importance of considering physiological plasticity on both long-term and short-term temporal scales in evaluating and forecasting vulnerability of organisms to climate change.

KEYWORDS

climate change, heat tolerance, intertidal bivalve, physiological plasticity, seasonal temperature variation

1 | INTRODUCTION

Ongoing climate change, manifested as increased average temperature and more frequent extreme weather, has had catastrophic consequences around the globe (IPCC, 2014). Understanding how organisms respond to warmer weather under different scenarios of climate change is important. Geographical shifts, genetically adaptation, physiological adjustments and behavioural

thermoregulation are significant options acting at different scales to allow organisms to respond to warm conditions (Fuller et al., 2010; Gilbert et al., 2017). However, for organisms that have a limited ability to migrate, the ability to alter their tolerance of high temperature is essential for long-term persistence (Harley, 2011; Walters et al., 2012). The heat tolerance of organisms can shift with environmental temperature variation over different time-scales, from short-term rapid adjustments (e.g. heat hardening) to long-term plastic responses (acclimation/acclimatization; acclimation refers to physiological adjustment following prolonged exposure to a certain temperature in the laboratory, whereas acclimatization refers to seasonal outdoor adjustments), and further to transgenerational evolutionary adaptation (Chown et al., 2010; Hoffmann, 2010). For individuals, short-term adjustments and long-term acclimatization are important because they can benefit survival within their lifetime and further drive or inhibit selection in evolutionary changes (Mladineo & Block, 2009; Sejerkilde et al., 2003; Van Heerwaarden et al., 2014). Short-term adjustments, especially heat hardening, can occur after acute exposure to a non-lethal heat event to temporarily increase heat tolerance, whereas long-term acclimatization to warm conditions results in a slower increase in heat tolerance over a longer time-scale (Angilletta, 2009; Bowler, 2005). Physiological responses of organisms to temperature variation and adaptive evolution during climate change depend on the extent to which different plastic processes (heat hardening and acclimatization) are triggered. This calls for an understanding of the relationship between these plastic responses whether they are employed simultaneously (a synchronous process) or not (an asynchronous process), especially in natural populations.

Frequent exposure to extreme situations results in a shift from an inducible response to a constitutive response that, in turn, reduces the inducible response (Stanton-Geddes et al., 2016; Zhang et al., 2019). For example, corals that frequently experience thermal stress show higher expression of genes involved in responses to heat stress, such as heat shock proteins or antioxidant enzymes, under control conditions and less upregulation during heat stress than conspecifics that less frequently encounter heat stress (Barshis et al., 2013). This may form an asynchronous process wherein acclimatization to warm conditions is accompanied by an attenuated heat hardening response. This asynchronous process means that the effect of heat hardening depends on the extent to which thermal stress can trigger inducible responses to heat stress (Stanton-Geddes et al., 2016). Therefore, in the asynchronous process, the temperature that induces a more effective heat hardening response may depend on basal heat tolerance that can modulate the extent to which a given temperature is perceived to be stressful. However, mounting a heat hardening response increases short-term survival of organisms but is costly due to the energy needed to generate and/or maintain proteins involved in heat stress responses and also affects other traits (Gilbert & Miles, 2019; Huang et al., 2007; Somero, 2002). To avoid inappropriate responses and cost, the heat hardening response should occur under conditions when frequent heat stress is more likely, such as during summer weather. This may lead to a process where acclimatization and heat hardening work synchronously. In the synchronous process, the heat hardening response should depend on the predictability of environmental temperatures to avoid inappropriate responses in unpredictable conditions (Phillips et al., 2016). Therefore, in this process, temperatures that induce effective heat hardening responses will match with the extremes that organisms experience in their habitat.

The intertidal zone is one of the most physiologically harsh habitats on earth wherein temperature is highly variable across different spatiotemporal scales. Temperature variation in the intertidal zone can vary between tidal cycles, across daily fluctuations and over seasons (Brahim et al., 2019; Dong et al., 2017). Studies have shown that various species in the intertidal zone live close to their upper thermal limits and are vulnerable to high temperature in hot summers and to future warming climates, and their heat tolerance, abundance and reproductive output are closely related to the environmental thermal predictability (Dong et al., 2017; Han et al., 2020; Somero, 2010; Stillman & Somero, 2000; Wang et al., 2017, 2020). Driven by global climate change, the geographical distribution and biological abundance of intertidal species, including those from rocky shores or mudflats, are rapidly changing (Saeedi et al., 2016; Schtickel & Kroencke, 2013). Intertidal species experience frequent thermal challenges within their lifetimes and their heat tolerance can be shaped by both long-term acclimatization to seasonal temperature variation and short-term heat hardening after sudden temperature rising. Therefore, the mix of tidal, daily or seasonal cycles of temperature variation makes intertidal species ideal for testing whether plastic responses over different time-scales are employed synchronously or not.

To reveal the relationship between seasonal acclimatization and short-term heat hardening in shaping heat tolerance, the present study measured the heat hardening response in a natural population of a burrowing bivalve, the razor clam *Sinonovacula constricta*, at different seasons and used heart rate as the indicator of heat tolerance (Dong et al., 2017; Marshall et al., 2011; Zhang et al., 2020). This clam inhabits intertidal mudflats of China, Japan and Korea and is sensitive to temperature variation, although its burrowing behaviours can provide a certain amount of physical relief from temperature extremes (Fan et al., 2002; He et al., 2017; Zhang et al., 2020). We hypothesized a synchronization of seasonal acclimatization and heat hardening responses in *S. constricta* that can benefit clams by increasing heat tolerance against high temperatures and predictable excursions into stressfully warm temperatures in summer. Different initial acute heat stresses relevant to the thermal lethal tolerance of *S. constricta* were used to induce heat hardening responses to determine (a) the relationship between heat hardening responses and the inducing temperature in different seasons and (b) how such relationships relate to seasonal acclimatization. In addition, heat hardening effects were measured after three recovery periods that were selected based on tidal

cycles or daily cycles, to ascertain the temporal dependence of the heat hardening response.

2 | MATERIALS AND METHODS

2.1 | In situ temperature measurement

Three roboclams (biomimetic temperature recorders simulated the morphology of *S. constricta*) were made using empty shells of *S. constricta* that contained iButtons (DS1922L, Maxim integrated) and vulcanized rubber (No. 2131, Minnesota Mining and Manufacturing) and were employed to assess in situ operative temperatures experienced by clams in the mudflat. These roboclams were anchored to three plastic pipes and the pipes were placed in a mudflat (24.6°N, 118.3°E) to bury the roboclams at the locations that were naturally inhabited by *S. constricta* (10–20 cm under the surface of mudflat), from April 2019 to April 2020. Measuring accuracy was set at 0.0625°C and the recording interval was set at 10 min or 20 min. The operative temperatures in the previous month and current month of sampling (2-month time window) were used to calculate the frequency of temperature within each 1°C interval to determine the temperature range of seasonal acclimatization. Average temperatures were calculated with operative temperatures in the sampling month; the maximum value among the daily 99th percentiles of operative temperature in the previous month and current month of sampling was used as the seasonal maximum temperature.

2.2 | Heat tolerance with seasonal acclimatization and heat hardening

To measure the heat tolerance of *S. constricta* with seasonal acclimatization and heat hardening, clams were obtained from a natural mudflat in Xiamen, Fujian Province, China (24.6°N, 118.3°E) in June ($n = 240$, mean mass = 13.41 ± 0.15 g), September ($n = 240$, mean mass = 11.87 ± 0.17 g), December 2019 ($n = 240$, mean mass = 12.06 ± 0.20 g) and April 2020 ($n = 240$, mean mass = 11.30 ± 0.14 g). Clams were first given a short acclimation of 3–6 days in aerated seawater at the approximate water temperature of the sampling season, pH of 8.4 and salinity of 28 psu with a stocking density of about 20 individuals per tank and natural photoperiod. After the short acclimation, initial heat stresses were conducted by heating clams in 250 ml beakers (one individual per beaker) filled with mud that was obtained from the sampling field (pre-sifted through a 40 mesh and with ~40% water content) at a heating rate of 0.1°C/min using a controllable water bath (TFX200, Grant). Temperatures were raised to 33°C, 35°C or 37°C, respectively, except for December 2019 samples that were exposed to 31°C, 33°C or 35°C, respectively (Figure 1). Maximum temperature was maintained for 1 min at the end of heating. These temperatures were based on similar gradient temperature differences to thermal lethal limits of clams in each season. In all, 12 clams were heated at a time and temperature in the mud was monitored by a temperature sensor (54-II, Fluke). After heat stress, clams were allowed to recover from high temperature exposure by immediately returning them to their initial short acclimation conditions for 3, 12 or 24 hr and then they were used for cardiac performance measurements. These three recovery durations were selected for the following reasons: (a) a 3-hr recovery duration was used to detect whether heat hardening response can increase heat tolerance after a relatively short recovery period and (b) the other two durations were related to tidal and daily cycle periods in the intertidal zone. Each group (one initial heat stress with one recovery period) included 24 clams in each season (Figure 1).

Cardiac performance was measured using a non-invasive method as described by Dong and Williams (2011). Briefly, the infrared heart beat signal was amplified (AMP03, Newshift),
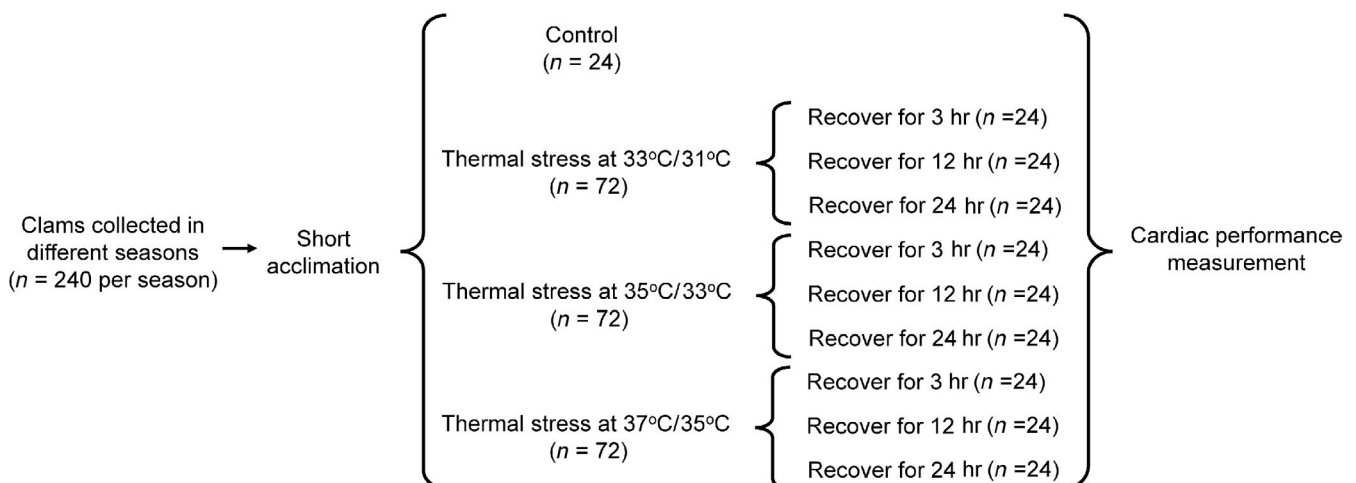

FIGURE 1 Experimental process for assessing the relationship between seasonal acclimatization and short-term heat hardening in shaping heat tolerance of *Sinonovacula constricta*

filtered, smoothed and recorded with a PowerLab AD converter (16/30, ADInstruments), and valid data were analysed with LabChart (v7.2). Clams without initial heat stress were used as controls for each of the four seasons. Heat tolerance of *S. constricta* was indicated by the Arrhenius break temperature (ABT), the sublethal temperature where heart rate increases with rising temperature but then drops rapidly, flatline temperature (FLT) and the lethal temperature where heart rate drops to zero (Zhang et al., 2020). Temperatures exceeding the ABT impair physiological functions and the response of organisms to rising temperature, and then results in mortality due to other biotic and abiotic stressors (Dong et al., 2017; Helmuth et al., 2014). Temperature exceeding FLT usually causes mortality of the clam. ABT values were calculated by segmented linear regression using Origin software (OriginLab Corp.).

2.3 | Data analysis and statistics

The ΔABT or ΔFLT was calculated as all ABT or FLT values minus the average ABT or FLT of clams from the control group in each season. The heat hardening response can be expected to reach a peak effect with increasing initial heat stress and then weakens when initial heat stresses excess certain upper high temperature limit. If the heat hardening response and seasonal acclimatization work as asynchronous processes, an initial heat stress closer to the basal heat tolerance of clams may induce a stronger heat hardening response, whereas an initial heat stress closer to certain ambient temperature characteristics (e.g. average or maximum values) may induce a stronger heat hardening response if these two plastic responses work in synchrony. Therefore, in each sampling season, initial heat stresses were categorized as three levels (close, intermediate and far) based on the absolute temperature difference between each initial heat stress and the seasonal temperature characteristics (seasonal maximum temperature or seasonal average temperature) or heat tolerance characteristics of razor clams from the control group (ABT or FLT), respectively. For example, based on the seasonal maximum temperature, initial heat stresses in June 2019 were marked with 'close, intermediate, far' with respect to the sequence of 33°C (close to the maximum temperature of 32.05°C), 35°C (intermediate) and 37°C (far from the maximum temperature) and initial heat stresses in September 2019 were categorized as 35°C (close to the maximum temperature of 35.42°C), 37°C (intermediate) and 33°C (far from the maximum temperature). We checked the model assumption for normality and variance homogeneity of our data. Thereafter, for ΔABT and ΔFLT, we used a generalized linear model with the following explanatory variables: seasons (represented by four months: June, September, December 2019 and April 2020), initial heat stresses (three levels plus the control), recovery times (control, 3, 12 and 24 hr) and all possible interactions between these three factors. Because initial heat stresses can be evaluated

based on four different characteristics (seasonal maximum temperature, seasonal average temperature, ABT or FLT), models were constructed for four cases with different initial heat stress levels, respectively. Afterwards, a t test or the Satterthwaite t test was employed based on an assumption for variance homogeneity to detect significant differences between the heat hardened ABT or FLT and comparable data from the control group at each season. Statistical analysis was conducted with SPSS software (v17.0, IBM).

Thermal safety margins (TSM) for sublethal (TSM-ABT) and lethal (TSM-FLT) tolerance were calculated as the difference between the ABT or FLT of individuals and the seasonal maximum operative temperature in June and September 2019. To assess the effects of heat hardening on TSMs, the ABT and FLT from individuals with or without heat hardening treatments followed by 24-hr recovery were employed in the comparison. The t test was used to compare the change in TSMs.

3 | RESULTS

Clams from warmer seasons showed stronger heat hardening capacity to increase both ABT and FLT (Table 1; Figure 2). The maximum ΔABT reached about 4.3°C, whereas maximum ΔFLT was 1.8°C. Heat hardening norms also linked with recovery time after initial heat stress (Table 1, effect of Recovery time). Generally, *S. constricta* showed higher ΔABT or ΔFLT after recovery for 24 hr (Figures 2 and 3a). Results from the generalized linear model indicated a statistically significant three-way interaction among seasons, initial heat stress and recovery time on both ΔABT and ΔFLT (Table 1). This interaction indicated that the heat hardening norms based on different initial heat stresses and recovery times varied across different seasons (Table 1; Figure 2). It is worth noting that, when initial heat stress levels were marked based on absolute temperature difference between heat stress and seasonal maximum temperature, initial heat stress levels significantly affected heat hardening effects on both ΔABT and ΔFLT across all seasons (Table 1, effect of IMT). Results showed that initial heat stress, which was closer to seasonal maximum temperature, induced higher ΔABT or ΔFLT values in all seasons (Figure 3b). The model also showed that, in general, the initial heat stress that was farther away from the ABT of the control group induced higher ΔABT values (Table 1, effect of IABT). However, in September 2019, the initial heat stress that was closer to ABT of the control group also induced higher ΔABT in *S. constricta*, indicating that the absolute temperature difference from initial heat stresses to ABT of the control group may not be conclusive for effects of heat hardening in all seasons (Figure 3c).

The thermal safety margin for ABT or FLT increased in June and September 2019 because the heat hardening effect can increase heat tolerance of *S. constricta* in these 2 months (all $p < 0.001$, Figure 4).

Number of samples for analysis: 722	ΔABT			ΔFLT		
	Wald chi square	df	p	Wald chi square	df	p
Season	87.311	3	<0.001	185.178	3	<0.001
Initial heat stress to Seasonal maximum temperature (IMT)	20.733	2	<0.001	6.652	2	0.036
Recovery time	8.769	2	0.012	24.785	2	<0.001
Season × IMT	6.877	6	0.332	8.941	6	0.177
Season × Recovery time	10.745	6	0.097	47.248	6	<0.001
Recovery time × IMT	16.457	4	0.002	41.641	4	<0.001
Season × IMT × Recovery time	26.86	12	0.008	49.444	12	<0.001
Season	87.311	3	<0.001	185.178	3	<0.001
Initial heat stress to Seasonal average temperature (IAT)	4.22	2	0.121	4.539	2	0.103
Recovery time	8.769	2	0.012	24.785	2	<0.001
Season × IAT	23.984	6	0.001	11.554	6	0.073
Season × Recovery time	10.745	6	0.097	47.248	6	<0.001
Recovery time × IAT	7.505	4	0.111	14.677	4	0.005
Season × IAT × Recovery time	37.396	12	<0.001	79.506	12	<0.001
Season	87.311	3	<0.001	185.178	3	<0.001
Initial heat stress to ABT (IABT)	12.642	2	0.002	4.931	2	0.085
Recovery time	8.769	2	0.012	24.785	2	<0.001
Season × IABT	15.055	6	0.02	11.132	6	0.084
Season × Recovery time	10.745	6	0.097	47.248	6	<0.001
Recovery time × IABT	3.358	4	0.5	16.761	4	0.002
Season × IABT × Recovery time	39.752	12	<0.001	72.481	12	<0.001
Season	87.311	3	<0.001	185.178	3	<0.001
Initial heat stress to FLT (IFLT)	4.22	2	0.121	4.539	2	0.103
Recovery time	8.769	2	0.012	24.785	2	<0.001
Season × IFLT	23.984	6	0.001	11.554	6	0.073
Season × Recovery time	10.745	6	0.097	47.248	6	<0.001
Recovery time × IFLT	7.505	4	0.111	14.677	4	0.005
Season × IFLT × Recovery time	37.396	12	<0.001	79.506	12	<0.001

TABLE 1 Results of generalized linear models testing the effects of seasons (represented by 4 months), initial heat stress (three levels according to seasonal temperature characteristics or thermal tolerance characteristics), recovery times (3, 12 and 24 hr) and their interactions on ΔABT and ΔFLT of *Sinonovacula constricta*. Values in bold indicate significant differences, $p < 0.05$
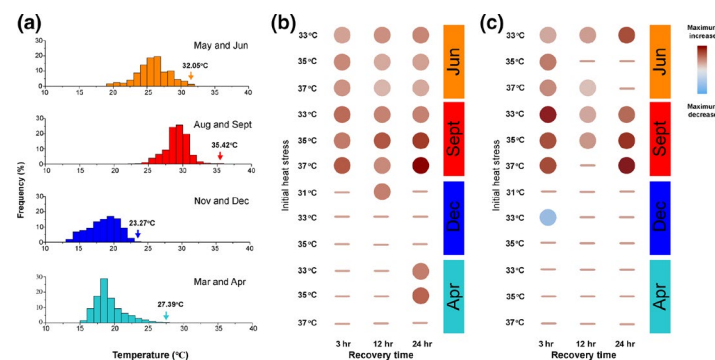

FIGURE 2 Relationship between heat hardening capacity of *Sinonovacula constricta* and seasonal temperature variation. (a) The frequency of operative temperatures that *S. constricta* experienced in the intertidal mudflat as measured by buried roboclams; arrows point to the maximum temperature recorded. (b) Effects of heat hardening in shifting the Arrhenius break temperature (ABT) of *S. constricta* after heat stress treatments of clams collected in June, September, December and April, respectively. (c) Comparable data for flatline temperature (FLT). Red circles indicate significant increases in ABT or FLT and the blue circle indicates a significant decrease. The red short line (–) indicates no significant change in ABT or FLT caused by heat hardening
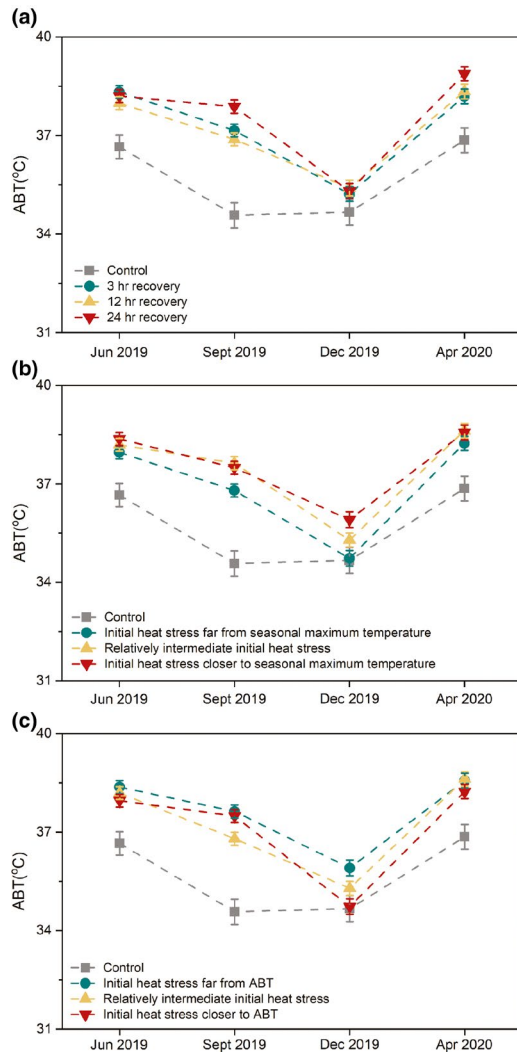
FIGURE 3 Effect of recovery periods (a), initial heat stresses categorized based on seasonal maximum temperature (b) and initial heat stresses categorized based on Arrhenius break temperature (ABT) of the control group (c) on ABT of *Sinonovacula constricta* at different seasons based on a generalized linear model. The difference in ABT of each group and the corresponding control group indicate ∆ABT. Error bars indicate standard errors

4 | DISCUSSION

Physiological plasticity is an important adaptive response to thermal variation that can reflect the sensitivity of organisms to high temperature and provide insight into how a warming climate could affect a species. Thermal plasticity can affect whether a species can endure extreme temperatures or how long species can survive hot conditions (Castañeda et al., 2015; Dong et al., 2010; Gu et al., 2018; Semsar-Kazerouni & Verberk, 2018). Understanding the adaptation or evolution of thermal plasticity requires determining how plastic responses at different time-scales are triggered, especially in the face of complex spatiotemporal temperature variation. The interaction of thermal plastic responses at different time-scales, from short-term heat hardening to long-term acclimatization, is unclear. In the present study, as we hypothesized, our results showed a synchronization of long-term seasonal acclimatization and short-term heat hardening to increase the heat tolerance of *S. constricta* in warm seasons.

With acclimatization in warm seasons, *S. constricta* showed a strong heat hardening capacity to increase heat tolerance over a short recovery time and to maintain higher heat tolerance for a long time, reflecting a better ability to endure sudden and extreme high temperature (Figure 2). Similar results were also observed for the Mediterranean fruit fly *Ceratitis capitata* acclimated to warmer environments (Terblanche et al., 2010). Warm seasons mean high average temperature and increased occurrence of heatwaves wherein heat hardening can offer important fitness benefits. This was supported by a field experiment that showed that higher recapture rates of flies that were pre-hardened in the laboratory occurred under hot conditions (Loeschcke & Hoffmann, 2007). Therefore, acclimatization during warm weather means that organisms will experience more repeated exposures to high temperatures, suggesting a greater selection for better heat hardening capacity in warm/hot than in cool/cold seasons (Phillips et al., 2016). The synchronization of seasonal acclimatization and heat hardening indicated that these two plastic responses may be triggered via different mechanisms or more subtle regulatory mechanisms. It is worth noting that, in our results, razor clams showed a relatively

FIGURE 4 The effect of heat hardening on the thermal safety margins (TSMs) of *Sinonovacula constricta* in June and September based on our data for Arrhenius break temperature (ABT) (a) and flatline temperature (FLT) (b). Points above the zero-line show that individuals have a physiological TSM for ABT or FLT; points below the zero-line show that individuals are threatened by high temperature
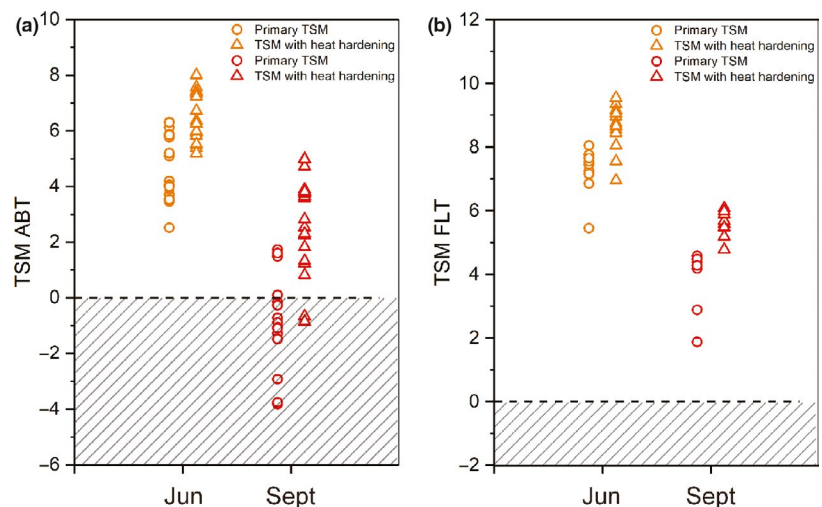
low basal sublethal heat tolerance in September 2019, a period with high ambient temperatures, and accordingly showed a stronger heat hardening response (Figures 2 and 3). The causes for the low basal sublethal heat tolerance may be various, such as large energy consumption to support reproduction which is going on during this period or a possible energy conservation strategy during frequent stressful heat events (Marshall & Mcquaid, 2011; Yan et al., 2010). This phenomenon further indicated the importance of quick heat hardening for *S. constricta* to endure stressful heat events in the warm summer. In cold seasons, heat hardening capacity of *S. constricta* was reduced. The cost of heat hardening or acclimatization to warm conditions may be higher in organisms acclimated to cold condition, such as that cold-acclimated flies were less likely to find food in hot environments than flies that were not cold-acclimated (Kristensen et al., 2008). This may be due to an extra energy cost in regulating levels of proteins or gene expression to increase heat resistance when organisms have been acclimated to cold conditions. Furthermore, maintenance of a strong heat hardening capacity in cold seasons does not match the reality that high temperature weather is rare at these times and the heat hardening response may bring greater physiological costs but no gain in fitness as mounting a heat hardening response can impair other performance issues, such as locomotion and fecundity (Gilbert & Miles, 2019; Huang et al., 2007).

When synchronously working with seasonal acclimatization, an optimal heat hardening response can increase heat tolerance of *S. constricta* by more than 2°C. This increase in heat tolerance was higher than that seen for damselfly nymphs, fruit flies or lizards (about 1°C; Gilbert & Miles, 2019; Phillips et al., 2016; Van Heerwaarden et al., 2016; Verberk & Calosi, 2012) but similar to that in the green-lipped mussel (Dunphy et al., 2018). This indicates that heat hardening is an important physiological function for intertidal species because they inhabit environments with dramatic temperature variations but they have limited ability to migrate, which promote a greater selection for mounting a short-term hardening response. Our results also showed a 24-hr temporal dependence of heat hardening in *S. constricta* because the maximum ΔABT and ΔFLT usually occurred after 24-hr recovery or can be maintained for at least 24 hr. This temporal dependence is different from that in fruit flies or lizards, whose heat tolerance peaked at about 6-hr recovery and then declined to baseline levels rapidly; however, the pattern in *S. constricta* is similar to that seen in intertidal mussels or sea cucumbers (Dong et al., 2010; Dunphy et al., 2018; Gilbert & Miles, 2019; Phillips et al., 2016). Over a long time-scale, temperature in the intertidal zone varies with the seasons but, on a short time-scale, it also changes with both daily and tidal cycles (Dong et al., 2017; Szathmary et al., 2009). High temperature in the intertidal zone usually occurs based on a day-to-day cycle. Therefore, long-term acclimatization can promote the adaptation of heat hardening in *S. constricta* to the diel fluctuation of temperature in the intertidal zone by forming a temporal dependence on 24 hr. This can help clams to deal effectively with high temperature reoccurring the next day in the presence of heat hardening responses.

Meanwhile, in warm seasons, a greater change in ΔABT was induced by heat hardening than in ΔFLT in *S. constricta*, indicating a better ability of *S. constricta* to shift thermal sublethal limits with synchronization of heat hardening and acclimatization. In fact, the thermal sublethal limit or lower heat tolerance traits show higher plasticity than the lethal limit in many species, including intertidal species (Han et al., 2013; Pasparakis et al., 2016; Semsar-Kazerouni & Verberk, 2018; Stenseng et al., 2005). Temperatures exceeding the thermal sublethal tolerance of organisms usually mean 'ecological death' because, at that time, organisms cannot avoid adverse effects of predation or a further rise in temperature due to activity restriction or thermal damage (Archambault et al., 2013; Dong et al., 2017). Intertidal species usually live close to their thermal sublethal limits and will experience more frequent challenges to their sublethal limits in warm seasons. Therefore, heat hardening is a further protection for organisms against adverse effects of high temperature by increasing their sublethal heat tolerance in addition to seasonal acclimatization.

As expected, synchronization of long-term acclimatization and short-term heat hardening lead to a close relationship between the temperature that can induce a stronger heat hardening response and the variability of environment temperatures. In the present study, an initial heat stress that was closer to the seasonal maximum temperature induced a stronger heat hardening response in *S. constricta* in all seasons. This suggests that the short-term hardening response is an important complementary mechanism that can combine with seasonal acclimatization to increase heat tolerance to deal with extreme situations. This complementary effect may further support that acclimatization and heat hardening are induced via different mechanisms or that there are complex regulatory processes in the clam to trigger these two forms of plasticity. The stronger heat hardening response induced by an initial heat stress closer to the seasonal maximum temperature also suggests that the short hardening effect may depend on the predictability of temperature variation, which can be obtained from long-term seasonal acclimatization. It has been reported that mounting a heat hardening response involves upregulation of multiple HSPs and regulation of energy metabolism after initial heat stresses (Dunphy et al., 2018; Gu et al., 2018). A heat hardening response that is beyond the actual demand or inappropriate will result in a loss of fitness due to the extra cost of energy, impaired locomotor activity or reduced fecundity (Gilbert & Miles, 2019; Huang et al., 2007; Jordan & Snell, 2002). Therefore, when heat stress occurs with a relatively short peak and can be predictable based on the range of long-term temperature variation, a heat hardening response will be promoted fully in *S. constricta* to benefit survival in high temperature. However, when heat stress is unpredictable or constant, *S. constricta* may reduce the investment in thermal plasticity, wherein heat hardening is positive but weakened. This may indicate a limit of unpredicted high temperature to the benefits of heat hardening.

Synchronization of the heat hardening response and seasonal acclimatization can lead to a shift in thermal safety margins for
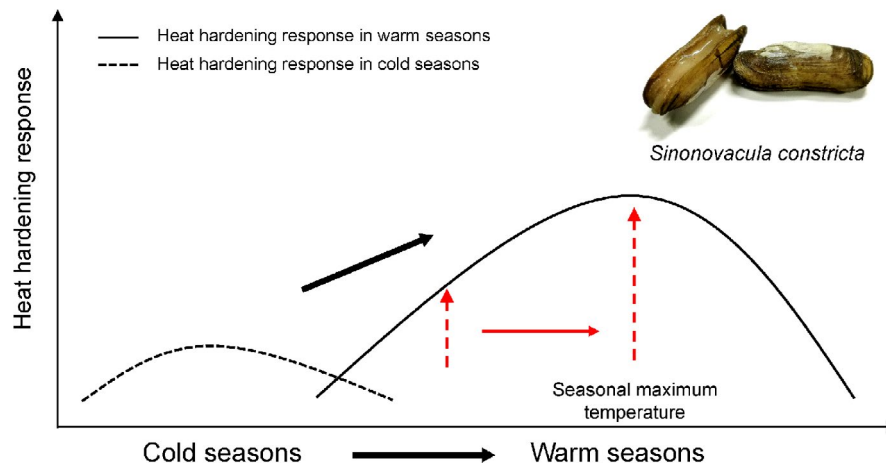
FIGURE 5 The heat hardening response of *Sinonovacula constricta* works synchronously with seasonal acclimation. Heat hardening response of *S. constricta* is enhanced as cold seasons change to warm seasons. In each season, when initial heat stress is in the range of seasonal temperature variation, *S. constricta* shows an appropriate heat hardening response until that initial heat stress reaches the seasonal maximum temperature and induces the strongest heat hardening effect (red solid line). When initial heat stress is beyond the seasonal maximum temperature, it induces a positive but weakened heat hardening response

S. constricta in hot summers. Thermal safety margins are an important way to quantify the sensitivity of organisms to variation in temperature (Deutsch et al., 2008). Extreme temperatures are more important than average ones for affecting long-term persistence of species (Sunday et al., 2014). With the synchronization of seasonal acclimatization and heat hardening, seasonal extreme temperatures induced larger shifts in ABT of *S. constricta* and then contributed to a greater TSM, which may help the clams to withstand a hot summer. Especially in September 2019, more individuals can obtain a positive thermal safety margin for sublethal heat tolerance with the heat hardening response (Figure 4). Our results indicate a crucial role of physiological plasticity in preventing warming effects on the clam *S. constricta*. In fact, the effect of heat hardening has rarely been considered in calculating TSM, although studies showed that a heat hardening response is common in many species (Dong et al., 2010; Dunphy et al., 2018; Phillips et al., 2016; Sejerkilde et al., 2003; Willot et al., 2017). The present research suggests that future studies should take the effect of the heat hardening response into consideration, since this may be valuable in allowing us to predict the vulnerability of a species or the change in geographical distribution of organisms during climate change.

In conclusion, the present study showed a synchronization of long-term seasonal acclimatization and short-term heat hardening to increase heat tolerance of *S. constricta* to high temperatures (Figure 5). The heat hardening response significantly increased the heat tolerance of *S. constricta* in the hot summer but caused few effects in cold winter. The scale and duration of the heat hardening response were affected by long-term seasonal acclimatization. In this synchronization, a short-term high temperature in the range of long-term temperature variation in warm seasons can induce an appropriate heat hardening response to promote a higher heat tolerance. However, this heat hardening response will be weakened when high temperature rises further to exceed the range of long-term temperature variation (Figure 3). Our study suggests that short-term

adjustment and long-term acclimatization can work jointly to increase heat tolerance in warm seasons and also reveals that the heat hardening response is potentially limited to predictability of long-term temperature variation.

CONFLICT OF INTERESTS

Authors declared that there was no conflict of interests.

AUTHORS' CONTRIBUTIONS

W.-y.Z. and Y.-w.D. conceived the study; W.-y.Z. collected and analyzed the data; W.-y.Z. and Y.-w.D. wrote the paper; Y.-w.D. and K.B.S. revised the paper.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.70rxwdbwq (Zhang et al., 2021).

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SUPPORTING INFORMATION

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