Journal of Thermal Biology 119 (2024) 103763

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Geography and developmental plasticity shape post-larval thermal tolerance in the golden star tunicate, *Botryllus schlosseri*

Zachary Tobias[a,b,c,*], Andrew Solow[d], Carolyn Tepolt[c]

[a] *MIT-WHOI Joint Program in Oceanography/Applied Ocean Science and Engineering, Cambridge and Woods Hole, MA, USA*
[b] *Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA*
[c] *Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA*
[d] *Marine Policy Center, Woods Hole Oceanographic Institution, Woods Hole, MA, USA*

ARTICLE INFO

Keywords:
Developmental plasticity
Local adaptation
Thermal tolerance
LT_{50}
Invasive species
Tunicate

ABSTRACT

Local adaptation and phenotypic plasticity play key roles in mediating organisms' ability to respond to spatiotemporal variation in temperature. These two processes often act together to generate latitudinal or elevational clines in acute temperature tolerance. Phenotypic plasticity is also subject to local adaptation, with the expectation that populations inhabiting more variable environments should exhibit greater phenotypic plasticity of thermal tolerance. Here we examine the potential for local adaptation and developmental plasticity of thermal tolerance in the widespread invasive tunicate *Botryllus schlosseri*. By comparing five populations across a thermal gradient spanning 4.4° of latitude in the northwest Atlantic, we demonstrate that warmer populations south of the Gulf of Maine exhibit significantly increased (~0.2 °C) post-larval temperature tolerance relative to the colder populations within it. We also show that *B. schlosseri* post-larvae possess a high degree of developmental plasticity for this trait, shifting their median temperature of survival (LT_{50}) upwards by as much as 0.18 °C per 1 °C increase in environmental temperature. Lastly, we found that populations vary in their degrees of developmental plasticity, with populations that experience more pronounced short-term temperature variability exhibiting greater developmental plasticity, suggesting the local adaptation of developmental plasticity. By comparing the thermal tolerance of populations across space and through time, we demonstrate how geography and developmental plasticity have shaped thermal tolerance in *B. schlosseri.* These results help inform our understanding of how species are able to adjust their thermal physiology in new environments, including those encountered during invasion and under increasingly novel climate conditions.

1. Introduction

Temperature exerts broad influence on biological systems, from setting rates of biochemical reactions to impacting the geographic distribution of species (Hochachka and Somero, 2002). Understanding how species cope with changes in temperature is a fundamental concern of ecophysiology and has broad implications in an era of global climate change (Chown et al., 2010; Somero, 2010). For sessile marine ectotherms, which have limited capacity for behavioral thermoregulation, the physiological effects of temperature can be especially pronounced (e.g. Hofmann and Somero, 1995). Nonetheless, many marine invertebrates have extensive geographic distributions and persist over broad spatial gradients in temperature. Many such organisms, especially those in coastal and intertidal environments, are subject to wide swings in temperature across a range of temporal scales, including tidal, diel, seasonal, and interannual (Deser et al., 2010; Hochachka and Somero, 2002). The persistence of marine ectotherms in the face of such spatiotemporal variation demonstrates their ability to tune their physiological performance to the prevailing temperature conditions.

Geographic variation in temperature is often reflected by clines in the acute thermal tolerance of a focal species across its range (Fangue et al., 2006; Kelly et al., 2012; Kuo and Sanford, 2009; Pereira et al., 2017; Sasaki and Dam, 2019; Tepolt and Somero, 2014; Zippay and Hofmann, 2010). For example, populations of the intertidal copepod *Tigriopus californicus* exhibit increasing thermal tolerance with decreasing latitude across a gradient spanning British Columbia to Baja California (Pereira et al., 2017). Such patterns can arise through two general mechanisms. First, provided sufficient heritable variation and

---

[*] Corresponding author. MIT-WHOI Joint Program in Oceanography/Applied Ocean Science and Engineering, Cambridge and Woods Hole, MA, USA.
E-mail address: ztobias@whoi.edu (Z. Tobias).

https://doi.org/10.1016/j.jtherbio.2023.103763
Received 27 September 2023; Received in revised form 26 October 2023; Accepted 19 November 2023

selective pressure, separate populations of a given species may evolve different thermophysiological phenotypes (Angilletta, 2009). This type of local adaptation, while historically considered to be rare in the marine environment due to the assumption of widespread connectivity, has now been demonstrated in a variety of systems (reviewed in Sanford and Kelly, 2011). By contrast, individual organisms are also often capable of altering their phenotypes depending on environmental conditions. Phenotypic plasticity, the ability of an individual genotype to produce multiple phenotypes depending on its environment (West-Eberhard, 2003), is pervasive in the ocean and is a key modality for coping with environmental variability (Foo and Byrne, 2016; Padilla and Savedo, 2013; Somero, 2010). Phenotypic plasticity is itself subject to natural selection, leading to the expectation that populations experiencing more variable environments should exhibit greater acclimatory capacity (Bradshaw, 1965; Levins, 1968).

Investigating how thermal tolerance varies within a species both across space and through time can inform us about how local adaptation and phenotypic plasticity are operating in natural populations. However, most studies of intraspecific variation of thermal tolerance in marine invertebrates focus on either the spatial or temporal dimension, but not both (but see Crickenberger et al., 2015; Morley et al., 2012). When the temporal scale of environmental temperature variation is less than the generation time of the focal species, temporal variation in thermal tolerance can generally be attributed to phenotypic plasticity. Spatial variation in thermal tolerance, however, can be shaped by both local adaptation and plasticity. By studying different populations both across space and through time, one can assess how thermal tolerance is shaped by both long-term temperature trends coincident with latitude (potentially via local adaptation) and short-term variation due to more rapid bouts of heating and cooling (via phenotypic plasticity). While definitively disentangling the relative contributions of local adaptation and plasticity to variation in a particular phenotype requires a reciprocal transplant or common garden approach, ideally across multiple generations, a comparison among wild individuals from populations along a latitudinal gradient can inform us of the *potential* for local adaptation. A spatiotemporal approach can also reveal how the capacity for phenotypic plasticity differs among populations, testing the prediction that populations experiencing more variable environments should exhibit greater acclimatization potential.

Temperature tolerance is often not fixed throughout the life of an individual, but rather can shift across ontogeny (Klockmann et al., 2017; Komoroske et al., 2014; Pincebourde and Casas, 2015; Ruthsatz et al., 2022). In general, it is early life history stages of marine species that are most vulnerable to temperature stress (Collin et al., 2021; Pandori and Sorte, 2019; Pineda et al., 2012; but see Peck et al., 2013; Tangwancharoen and Burton, 2014). Given the biphasic life history of many marine organisms and the importance of larval dispersal, studying thermal tolerance at early stages in development is critical for assessing the sensitivity of marine species to climatic changes (Pandori and Sorte, 2019; Pankhurst and Munday, 2011; Przeslawski et al., 2015). Importantly, temperature tolerance of later life stages can be affected by the thermal environment experienced by individuals earlier in ontogeny. Developmental plasticity is a form of phenotypic plasticity and is a critical means of coping with variability in the thermal environment, especially for marine and aquatic organisms (Pottier et al., 2022). Studies that simultaneously investigate how local adaptation and developmental plasticity affect thermal tolerance in natural populations of marine animals are rare (but see Pereira et al., 2017; Sasaki and Dam, 2020, 2019) but important for assessing species' ability to cope with increasing environmental stress. Here we use cross-population comparisons and natural temporal variability in environmental temperature to investigate the potential for local adaptation and developmental plasticity of thermal tolerance in post-larvae of the colonial tunicate *Botryllus schlosseri*.

Botryllus schlosseri (Pallas, 1776) is an invasive ascidian that has successfully colonized diverse habitats around the globe (invasions.si.edu/nemesis/species_summary/159373). In the northwest Atlantic, where it is considered cryptogenic, *B. schlosseri*'s range extends from Virginia, USA to Newfoundland, Canada. *Botryllus schlosseri* is thus subject to a wide gradient of temperatures across this range, exceeding 30 °C in the summer in the south and reaching sub-zero temperatures during the northern winters. Its persistence across this broad temperature gradient suggests the potential for local adaptation and/or phenotypic plasticity of thermal tolerance. *Botryllus schlosseri* larvae are incredibly short-lived, spending mere hours in the water column, which severely restricts their capacity for larval dispersal (Grosberg, 1987). Instead, most long-distance dispersal is thought to be through relatively infrequent anthropogenic translocation events or rafting on natural substrates (Lacoursière-Roussel et al., 2012; Thiel and Gutow, 2005; Worcester, 1994). This results in limited gene flow and a high degree of genetic differentiation among populations (Grosberg, 1987) (Tobias and Tepolt, in prep), thus contributing to the high potential for local adaptation in this species. Furthermore, ascidians are known to be extremely fast-evolving at the molecular level, exhibiting high mutation rates (Tsagkogeorga et al., 2010, 2012; Vinson et al., 2005), which may further potentiate adaptive divergence of thermal tolerance among populations. In addition to its high potential for local adaptation, *B. schlosseri* exhibits a high degree of physiological flexibility. Prior studies have demonstrated pronounced plasticity for traits including growth and reproductive effort in response to changes in temperature (Newlon et al., 2003; Rinkevich et al., 1998). *Botryllus schlosseri* can also establish in extremely thermally variable environments, for example, at the mouths of large estuaries, where temperatures can fluctuate in excess of 10 °C over the 6 h of a tidal period (AUTHOR, pers. obs.). This suggests that individuals must be capable of tuning their physiology over short temporal scales. This, coupled with its high potential for adaptive divergence, make *B. schlosseri* an excellent system for testing predictions about the evolution of phenotypic plasticity by comparing populations experiencing differing levels of short-term temperature variability.

In the present study, we conduct experiments across a latitudinal and temporal gradient in temperature to address three questions: 1) how does local adaptation potentially shape thermal tolerance across populations? 2) how does short-term temperature history affect subsequent thermal tolerance through developmental plasticity? and 3) how does the degree of developmental plasticity vary among populations, potentially through local adaptation of phenotypic plasticity?

2. Materials and Methods

2.1. Sites

Lethal tolerance of 50% survival (LT_{50}) experiments were conducted during the summer of 2022 at five sites in the northeastern USA: Rutgers University Marine Field Station, Tuckerton, NJ (RUTG), Falmouth Harbor, Falmouth, MA (FALM), Sandwich Marina, Sandwich, MA (SAND), Chatham Fish Pier, Chatham, MA (CHAT), and Darling Marine Center, Walpole, ME (DARL) (Fig. 1a, Table 1). For the three Massachusetts sites, all experiments were conducted at Woods Hole Oceanographic Institution. For RUTG and DARL, experiments were conducted over 2 weeks at the each field station.

2.2. Environmental temperature

Short-term environmental temperature data from all sites were collected with HOBO loggers while experiments were being run. Long-term field temperatures were obtained with HOBO loggers (for FALM and SAND) or from publicly available sources (for CHAT, DARL, RUTG) (see Supplementary Materials).

2.3. Experimentation

The day prior to each experiment, 24 adult *B. schlosseri* colonies were

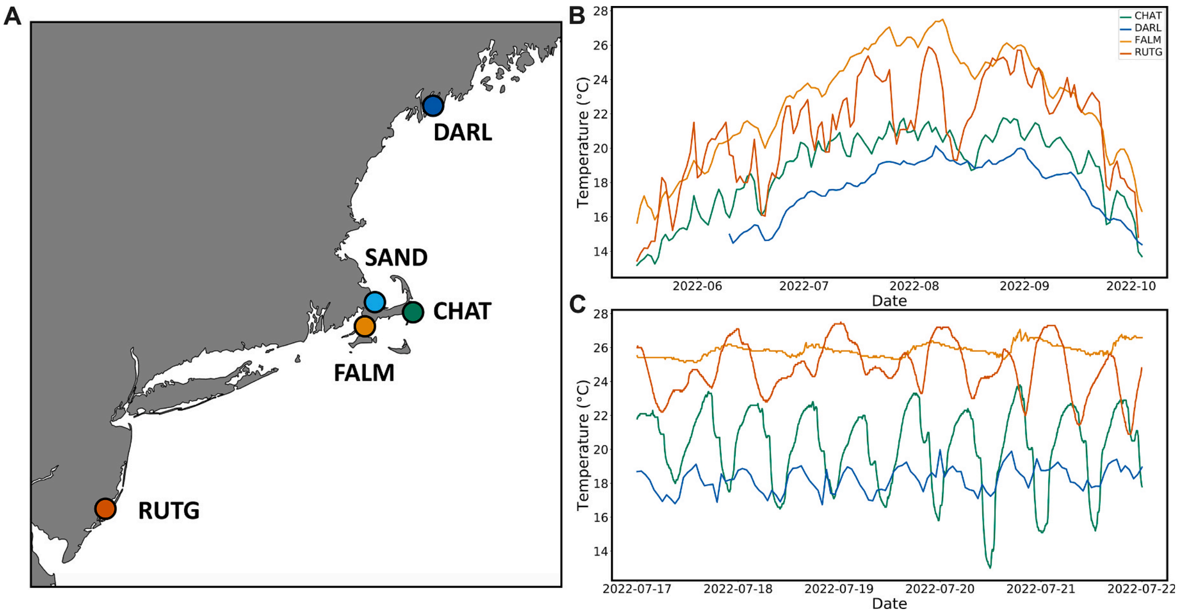

Fig. 1. Sites differ in mean summer temperature trends and the extent of daily variability. (A) Map of sites. (B) Mean daily environmental temperatures at study sites during the late spring, summer, and early fall of 2022. (C) Environmental temperature data during a five-day period from July 17–22, 2022. Note the differences in temperature variability among sites. Temperature data for SAND is missing due to temperature logger failure.

Table 1
Collection site information, with LT_{50} and the lower and upper bounds of 95% confidence intervals.

Site	Code	Lat	Lon	Oozooids	Clutches	LT_{50} (°C)	Lower	Upper
Tuckerton, NJ	RUTG	39.509	−74.325	900	24	32.21	32.15	32.28
Falmouth, MA	FALM	41.548	−70.603	769	18	32.21	32.15	32.27
Chatham, MA	CHAT	41.689	−69.951	731	10	32.24	32.17	32.32
Sandwich, MA	SAND	41.772	−70.503	1003	22	32.01	31.95	32.06
Walpole, ME	DARL	43.935	−69.581	325	19	32.06	32.00	32.12

collected by hand from the underside of floating docks and other substrates. Colonies were transported back to the laboratory and placed into individual 3.5 x 2 x 2″ wells of polycarbonate compartment boxes with ~200 ml of local seawater. Each well was fitted with five 3 x 2″ extra-thick microscope slides (Fisherbrand, Pittsburgh, PA, USA) along the bottom and side walls. Colonies were left overnight at room temperature and allowed to release larvae.

The following morning, each well was checked for larval release and settlement on the slides. To facilitate counting, oozooids (settled and metamorphosed larvae) were thinned to a maximum of 35 individuals per slide. Oozooids were then censused under a Stemi dissecting stereomicroscope (Zeiss, Oberkochen, Germany) at 50× magnification and examined for a heartbeat and typical development. All those showing slowed (tail still present) or abnormal development were removed prior to the experiment.

The five slides from each clutch (offspring of a single clonal colony) were partitioned across five temperature treatments, consisting of set temperatures of 30.0, 30.6, 31.1, 31.7, and 32.2 °C. These temperatures were selected based on pilot experiments run in the summer of 2021 in Woods Hole, MA and were chosen to yield approximately 100% survival at the lower end, 100% mortality at the higher end, and intermediate survivorship in between. The heat exposures were performed in polycarbonate 4″-deep 1/3 pans (Cambro, Huntington Beach, CA, USA) equipped with programmable 25 W glass aquarium heaters (YOFOTHS, Shenzhen, Guangdong, China) and HOBO Pendant temperature loggers (Onset, Bourne, MA, USA). While temperatures were set using the programmable aquarium heaters, the temperatures used for downstream analysis were those recorded by the temperature loggers.

Heat exposures lasted for 20 h, starting from the ambient temperature of local seawater with a ramp speed of approximately 4 °C/h. After exposure, survivorship was assessed through a second census. All individuals without a detectable heartbeat were considered dead.

LT_{50} experiments were repeated through time across the summer at each site with the exception of CHAT. Because environmental temperature varied across the summer, even during the course of the two-week-long field excursions at RUTG and DARL, we used temporal variation in environmental temperature as a proxy for developmental temperature (see *2.4. Data analysis*), essentially providing pseudo-developmental temperature treatments. While our design does not directly manipulate developmental temperature, by investigating how heat tolerance varies across short-term changes in environmental temperature we are able to make inferences about how developmental plasticity is operating in natural populations.

2.4. Data analysis

All data analysis was performed in R v. 4.1.2 (R Core Team, 2013). To investigate differences in thermal tolerance among sites, we first used the R package *drc* v. 3.0.1 (Ritz et al., 2015) to derive LT_{50} estimates for each population. Two-parameter (slope and midpoint [i.e. LT_{50}]) log-logistic curves were fit to the binomial survivorship data. To test for significance of population-level differences in LT_{50}, a model with separately estimated LT_{50} values for each population was compared to a jointly-estimated LT_{50} model using a likelihood ratio test. Post-hoc comparisons between population pairs were made using the same approach, using Bonferroni-corrected p-values to account for multiple comparisons.

2.4.1. *Model*

While the package *drc* allows for a comparison of response curves among categorical variables (i.e. population), it does not contain an accessible functionality for investigating the effect of continuous variables (i.e. environmental temperature). In order to explore the effect of short-term temperature history on thermal tolerance through developmental plasticity, we created a separate statistical model, reparameterizing a conventional logistic regression model in terms of LT_{50}.

We defined $p_{jk}(x)$ as the survival probability at experimental temperature x of individuals collected at site j ($j = 1, 2, \ldots, J$) on day k ($k = 1, 2, \ldots, n_j$). The analysis was performed under the general model:

$$p_{jk}(x) = \frac{e^{a_{jk} \bullet \left(1 - \frac{x}{g_{jk}}\right)}}{1 + e^{a_{jk} \bullet \left(1 - \frac{x}{g_{jk}}\right)}} \tag{1}$$

where g_{jk} is the LT_{50} at site j for individuals collected on day k. Under this model, the parameters a_{jk} and g_{jk} represent the slope and LT_{50}, respectively (Fig. S1). This is analogous to the model used above in the R package *drc*. Indeed, both approaches yield near identical LT_{50} values (Fig. S2).

To assess the effect of environmental temperature on survival probability through developmental plasticity, we introduced a linear dependence of g (LT_{50}) on environmental temperature:

$$g_{jk} = c_{0j} + c_{1j} \bullet T_{jk} \tag{2}$$

such that g_{jk} is modulated by a constant c_{0j}, a plasticity parameter c_{1j}, and environmental temperature T_{jk}. c_1 is referred to as the plasticity parameter, as it relates the environmental temperature experienced by oozooids as embryos during development to heat tolerance post-settlement. Greater values of c_1 indicate greater levels of plasticity, with each increment in developmental temperature inducing a greater upward shift in thermal tolerance. c_0 has a limited biological interpretation (i.e. basal thermal tolerance at a developmental temperature of 0 °C) and is not a focus of our study. We defined T_{jk} as the mean temperature of the day prior to collection and chose this rather than an earlier day or longer time period because it would be less likely to impose biases in developmental stage among sites/collection days due to the temperature-dependence of development. Thus, the full model can be written as:

$$p_{jk}(x) = \frac{e^{a_{jk} \bullet \left(1 - \frac{x}{c_{0j} + c_{1j} \bullet T_{jk}}\right)}}{1 + e^{a_{jk} \bullet \left(1 - \frac{x}{c_{0j} + c_{1j} \bullet T_{jk}}\right)}} \tag{3}$$

We used a maximum likelihood approach to fit this model, estimating the parameters a_{jk}, c_{0jk}, and c_{1jk} for each combination of site and collection day. Because clutches born from adults collected on the same day experienced the same field temperatures, grouping by collection day provides the most granular perspective. We used the R package *bbmle* (Bolker, 2022) to perform maximum likelihood estimation, using its core function *mle2* with default parameters, apart from using Nelder-Mead as the optimization algorithm (Nelder and Mead, 1965).

Clutches from adults collected from CHAT and a small number of additional clutches were excluded from these analyses. Because CHAT is only represented by a single collection date, oozooids from this site all experienced the same field temperatures, precluding an analysis of how thermal tolerance is influenced by developmental temperature. Oozooids belonging to one clutch from an adult collected at RUTG on June 5th exhibited 100% survival across the experimental temperatures, precluding fitting a survivorship curve. Two clutches from adults collected at DARL on August 29th were also excluded, as there was only one experimental temperature of the five with intermediate survivorship (not 100% survival/mortality). This results in an inability to accurately estimate the *a* and *g* parameters. See Figs. S3–S4 for plots of raw data for these two collection dates. With these clutches excluded from the dataset, we used three separate tests to evaluate 1) the potential for local adaptation of thermal tolerance among sites, 2) whether short-term temperature history affects temperature tolerance through developmental plasticity, and 3) how the degree of developmental plasticity varies among sites. See Supplementary Materials and Fig. 2 for detail on null models used for these tests.

3. Results

3.1. *Environmental temperature*

While data was missing from SAND for 2022, sites mostly followed the expected latitudinal trend in environmental temperature, with more southern sites exhibiting higher mean daily temperatures over the course of the summer (Fig. 1b). The exception to this pattern is RUTG showing lower daily mean temperature than expected, falling in between two more northern populations on Cape Cod, FALM and CHAT.

Sites also differed in their degree of short-term (tidal or diel) temperature variability (Fig. 1c). Both CHAT and RUTG exhibit pronounced tidal fluctuations in temperature across the summer months, with mean daily ranges of 5.24 and 4.05 °C, respectively. DARL showed some tidal variation in temperature, though to a lesser extent (mean daily range of 1.84 °C). FALM exhibited the least short-term variability, fluctuating slightly over a diel period (mean daily range of 1.05 °C). SAND, while missing from the 2022 temperature data, showed pronounced tidal variability in the summer of 2021 (mean daily range of 3.42 °C), a pattern that is likely to hold across years (Fig. S5).

3.2. *Thermal tolerance experiment*

In total across the five sites, 3728 oozooids from 93 clutches were

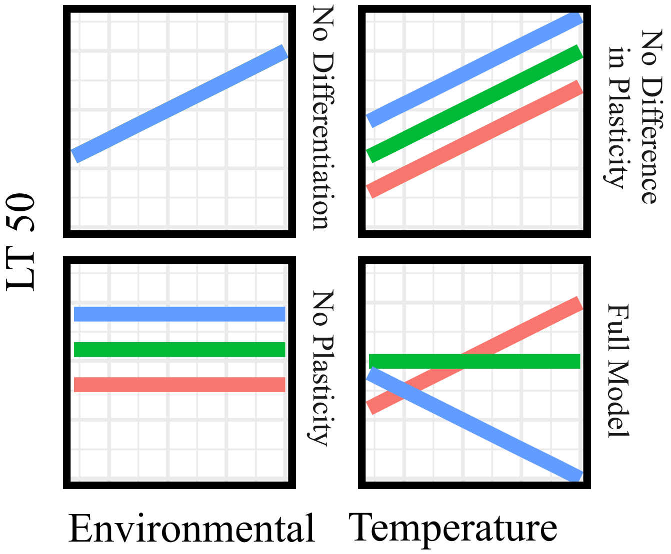

Fig. 2. Schematic of null and general statistical models. Upper left represents the null hypothesis that there is no differentiation among populations (i.e. single, global estimates of c_0 and c_1), and is depicted by three identical, overlapping lines. Lower left represents the null hypothesis that there is no phenotypic plasticity for thermal tolerance (i.e. population-specific estimates of c_0, c_1 globally fixed at 0). Upper right represents the null hypothesis that there is no difference among populations in their degree of phenotypic plasticity (i.e. population-specific estimates of c_0, a single, global estimate of c_1). The lower right depicts the general model, which allows each population to vary with respect to model parameters (population-specific estimates of c_0 and c_1). See Supplemental Materials for additional information on null model design.

included in 24 experiments across the summer (Table 1, Fig. S6). Using the R package *drc*, the LT_{50} estimates at each site ranged from 32.01 to 32.24 °C. A model including separate LT_{50} estimates for each population explained the data significantly better than a model with a jointly estimated LT_{50} value ($\chi^2(4) = 77.30$, $p = 6.66 \times 10^{-16}$), indicating a significant effect of site. Post-hoc comparisons revealed that sites in the Gulf of Maine (DARL and SAND) exhibited significantly lower LT_{50} values than sites further to the south (CHAT, FALM, and RUTG) (Fig. 3, Table S1).

After removing clutches from CHAT and from adults collected on June 5th at RUTG and August 29th at DARL, 2907 oozooids from 80 clutches across 21 collection days at four sites remained for investigating the effect of environmental temperature history on thermal tolerance using our statistical model. Plotting the estimated LT_{50} for each collection day against the mean environmental temperature the day prior to collection demonstrated a clear relationship between thermal tolerance and the temperature experienced during development (Fig. 4). All sites exhibited some degree of developmental plasticity, with warmer field temperatures prior to collection resulting in higher LT_{50} values. However, the degree of developmental plasticity, represented by the c_1 plasticity parameter in the model, varied by site (Fig. 5). Sites that experienced more short-term temperature variability exhibited higher estimates for c_1 (RUTG $c_1 = 0.184$; SAND $c_1 = 0.187$) and thus greater developmental plasticity for thermal tolerance. FALM, the site with the least short-term temperature variability ($c_1 = 0.070$), correspondingly exhibited the least developmental plasticity. DARL, with intermediate temperature variability, showed intermediate plasticity ($c_1 = 0.142$), though this estimate is subject to great uncertainty due to small sample size and a small range of field temperatures during the experimental period. In addition to LT_{50} being sensitive to environmental temperatures, survivorship curves also became steeper with increasing developmental temperatures, as indicated by upward shifts in the *a* parameter (Fig. 6).

A likelihood ratio test comparing our full model against a “no differentiation” null model (Fig. 2, upper left) in which c_0 and c_1 are fixed across populations revealed a significant effect of site ($\chi^2(6) = 227.43$, $p = 2.71 \times 10^{-46}$), indicating that each population’s thermal tolerances responded differently to environmental temperature. A second likelihood ratio test comparing our full model against a “no plasticity” null model (Fig. 2, lower left) in which c_0 is allowed to vary by population but c_1 is fixed at zero revealed a significant effect of environmental temperature history on thermal tolerance ($\chi^2(4) = 1072.58$, $p = 3.33 \times 10^{-231}$), indicating the presence of developmental plasticity. A third likelihood ratio test comparing our full model against a “no difference in plasticity” null model (Fig. 2, upper right) in which c_0 is allowed to vary by population but c_1 is shared among sites revealed that sites significantly differ in their degree of developmental plasticity ($\chi^2(3) = 373.24$, $p = 1.28 \times 10^{-80}$).

It is possible that our experimental design allowed for some preselection of embryos to occur. If elevated environmental temperatures were lethal to some heat-sensitive embryos during development, this would have biased our study to include more heat-tolerant individuals. Thus, it is conceivable that the observed pattern of higher thermal tolerance in response to greater environmental temperatures could be a result of environmentally imposed selection prior to experimentation. However, we find this unlikely, as we typically observed 100% survivorship at our lowest experimental temperature (~30.5 °C), which was significantly warmer than field temperatures.

4. Discussion

How local adaptation and phenotypic plasticity contribute to species’ ability to cope with spatiotemporal variation in temperature has broad implications for persistence in a rapidly changing environment (Donelson et al., 2019; Gunderson and Stillman, 2015; Hoffmann and Sgró, 2011; Seebacher et al., 2015; Somero, 2010). Here, we demonstrate that post-larvae (oozooids) of the invasive tunicate *Botryllus schlosseri* exhibit differentiation of thermal tolerance among populations and possess pronounced developmental plasticity. We also show that populations vary in their degrees of developmental plasticity and that this trend coincides with the extent of short-term environmental temperature variability, with populations in more variable environments exhibiting greater developmental plasticity. This observation demonstrates the potential for local adaptation of phenotypic plasticity in this system.

Species persistence in new or changing environments relies in large part upon an ability to shift ecologically relevant phenotypes through local adaptation, phenotypic plasticity, or both (Somero, 2010). However, how local adaptation and phenotypic plasticity interact to promote adaptive responses to novel conditions remains poorly understood (Ghalambor et al., 2007). Given the importance of thermal tolerance in shaping the biogeographic distributions of species (Sunday et al., 2012), our study highlights the importance of understanding the potential contributions of thermal adaptation and plasticity in the context of

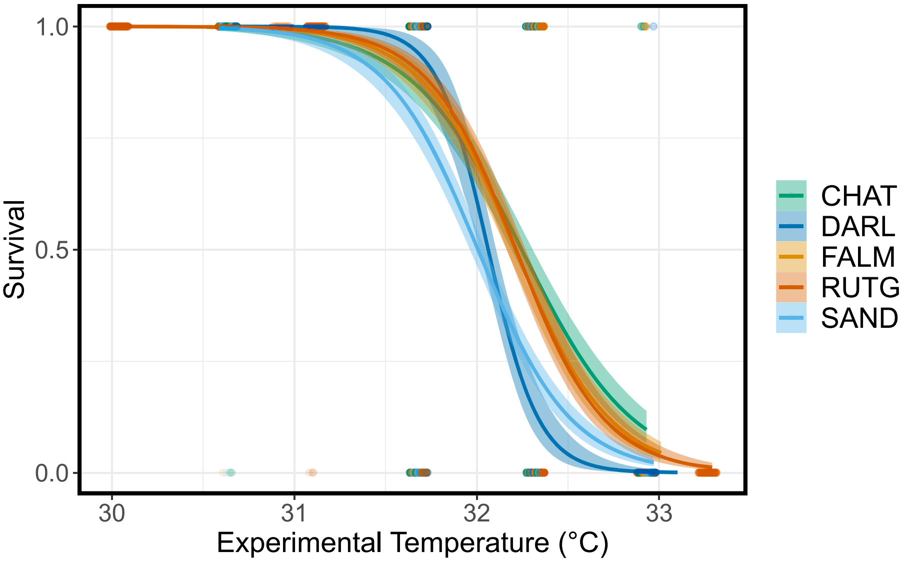

Fig. 3. Sites differ in thermal tolerance. Survivorship curves are displayed for each population, aggregated across all collection days. Curves derived by logistic regression. Shaded areas represent standard error.

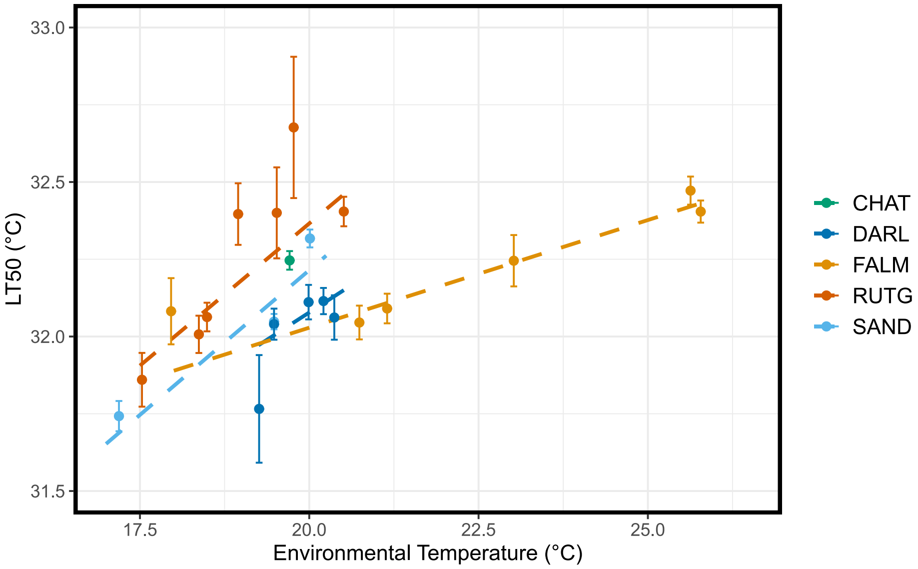

Fig. 4. Developmental plasticity results in upward shifts in thermal tolerance in response to higher environmental temperatures. LT_{50} is plotted against mean temperature of day prior to collection. Points represent the LT_{50} estimate across all clutches born on a particular day. Error bars represent 95% confidence intervals. For each population except CHAT, dashed lines represent the response of LT_{50} to environmental temperature, as given by $g = c_{0jk} + c_{1jk} * T_{jk}$, where j represents population, k represents collection day, and T_{jk} represents environmental temperature at site j on day k (see 2. Materials and Methods).

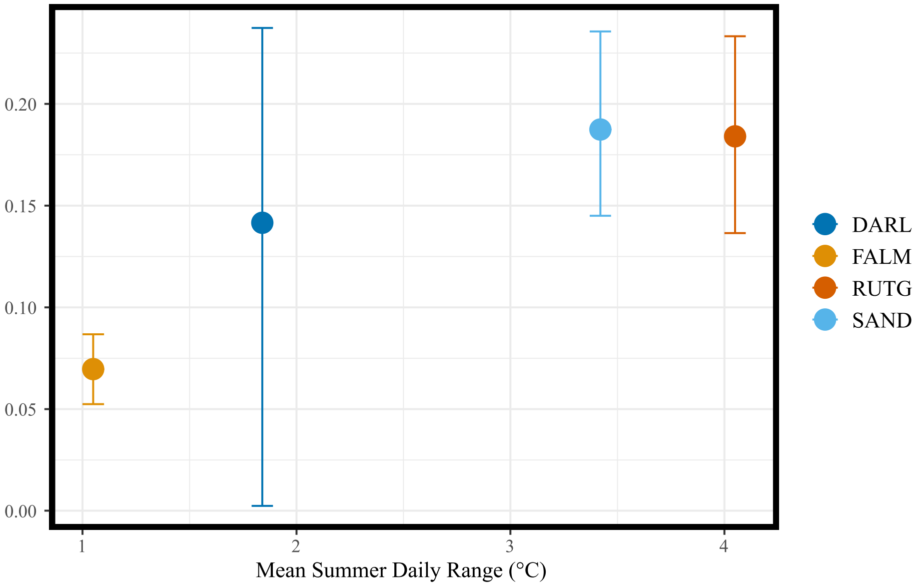

Fig. 5. The degree of developmental plasticity varies across sites according to degree of temperature variability. Estimates for c_1 parameter (plasticity) plotted as a function of mean daily range of summer temperatures at each site. Error bars represent 95% confidence intervals.

species invasions and global change.

4.1. Spatial variation of thermal tolerance

We found that populations differed in their thermal tolerance, and that these differences were broadly consistent with latitude. Populations south of the Gulf of Maine (CHAT, FALM, and RUTG) possessed higher LT_{50} values than those within it (SAND and DARL), matching prevailing environmental temperatures: the Gulf of Maine, bounded by Cape Cod and Nova Scotia, harbors cooler waters than those further south (Fig. 1). That we observe differentiation in thermal tolerance across this break, with populations clustering according to their latitudinal position relative to Cape Cod, may suggest local adaptation to temperature among populations of *B. schlosseri*. However, because individuals were taken from the wild and not lab-reared in a common garden, we cannot rule out an effect of environment. Indeed, our temporal data demonstrate a high degree of phenotypic plasticity for this trait (see below). Thus, the differences in the environmental temperatures at the times of collection at each site have likely also contributed to the differences we observe in temperature tolerance among populations.

There has been somewhat limited investigation of the thermal tolerance of *B. schlosseri*, with most studies focusing on a single population and time of year (Brunetti et al., 1980; Epelbaum et al., 2009). Sorte et al. (2011) found that adult colonies of *B. schlosseri* from Massachusetts had greater heat tolerance than those from California, consistent with higher summer temperatures in the northwest Atlantic. Interestingly, for both of their populations, adult LT_{50} values were lower than those reported here (Sorte et al., 2011). This suggests that adults

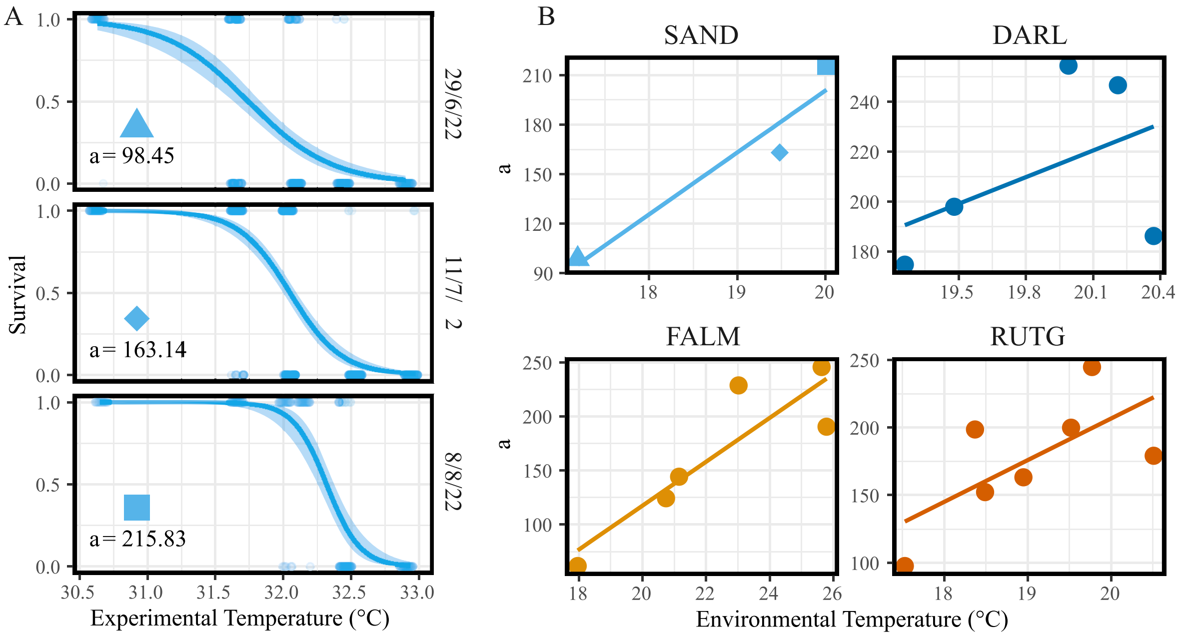

Fig. 6. Survivorship curves become steeper with increasing environmental temperature, as represented by upward shifts in the *a* parameter. (A) Survivorship curves for the three days of collection at SAND. As the summer progresses and temperatures rise, the curves steepen. Symbols (triangle, diamond, and square) denote each day of collection and are repeated in panel B. (B) Estimates for *a* increase with increasing environmental temperatures. Line represents linear regression of *a* against environmental temperature. Different axis scales have been used for each panel to highlight relationships within each site.

may be more sensitive to heat than oozooids, which is contrary to expectations (Pandori and Sorte, 2019). However, methodological differences, such as the length of heat exposure, which was slightly shorter in our study (20 vs. 24 h), could also contribute to the observed differences.

While the geographic differentiation of thermal tolerance could be an effect of the environment, *B. schlosseri* and ascidians more generally possess several characteristics that promote local adaptation. First, tunicates are considered to have extremely high rates of molecular evolution (Berna and Alvarez-Valin, 2014; Denoeud et al., 2010; Tsagkogeorga et al., 2010, 2012; Vinson et al., 2005). Second, ascidian populations tend to harbor remarkable levels of genetic diversity, even in putatively bottlenecked invasive populations (Reem et al., 2013; Rius et al., 2008; Zhan et al., 2015) (Tobias and Tepolt, in prep.). Such standing genetic diversity can serve as a pool of adaptive variation upon which natural selection can act to drive local adaptation (Barrett and Schluter, 2008). Third, ascidians tend to exhibit extremely restricted larval dispersal, with *B. schlosseri* larvae typically only settling on the scale of a few meters from their parent colony (Grosberg, 1987). This can result in markedly high levels of genetic differentiation across small spatial scales (Grosberg, 1987; Yund and O'Neil, 2000) (Tobias and Tepolt, in prep.), contributing to the high potential for evolutionary divergence of thermal tolerance in *B. schlosseri*.

Despite historical assumptions of the limited potential for local adaptation in marine systems, many recent studies have demonstrated local adaptation in marine organisms (DuBois et al., 2022; Kuo and Sanford, 2009; Pereira et al., 2017; reviewed in Sanford and Kelly, 2011). For example, in a multigenerational common garden experiment using the intertidal snail *Nucella canaliculata*, Kuo and Sanford (2009) demonstrated evolutionary divergence of upper thermal limits among populations. Beyond this single example, recent work has shown that the evolutionary differentiation of thermal limits is more pervasive in the marine environment than on land (Sasaki et al., 2022). Marine organisms, especially those that are sessile, generally have more limited capacity for behavioral thermoregulation and more restricted access to thermal refugia than terrestrial species (Antão et al., 2020; Pinsky et al., 2019; Sunday et al., 2015). This likely renders them more susceptible to natural selection, driving local adaptation of thermal physiology (Sasaki et al., 2022). This greater potential for thermal adaptation in the ocean has implications for marine species persistence under a changing climate. Evolutionary differences in thermal physiology among populations can confound predictions of distributional shifts under future climate scenarios, and render highly locally adapted species more susceptible to climatic changes (Bennett et al., 2019). Conversely, local adaptation to heterogeneous environments can promote persistence by preserving adaptive variation that can be redistributed via natural dispersal or programs of assisted gene flow (Aitken and Whitlock, 2013; Garant et al., 2007). Clearly, the consequences of local adaptation in the ocean for species persistence are complex and rely heavily on the potential for species to adapt on contemporary time scales.

4.2. *Developmental plasticity of thermal tolerance*

In addition to differences among sites, we found that the mean temperature of the day prior to collection had a strong effect on the measured thermal tolerance of *B. schlosseri* oozooids (Fig. 4). This demonstrates a clear effect of environment on thermal tolerance and suggests the presence of developmental plasticity in this system. Developmental plasticity may thus be a key mechanism by which *B. schlosseri* oozooids cope with temperature stress.

Developmental plasticity of thermal tolerance has been demonstrated in a wide variety of taxa and is especially pervasive in marine and aquatic organisms (Pottier et al., 2022). In a recent meta-analysis, Pottier et al. (2022) synthesized the findings of 150 experimental studies investigating developmental plasticity of thermal tolerance. Using acclimation response ratios (ARRs), or the change in thermal tolerance for a 1 °C increase in developmental temperature, they found that marine and aquatic animals tend to be much more plastic than terrestrial animals (mean ARR of 0.209 vs. 0.051). Our estimates of c_1, which is analogous to ARR, were 0.184 and 0.187 for RUTG and SAND, respectively, slightly lower but comparable to the mean ARR for marine and aquatic organisms found by Pottier et al. (2022). Given the pervasiveness of developmental plasticity of temperature tolerance in the marine realm, it is not altogether surprising that we observe this in *B. schlosseri*. However, our study represents an important first step in understanding this phenomenon in ascidians.

To our knowledge, our study comprises the first investigation of developmental plasticity of tolerance to any abiotic stressor, including temperature, in an ascidian. However, previous studies have investigated other types of phenotypic plasticity in ascidians (Chadwick-Furman and Weissman, 1995; Newlon et al., 2003; Renborg et al., 2014). For example, Renborg et al. (2014) demonstrated that sensitivity to salinity in embryos of *Ciona intestinalis* depended on the salinity of the parental environment prior to embryogenesis, establishing transgenerational plasticity of salinity tolerance. Given the long-standing consideration of the role for phenotypic plasticity in species invasions (see below) and the remarkable success of ascidians as an invasive taxon (Zhan et al., 2015), further study of phenotypic plasticity in ascidians is warranted.

Phenotypic plasticity has long been thought to play a major role in the success of invasive species (Baker, 1965; Davidson et al., 2011; Richards et al., 2006). Generalist species with broad ecological tolerances and pronounced phenotypic plasticity have been assumed to be ideal invaders (Daehler, 2003; Higgins and Richardson, 2014). This extends to thermophysiological phenotypes. For example, the widespread invasive European green crab, *Carcinus maenas*, has extreme thermal breadth, with limits far surpassing those of co-occurring native species (Tepolt and Somero, 2014). Additionally, it exhibits pronounced thermal phenotypic plasticity, shifting its upper and lower limits substantially in response to short-term acclimation. While its invasive status in unclear in the northwest Atlantic, *B. schlosseri* is an invasive species elsewhere and has established in waters spanning a vast thermal gradient. Its persistence across this broad gradient suggests a pronounced ability to cope with spatially variable environmental temperatures, whether through local adaptation, phenotypic plasticity, or both. Given our observations, it is likely that developmental plasticity has played some role in its ability to thrive across diverse habitats. Further study is warranted to investigate how other types of phenotypic plasticity, for example, acclimatization in adults or transgenerational plasticity, may also shape the thermal tolerance of this species.

While we ascribe temporal variation in thermal tolerance to phenotypic plasticity, it is worth noting that there may be a hereditary component as well. Fluctuating selection by environmental temperature may result in shifts in the genotypic composition of *B. schlosseri* populations, with more heat-sensitive genotypes produced earlier in the summer and more heat-tolerant genotypes later in the summer. While this may contribute to the patterns described at sites sampled over many months (e.g. FALM), because in some cases we are describing variation across limited duration (e.g. less than 2 weeks at RUTG), we consider it more likely that phenotypic plasticity plays a dominant role.

4.3. Potential for local adaptation of developmental plasticity

Theory predicts that populations in more variable environments should evolve greater phenotypic plasticity (Bradshaw, 1965; Levins, 1968; Schlichting, 1986). While evidence for this prediction for thermal tolerance is equivocal (see meta-analysis by Barley et al., 2021), we found that sites experiencing more short-term temperature variability harbored populations with greater degrees of developmental plasticity, consistent with local adaptation of phenotypic plasticity. Again, because the individuals used in these experiments were born from wild-collected adults, it is impossible to disentangle the effects of environment from heredity with our data. Nonetheless, given the high potential for local adaptation in this system (see above), it is likely that it plays some role in generating the patterns observed here.

Despite predictions for the evolution of phenotypic plasticity in variable environments, there are surprisingly few studies demonstrating local adaptation of phenotypic plasticity (Hendry, 2016). Many, like ours, relate differentiation in phenotypic plasticity to the degree of environmental heterogeneity experienced by populations (De Meester, 1996; Diaz et al., 2021; Gianoli and González-Teuber, 2005; Phillips et al., 2016; Reger et al., 2018; Richter-Boix et al., 2015). Others use experimental evolution approaches, comparing lineages reared in constant conditions to those reared in a variable environment (Condon et al., 2014; Leggett et al., 2013; Reboud and Bell, 1997). While not definitive, our results add to a growing body of literature demonstrating the potential for local adaptation of phenotypic plasticity in natural populations.

Notably, plasticity in one life stage can affect plasticity at later life stages (Beaman et al., 2016). For example, Healy et al. (2019) demonstrated that developmental temperature can affect acclimatory capacity at later life stages in the intertidal copepod *Tigriopus californicus*. Similarly, this cascading effect can also apply across generations through transgenerational plasticity (Donelson et al., 2018). If the adult *B. schlosseri* colonies from which larvae were collected experienced a warmer or more variable thermal environment during their lifetime, it may be advantageous for their larvae to exhibit developmental plasticity for temperature tolerance, generating a pattern like that which we found in our study. Reaction norm shape in offspring has been shown to depend in part on parental environment (Cavieres et al., 2019; Donelson et al., 2016; Salinas et al., 2013; Stein et al., 2018; Sturiale and Bailey, 2021; Wadgymar et al., 2018). Despite these examples, the relationship between transgenerational plasticity and within-generation plasticity remains understudied (Donelan et al., 2020; Donelson et al., 2018), but interactions between the two could play some role in our observation of increased developmental plasticity at sites with more short-term temperature variability. Manipulative multi-generational experiments would have to be performed to test this potential in more detail.

4.4. Steepening of survivorship curves and the limits of thermal tolerance

While the main metric of interest in our study was LT_{50}, we have also shown that the steepness of the survivorship curves, as contained within the *a* parameter in our model, increases with greater developmental temperature (Fig. 6). This steepening effect suggests that 1) development at elevated temperatures reduces inter-individual variation in thermal tolerance and 2) there may be relatively hard upper limits for thermal tolerance in this system. There is undoubtedly variation for thermal tolerance within populations of *B. schlosseri*: some oozooids survive at a particular experimental temperature while others die. It is apparent from this steepening observation that the capacity for developmental plasticity is not equal across individuals. Higher developmental temperatures seem to allow those oozooids with lower basal thermal tolerance to shift their probability of survival upwards, while those with higher basal thermal tolerance are more constrained, leading to a steepening of the survivorship curve. This observation is consistent with studies that demonstrate a negative relationship between basal thermal tolerance and thermal tolerance plasticity (Faulkner et al., 2014; Kelly et al., 2017; Morgan et al., 2020; Phillips et al., 2016; Sasaki and Dam, 2021, 2019; Stillman, 2003; but see Calosi et al., 2008), which may be indicative of a trade-off (Barley et al., 2021; van Heerwaarden and Kellermann, 2020).

Constraints on the plasticity and evolution of upper thermal limits appear to be common across taxa, while lower limits appear to be more labile (Gaston and Chown, 1999; Munoz et al., 2014; Phillips et al., 2016; van Heerwaarden et al., 2016). For example, in the tsetse fly, *Glossina pallidipes*, acclimation temperature had a pronounced effect on tolerance to cold temperatures, but upper thermal limits were less flexible (Terblanche and Chown, 2006). The meta-analysis by Pottier et al. (2022) found that while marine and aquatic organisms exhibit greater levels of developmental plasticity for heat tolerance, this shift is unlikely to perfectly compensate for anticipated levels of warming (Gunderson and Stillman, 2015). On an evolutionary scale, there may be strong phylogenetic constraints on upper thermal limit evolution (Kellermann et al., 2012). Several meta-analyses have demonstrated that while lower thermal limits vary with latitude, as would be expected through directional selection on temperature tolerance, upper thermal limits appear to be rather inflexible, again pointing towards the potential importance of evolutionary constraints on upper thermal limits (Araújo et al., 2013; Hoffmann et al., 2013; Sunday et al., 2019).

While we are not aware of studies that explicitly discuss this pattern of survivorship curve steepening, several have demonstrated this effect as a component of plasticity and/or adaptation of heat tolerance (Pereira et al., 2017; Rebolledo et al., 2021; Sasaki and Dam, 2019, 2021). For example, Sasaki and Dam (2019) compared thermal tolerance and its plasticity across populations of the coastal and estuarine copepod *Acartia tonsa*. At increased developmental temperatures, not only did LT_{50} increase, but so did the steepness of the survivorship curves (p. 4153, Fig. 2). Correspondingly, they showed that thermal limits (as reflected in LT_{10}, or the temperature inducing 10% survival) were less labile than LT_{50} (their Fig. S2); if limits shift less than midpoints in response to higher developmental temperatures, curves necessarily become steeper. This pattern was also observed in a related study investigating adaptation of thermal tolerance in *A. tonsa* via experimental evolution (Sasaki and Dam, 2021), suggesting that both plasticity and evolutionary adaptation can have this effect. While it seems there has been minimal attention on this phenomenon on survivorship curve steepening, these examples indicate that it may be widespread. Given its potential implications for the evolution and plasticity of upper thermal limits, further investigation is warranted.

5. Conclusions

We have shown that temperature tolerance in *B. schlosseri* oozooids varies by population across a latitudinal gradient, that tolerance is influenced environmental temperature through developmental plasticity, and that the degree of developmental plasticity varies according to the level of short-term temperature variability at each site. Together, these results demonstrate how local adaptation and phenotypic plasticity may underly spatiotemporal variation of temperature tolerance in this system. Importantly, our findings suggest the potential for local adaptation of developmental plasticity, with populations inhabiting more thermally variable sites exhibiting greater levels of plasticity. While further investigation using a multigenerational approach would be necessary to fully disentangle the relative contributions of heritable versus environmental effects, by taking advantage of natural spatiotemporal variability in temperature, our study documents important patterns of temperature tolerance that could have implications for species invasions and population persistence in an era of global change.

Organisms in all habitats are under increasing threat of climatic changes, but it is marine ectotherms that are considered most at risk with an increase in global temperatures (Pinsky et al., 2019). Many marine species are living near their upper thermal limits (Pinsky et al., 2019), and the rate of warming may exceed the ability of evolution and/or phenotypic plasticity to buffer species against its effects (Bennett et al., 2021; Gunderson and Stillman, 2015). Despite some ability to shift thermal tolerance upward in response to elevated temperatures, the steepening of survivorship curves observed here and in other studies suggests that there may be strong constraints on the plasticity and evolution of upper thermal limits in the ocean. In the absence of widespread adjustments to thermal niches, we may expect to see increasing rates of marine extirpation and range shifts (Donelson et al., 2019; Sunday et al., 2012). Indeed, it has been demonstrated that distributional shifts in the ocean track climate velocities more closely than on land (Lenoir et al., 2020). Understanding the interplay between evolutionary adaptation, phenotypic plasticity, and range shifts in response to climate warming is key to making predictions about species persistence in the modern ocean (Donelson et al., 2019).

Funding sources

This study was funded by a Woods Hole Oceanographic Institution Ocean Ventures Fund award to Z Tobias, an American Philosophical Society Lewis and Clark Fund for Exploration and Field Research grant to Z Tobias, and a National Defense Science and Engineering Graduate Fellowship awarded to Z Tobias.

CRediT authorship contribution statement

Zachary Tobias: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Andrew Solow:** Formal analysis, Writing – review & editing. **Carolyn Tepolt:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

We would like to thank all staff at the Rutgers University Marine Field Station and Darling Marine Center, in particular Roland Hagan and Tim Miller, for accommodating our research. We also thank Ann Tarrant for providing helpful comments on an earlier version of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103763.

References

Aitken, S.N., Whitlock, M.C., 2013. Assisted gene flow to facilitate local adaptation to climate change. Annu. Rev. Ecol. Evol. Syst. 44, 367–388. https://doi.org/10.1146/annurev-ecolsys-110512-135747.

Angilletta, M., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, Oxford. https://doi.org/10.1093/acprof:oso/9780198570875.001.1.

Antão, L.H., Bates, A.E., Blowes, S.A., Waldock, C., Supp, S.R., Magurran, A.E., Dornelas, M., Schipper, A.M., 2020. Temperature-related biodiversity change across temperate marine and terrestrial systems. Nat. Ecol. Evol. 4, 927–933. https://doi.org/10.1038/s41559-020-1185-7.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F., Chown, S.L., 2013. Heat freezes niche evolution. Ecol. Lett. 16, 1206–1219. https://doi.org/10.1111/ele.12155.

Baker, H.G., 1965. Characteristics and modes of origin of weeds. In: Stebbins, G.L., Baker, H.G. (Eds.), The Genetics of Colonizing Species. Academic Press, New York, pp. 147–172.

Barley, J.M., Cheng, B.S., Sasaki, M., Gignoux-Wolfsohn, S., Hays, C.G., Putnam, A.B., Sheth, S., Villeneuve, A.R., Kelly, M., 2021. Limited plasticity in thermally tolerant ectotherm populations: evidence for a trade-off. Proc. R. Soc. B Biol. Sci. 288 https://doi.org/10.1098/rspb.2021.0765.

Barrett, R.D.H., Schluter, D., 2008. Adaptation from standing genetic variation. Trends Ecol. Evol. 23, 38–44. https://doi.org/10.1016/j.tree.2007.09.008.

Beaman, J.E., White, C.R., Seebacher, F., 2016. Evolution of plasticity: mechanistic link between development and reversible acclimation. Trends Ecol. Evol. 31, 237–249.

Bennett, J.M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., Araújo, M.B., Algar, A.C., Clusella-Trullas, S., Hawkins, B.A., Keith, S.A., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Morales-Castilla, I., Olalla-Tárraga, M.Á., 2021. The evolution of critical thermal limits of life on Earth. Nat. Commun. 12, 1–9. https://doi.org/10.1038/s41467-021-21263-8.

Bennett, S., Duarte, C.M., Marbà, N., Wernberg, T., 2019. Integrating within-species variation in thermal physiology into climate change ecology. Philos. Trans. R. Soc. B Biol. Sci. 374 https://doi.org/10.1098/rstb.2018.0550.

Berna, L., Alvarez-Valin, F., 2014. Evolutionary genomics of fast evolving tunicates. Genome Biol. Evol. 6, 1724–1738.

Bolker, B., 2022. Bbmle: Tools for General Maximum Likelihood Estimation. R Packag version 1.0.25.

Bradshaw, A.D., 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13, 115–155.

Brunetti, R., Beghi, L., Bressan, M., Marin, M., 1980. Combined effects of temperature and salinity on colonies of *Botryllus schlosseri* and *Botrylloides leachi* (Ascidiacea) from the Venetian Lagoon. Mar. Ecol. Prog. Ser. 2, 303–314. https://doi.org/10.3354/meps002303.

Calosi, P., Bilton, D.T., Spicer, J.I., 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biol. Lett. 4, 99–102. https://doi.org/10.1098/rsbl.2007.0408.

Cavieres, G., Alruiz, J.M., Medina, N.R., Bogdanovich, J.M., Bozinovic, F., 2019. Transgenerational and within-generation plasticity shape thermal performance curves. Ecol. Evol. 9, 2072–2082.

Chadwick-Furman, N.E., Weissman, I.L., 1995. Life history plasticity in chimaeras of the colonial ascidian Botryllus schlosseri. Proc. R. Soc. B Biol. Sci. 262, 157–162. https://doi.org/10.1098/rspb.1995.0190.

Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta, M.J., Stenseth, N.C., Pertoldi, C., 2010. Adapting to climate change: a perspective from evolutionary physiology. Clim. Res. 43, 3–15. https://doi.org/10.3354/cr00879.

Collin, R., Rebolledo, A.P., Smith, E., Chan, K.Y.K., 2021. Thermal tolerance of early development predicts the realized thermal niche in marine ectotherms. Funct. Ecol. 35, 1679–1692. https://doi.org/10.1111/1365-2435.13850.

Condon, C., Cooper, B.S., Yeaman, S., Angilletta Jr., M.J., 2014. Temporal variation favors the evolution of generalists in experimental populations of Drosophila melanogaster. Evolution 68, 720–728.

Crickenberger, S., Walther, K., Marchant, S., Marko, P.B., Moran, A.L., 2015. Population-dependent acclimatization capacity of thermal tolerance in larvae of the rocky- shore barnacle *Pollicipes elegans*. Invertebr. Biol. 134, 291–302.

Daehler, C.C., 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annu. Rev. Ecol. Evol. Syst. 34, 183–211.

Davidson, A.M., Jennions, M., Nicotra, A.B., 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol. Lett. 14, 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x.

De Meester, L., 1996. Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, Daphnia magna. Evolution 50, 1293–1298.

Denoeud, F., Henriet, S., Mungpakdee, S., Aury, J.-M., Da Silva, C., Brinkmann, H., Mikhaleva, J., Olsen, L.C., Jubin, C., Cañestro, C., others, 2010. Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science 330, 1381–1385.

Deser, C., Alexander, M.A., Xie, S.-P., Phillips, A.S., 2010. Sea surface temperature variability: patterns and mechanisms. Ann. Rev. Mar. Sci 2, 115–143.

Diaz, F., Kuijper, B., Hoyle, R.B., Talamantes, N., Coleman, J.M., Matzkin, L.M., 2021. Environmental predictability drives adaptive within-and transgenerational plasticity of heat tolerance across life stages and climatic regions. Funct. Ecol. 35, 153–166.

Donelan, S.C., Hellmann, J.K., Bell, A.M., Luttbeg, B., Orrock, J.L., Sheriff, M.J., Sih, A., 2020. Transgenerational plasticity in human-altered environments. Trends Ecol. Evol. 35, 115–124. https://doi.org/10.1016/j.tree.2019.09.003.

Donelson, J.M., Salinas, S., Munday, P.L., Shama, L.N.S., 2018. Transgenerational plasticity and climate change experiments: where do we go from here? Global Change Biol. 24, 13–34. https://doi.org/10.1111/gcb.13903.

Donelson, J.M., Sunday, J.M., Figueira, W.F., Gaitán-Espitia, J.D., Hobday, A.J., Johnson, C.R., Leis, J.M., Ling, S.D., Marshall, D., Pandolfi, J.M., Pecl, G., Rodgers, G.G., Booth, D.J., Munday, P.L., 2019. Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. Philos. Trans. R. Soc. B Biol. Sci. 374 https://doi.org/10.1098/rstb.2018.0186.

Donelson, J.M., Wong, M., Booth, D.J., Munday, P.L., 2016. Transgenerational plasticity of reproduction depends on rate of warming across generations. Evol. Appl. 9, 1072–1081.

DuBois, K., Pollard, K.N., Kauffman, B.J., Williams, S.L., Stachowicz, J.J., 2022. Local adaptation in a marine foundation species: implications for resilience to future global change. Global Change Biol. 28, 2596–2610.

Epelbaum, A., Herborg, L.M., Therriault, T.W., Pearce, C.M., 2009. Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. J. Exp. Mar. Biol. Ecol. 369, 43–52. https://doi.org/10.1016/j.jembe.2008.10.028.

Fangue, N.A., Hofmeister, M., Schulte, P.M., 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. J. Exp. Biol. 209, 2859–2872.

Faulkner, K.T., Clusella-Trullas, S., Peck, L.S., Chown, S.L., 2014. Lack of coherence in the warming responses of marine crustaceans. Funct. Ecol. 28, 895–903. https://doi.org/10.1111/1365-2435.12219.

Foo, S.A., Byrne, M., 2016. Acclimatization and adaptive capacity of marine species in a changing ocean. In: Advances in Marine Biology, first ed. Elsevier Ltd. https://doi.org/10.1016/bs.amb.2016.06.001.

Garant, D., Forde, S.E., Hendry, A.P., 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Funct. Ecol. 434–443.

Gaston, K.J., Chown, S.L., 1999. Elevation and climatic tolerance: a test using dung beetles. Oikos 584–590.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21, 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x.

Gianoli, E., González-Teuber, M., 2005. Environmental heterogeneity and population differentiation in plasticity to drought in Convolvulus chilensis (Convolvulaceae). Evol. Ecol. 19, 603–613.

Grosberg, R.K., 1987. Limited dispersal and proximity-dependent mating success in the colonial ascidian Botryllus schlosseri. Evolution 41, 372–384. https://doi.org/10.2307/2409145.

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. R. Soc. B Biol. Sci. 282 https://doi.org/10.1098/rspb.2015.0401.

Healy, T.M., Bock, A.K., Burton, R.S., 2019. Variation in developmental temperature alters adulthood plasticity of thermal tolerance in Tigriopus californicus. J. Exp. Biol. 222 https://doi.org/10.1242/jeb.213405.

Hendry, A.P., 2016. Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. J. Hered. 107, 25–41.

Higgins, S.I., Richardson, D.M., 2014. Invasive plants have broader physiological niches. Proc. Natl. Acad. Sci. U.S.A. 111, 10610–10614. https://doi.org/10.1073/pnas.1406075111.

Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press.

Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct. Ecol. 27, 934–949. https://doi.org/10.1111/j.1365-2435.2012.02036.x.

Hoffmann, A.A., Sgró, C.M., 2011. Climate change and evolutionary adaptation. Nature 470, 479–485. https://doi.org/10.1038/nature09670.

Hofmann, G., Somero, G., 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel Mytilus trossulus. J. Exp. Biol. 198, 1509–1518. https://doi.org/10.1242/jeb.198.7.1509.

Kellermann, V., Overgaard, J., Hoffmann, A.A., Fljøgaard, C., Svenning, J.C., Loeschcke, V., 2012. Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. Proc. Natl. Acad. Sci. U.S.A. 109, 16228–16233. https://doi.org/10.1073/pnas.1207553109.

Kelly, M.W., Pankey, M.S., DeBiasse, M.B., Plachetzki, D.C., 2017. Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. Funct. Ecol. 31, 398–406. https://doi.org/10.1111/1365-2435.12725.

Kelly, M.W., Sanford, E., Grosberg, R.K., 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proc. R. Soc. B Biol. Sci. 279, 349–356. https://doi.org/10.1098/rspb.2011.0542.

Klockmann, M., Günter, F., Fischer, K., 2017. Heat resistance throughout ontogeny: body size constrains thermal tolerance. Global Change Biol. 23, 686–696. https://doi.org/10.1111/gcb.13407.

Komoroske, L.M., Connon, R.E., Lindberg, J., Cheng, B.S., Castillo, G., Hasenbein, M., Fangue, N.A., 2014. Ontogeny influences sensitivity to climate change stressors in an endangered fish. Conserv. Physiol. 2, 1–13. https://doi.org/10.1093/conphys/cou008.

Kuo, E.S.L., Sanford, E., 2009. Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. Mar. Ecol. Prog. Ser. 388, 137–146. https://doi.org/10.3354/meps08102.

Lacoursière-Roussel, A., Bock, D.G., Cristescu, M.E., Guichard, F., Girard, P., Legendre, P., McKindsey, C.W., 2012. Disentangling invasion processes in a dynamic shipping-boating network. Mol. Ecol. 21, 4227–4241. https://doi.org/10.1111/j.1365-294X.2012.05702.x.

Leggett, H.C., Benmayor, R., Hodgson, D.J., Buckling, A., 2013. Experimental evolution of adaptive phenotypic plasticity in a parasite. Curr. Biol. 23, 139–142.

Lenoir, J., Bertrand, R., Comte, L., Bourgeaud, L., Hattab, T., Murienne, J., Grenouillet, G., 2020. Species better track climate warming in the oceans than on land. Nat. Ecol. Evol. 4, 1044–1059. https://doi.org/10.1038/s41559-020-1198-2.

Levins, R., 1968. Evolution in Changing Environments: Some Theoretical Explorations. Princeton University Press.

Morgan, R., Finnøen, M.H., Jensen, H., Pélabon, C., Jutfelt, F., 2020. Low potential for evolutionary rescue from climate change in a tropical fish. Proc. Natl. Acad. Sci. U.S.A. 117, 33365–33372. https://doi.org/10.1073/PNAS.2011419117.

Morley, S.A., Martin, S.M., Bates, A.E., Clark, M.S., Ericson, J., Lamare, M., Peck, L.S., 2012. Spatial and temporal variation in the heat tolerance limits of two abundant Southern Ocean invertebrates. Mar. Ecol. Prog. Ser. 450, 81–92. https://doi.org/10.3354/meps09577.

Munoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A., Bakken, G.S., Losos, J.B., 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proc. R. Soc. B Biol. Sci. 281, 20132433.

Nelder, J.A., Mead, R., 1965. A simplex method for function minimization. Comput. J. 7, 308–313.

Newlon, A.W., Yund, P.O., Stewart-Savage, J., 2003. Phenotypic plasticity of reproductive effort in a colonial ascidian, Botryllus schlosseri. J. Exp. Zool. Part A Comp. Exp. Biol. 297, 180–188. https://doi.org/10.1002/jez.a.10244.

Padilla, D.K., Savedo, M.M., 2013. A systematic review of phenotypic plasticity in marine invertebrate and plant systems. Adv. Mar. Biol. 65, 67–94. https://doi.org/10.1016/B978-0-12-410498-3.00002-1.

Pandori, L.L.M., Sorte, C.J.B., 2019. The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. Oikos 128, 621–629. https://doi.org/10.1111/oik.05886.

Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. Mar. Freshw. Res. 62, 1015–1026.

Peck, L.S., Souster, T., Clark, M.S., 2013. Juveniles are more resistant to warming than adults in 4 species of antarctic marine invertebrates. PLoS One 8. https://doi.org/10.1371/journal.pone.0066033.

Pereira, R.J., Sasaki, M.C., Burton, R.S., 2017. Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. Proc. R. Soc. B Biol. Sci. 284 https://doi.org/10.1098/rspb.2017.0236.

Phillips, B.L., Muñoz, M.M., Hatcher, A., Macdonald, S.L., Llewelyn, J., Lucy, V., Moritz, C., 2016. Heat hardening in a tropical lizard: geographic variation explained by the predictability and variance in environmental temperatures. Funct. Ecol. 30, 1161–1168. https://doi.org/10.1111/1365-2435.12609.

Pincebourde, S., Casas, J., 2015. Warming tolerance across insect ontogeny: influence of joint shifts in microclimates and thermal limits. Ecology 96, 986–997. https://doi.org/10.1890/14-0744.1.

Pineda, M.C., McQuaid, C.D., Turon, X., López-Legentil, S., Ordóñez, V., Rius, M., 2012. Tough adults, frail babies: an analysis of stress sensitivity across early life-history stages of widely introduced marine invertebrates. PLoS One 7. https://doi.org/10.1371/journal.pone.0046672.

Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4.

Pottier, P., Burke, S., Zhang, R.Y., Noble, D.W.A., Schwanz, L.E., Drobniak, S.M., Nakagawa, S., 2022. Developmental plasticity in thermal tolerance: ontogenetic variation, persistence, and future directions. Ecol. Lett. 25, 2245–2268. https://doi.org/10.1111/ele.14083.

Przeslawski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Global Change Biol. 21, 2122–2140.

R Core Team, 2013. R: A Language and Environment for Statistical Computing.

Rebolledo, A.P., Sgrò, C.M., Monro, K., 2021. Thermal performance curves are shaped by prior thermal environment in early life. Front. Physiol. 12 https://doi.org/10.3389/fphys.2021.738338.

Reboud, X., Bell, G., 1997. Experimental evolution in Chlamydomonas. III. Evolution of specialist and generalist types in environments that vary in space and time. Heredity 78, 507–514.

Reem, E., Douek, J., Katzir, G., Rinkevich, B., 2013. Long-term population genetic structure of an invasive urochordate: the ascidian *Botryllus schlosseri*. Biol. Invasions 15, 225–241. https://doi.org/10.1007/s10530-012-0281-2.

Reger, J., Lind, M.I., Robinson, M.R., Beckerman, A.P., 2018. Predation drives local adaptation of phenotypic plasticity. Nat. Ecol. Evol. 2, 100–107.

Renborg, E., Johannesson, K., Havenhand, J., 2014. Variable salinity tolerance in ascidian larvae is primarily a plastic response to the parental environment. Evol. Ecol. 28, 561–572. https://doi.org/10.1007/s10682-013-9687-2.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J., Pigliucci, M., 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol. Lett. 9, 981–993. https://doi.org/10.1111/j.1461-0248.2006.00950.x.

Richter-Boix, A., Katzenberger, M., Duarte, H., Quintela, M., Tejedo, M., Laurila, A., 2015. Local divergence of thermal reaction norms among amphibian populations is affected by pond temperature variation. Evolution 69, 2210–2226.

Rinkevich, B., Porat, R., Goren, M., 1998. On the development and reproduction of Botryllus Schlosseri (Tunicata) colonies from the eastern mediterranean sea: plasticity of life history traits. Invertebr. Reprod. Dev. 34, 207–218. https://doi.org/10.1080/07924259.1998.9652655.

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, e0146021.

Rius, M., Pascual, M., Turon, X., 2008. Phylogeography of the widespread marine invader Microcosmus squamiger (Ascidiacea) reveals high genetic diversity of introduced populations and non-independent colonizations. Divers. Distrib. 14, 818–828.

Ruthsatz, K., Dausmann, K.H., Peck, M.A., Glos, J., 2022. Thermal tolerance and acclimation capacity in the European common frog (Rana temporaria) change throughout ontogeny. J. Exp. Zool. Part A Ecol. Integr. Physiol. 337, 477–490. https://doi.org/10.1002/jez.2582.

Salinas, S., Brown, S.C., Mangel, M., Munch, S.B., 2013. Non-genetic inheritance and changing environments. Non-Genetic Inherit. 1.

Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Ann. Rev. Mar. Sci 3, 509–535. https://doi.org/10.1146/annurev-marine-120709-142756.

Sasaki, M., Barley, J.M., Gignoux-Wolfsohn, S., Hays, C.G., Kelly, M.W., Putnam, A.B., Sheth, S.N., Villeneuve, A.R., Cheng, B.S., 2022. Greater evolutionary divergence of thermal limits within marine than terrestrial species. Nat. Clim. Change 1–6.

Sasaki, M.C., Dam, H.G., 2021. Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. Evol. Appl. 14, 2114–2123. https://doi.org/10.1111/eva.13270.

Sasaki, M.C., Dam, H.G., 2020. Genetic differentiation underlies seasonal variation in thermal tolerance, body size, and plasticity in a short-lived copepod. Ecol. Evol. 10, 12200–12210. https://doi.org/10.1002/ece3.6851.

Sasaki, M.C., Dam, H.G., 2019. Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. Global Change Biol. 25, 4147–4164. https://doi.org/10.1111/gcb.14811.

Schlichting, C.D., 1986. The evolution of phenotypic plasticity in plants. Annu. Rev. Ecol. Systemat. 17, 667–693.

Seebacher, F., White, C.R., Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat. Clim. Change 5, 61–66.

Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine “winners” and “losers.”. J. Exp. Biol. 213, 912–920. https://doi.org/10.1242/jeb.037473.

Sorte, C.J.B., Jones, S.J., Miller, L.P., 2011. Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. J. Exp. Mar. Biol. Ecol. 400, 209–217. https://doi.org/10.1016/j.jembe.2011.02.009.

Stein, L.R., Bukhari, S.A., Bell, A.M., 2018. Personal and transgenerational cues are nonadditive at the phenotypic and molecular level. Nat. Ecol. Evol. 2, 1306–1311. https://doi.org/10.1038/s41559-018-0605-4.

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.

Sturiale, S.L., Bailey, N.W., 2021. Within-generation and Transgenerational Social Plasticity Interact during Rapid Adaptive Evolution bioRxiv 2010–2021.

Sunday, J., Bennett, J.M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A.L., Leiva, F.P., Verberk, W.C.E.P., Olalla-Tárraga, M.Á., Morales-Castilla, I., 2019. Thermal tolerance patterns across latitude and elevation. Philos. Trans. R. Soc. B Biol. Sci. 374 https://doi.org/10.1098/rstb.2019.0036.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. Nat. Clim. Change 2, 686–690. https://doi.org/10.1038/nclimate1539.

Sunday, J.M., Pecl, G.T., Frusher, S., Hobday, A.J., Hill, N., Holbrook, N.J., Edgar, G.J., Stuart-Smith, R., Barrett, N., Wernberg, T., Watson, R.A., Smale, D.A., Fulton, E.A., Slawinski, D., Feng, M., Radford, B.T., Thompson, P.A., Bates, A.E., 2015. Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. Ecol. Lett. 18, 944–953. https://doi.org/10.1111/ele.12474.

Tangwancharoen, S., Burton, R.S., 2014. Early life stages are not always the most sensitive: heat stress responses in the copepod Tigriopus californicus. Mar. Ecol. Prog. Ser. 517, 75–83. https://doi.org/10.3354/meps11013.

Tepolt, C.K., Somero, G.N., 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas. J. Exp. Biol. 217, 1129–1138. https://doi.org/10.1242/jeb.093849.

Terblanche, J.S., Chown, S.L., 2006. The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, Glossina pallidipes (Diptera, Glossinidae). J. Exp. Biol. 209, 1064–1073. https://doi.org/10.1242/jeb.02129.

Thiel, M., Gutow, L., 2005. The ecology of rafting in the marine environment. II. The rafting organisms and community. Oceanogr. Mar. Biol. https://doi.org/10.1201/9781420037449.ch7.

Tsagkogeorga, G., Cahais, V., Galtier, N., 2012. The population genomics of a fast evolver: high levels of diversity, functional constraint, andmolecular adaptation in the tunicate Ciona intestinalis. Genome Biol. Evol. 4, 740–749. https://doi.org/10.1093/gbe/evs054.

Tsagkogeorga, G., Turon, X., Galtier, N., Douzery, E.J.P., Delsuc, F., 2010. Accelerated evolutionary rate of housekeeping genes in tunicates. J. Mol. Evol. 71, 153–167.

van Heerwaarden, B., Kellermann, V., 2020. Does plasticity trade off with basal heat tolerance? Trends Ecol. Evol. 35, 874–885. https://doi.org/10.1016/j.tree.2020.05.006.

van Heerwaarden, B., Kellermann, V., Sgrò, C.M., 2016. Limited scope for plasticity to increase upper thermal limits. Funct. Ecol. 30, 1947–1956. https://doi.org/10.1111/1365-2435.12687.

Vinson, J.P., Jaffe, D.B., O'Neill, K., Karlsson, E.K., Stange-Thomann, N., Anderson, S., Mesirov, J.P., Satoh, N., Satou, Y., Nusbaum, C., others, 2005. Assembly of polymorphic genomes: algorithms and application to Ciona savignyi. Genome Res. 15, 1127–1135.

Wadgymar, S.M., Mactavish, R.M., Anderson, J.T., 2018. Transgenerational and within-generation plasticity in response to climate change: insights from a manipulative field experiment across an elevational gradient. Am. Nat. 192, 698–714.

West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution. Oxford University Press, Oxford, U.K.

Worcester, S.E., 1994. Adult rafting versus larval swimming: dispersal and recruitment of a botryllid ascidian on eelgrass. Mar. Biol. 121, 309–317.

Yund, P.O., O'Neil, P.G., 2000. Microgeographic genetic differentiation in a colonial ascidian (*Botryllus schlosseri*) population. Mar. Biol. 137, 583–588. https://doi.org/10.1007/s002270000378.

Zhan, A., Briski, E., Bock, D.G., Ghabooli, S., MacIsaac, H.J., 2015. Ascidians as models for studying invasion success. Mar. Biol. 162, 2449–2470. https://doi.org/10.1007/s00227-015-2734-5.

Zippay, M.L., Hofmann, G.E., 2010. Physiological tolerances across latitudes: thermal sensitivity of larval marine snails (Nucella spp.). Mar. Biol. 157, 707–714. https://doi.org/10.1007/s00227-009-1354-3.