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Micro-scale environmental variation amplifies physiological variation among individual mussels

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The contributions of temporal and spatial environmental variation to physiological variation remain poorly resolved. Rocky intertidal zone populations are subjected to thermal variation over the tidal cycle, superimposed with micro-scale variation in individuals' body temperatures. Using the sea mussel (*Mytilus californianus*), we assessed the consequences of this micro-scale environmental variation for physiological variation among individuals, first by examining the latter in field-acclimatized animals, second by abolishing micro-scale environmental variation via common garden acclimation, and third by restoring this variation using a reciprocal outplant approach. Common garden acclimation reduced the magnitude of variation in tissue-level antioxidant capacities by approximately 30% among mussels from a wave-protected (warm) site, but it had no effect on antioxidant variation among mussels from a wave-exposed (cool) site. The field-acclimatized level of antioxidant variation was restored only when protected-site mussels were outplanted to a high, thermally stressful site. Variation in organismal oxygen consumption rates reflected antioxidant patterns, decreasing dramatically among protected-site mussels after common gardening. These results suggest a highly plastic relationship between individuals' genotypes and their physiological phenotypes that depends on recent environmental experience. Corresponding context-dependent changes in the physiological mean–variance relationships within populations complicate prediction of responses to shifts in environmental variability that are anticipated with global change.

1. Introduction

Understanding species' physiological capacities to cope with variation in environmental conditions is pivotal when predicting responses to global change [1]. Mean temperatures and the frequency of extreme weather events are predicted to continue increasing [2,3], likely reducing the mean performance of species [4,5]. Such global trends will interact with regional factors, such as spatial variation in climatic patterns or the timing of low tides [6,7], in sometimes complex and non-linear ways [8]. In addition, evolutionary responses to environmental change can act over short time scales once thought to be the exclusive realm of ecological interactions and/or phenotypic plasticity [9,10], with important implications for species' abilities to resist and adapt to global change. Further complexity arises when considering the often substantial degree of micro-scale variation in how individual organisms experience their environment [4,11], a pattern that likely interacts with genetic variation to contribute to variation among individuals in the physiological capacity to cope with environmental variation. In the context of thermal stress, such considerations have fostered a renewed emphasis on the causes and consequences of spatial and temporal patterns of variation in body temperature *per se*, in lieu of reliance on habitat temperatures and 'climate envelopes' [12,13].

Progress in delineating the consequences of micro-scale environmental variation requires functional analysis at the appropriate scale of individual organisms

[14,15], because the outcome for each individual likely depends on how its unique microhabitat conditions and past experience interact with the genomic, physiological and behavioural tools it has at its disposal [16]. At the most fundamental level, micro-scale environmental variation represents a potentially critical caveat to the conclusion that a number of species currently live close to their thermal tolerance limits [17]; rather, only a small subset of individuals are likely to do so [18]. It is also reasonable to expect that this variation could help to maintain expression of physiological variation within populations [11,19]. Particularly important from an evolutionary perspective is a deeper understanding of the amount of functional (i.e. physiological) variation within populations and the relative contributions of fixed effects (i.e. genetics or developmental constraint) as opposed to plastic phenotypic responses [20,21].

Rocky intertidal zone populations offer one tractable system for addressing these issues [22]. Inhabitants of this zone are routinely subjected to episodic environmental stress at low tide, including thermal stress, hypoxia and desiccation. This dynamic variation is superimposed by significant within-site variation in body temperatures [23]. For example, variation in body temperature among mussels (*Mytilus californianus*) within a single bed often exceeds between-bed and latitudinal-scale mean differences [18]. Ranges in body temperature of 10–15°C have been recorded over 1–2 m^2 of intertidal rock on the warmest days, and the average range of maximum daily body temperatures exceeds 5°C [18]. The consequences of this persistent, micro-scale environmental variation for individuals' physiological status remain largely under-studied. Notably, comparisons of populations from throughout the range of *M. californianus* have generated little genetic evidence for large-scale population structure [24,25], but instead indicate substantial variation in thermal physiology within populations. For example, within-population ranges of more than 8°C in critical heart rate temperature persist after common gardening [26]. Such small-scale physiological variation could arise from fixed genetic differences and/or microhabitat-induced developmental effects, and it is likely modified via acclimatization to recent environmental variation (i.e. physiological plasticity). Mussels are notable for their plasticity, at time scales ranging from hours to months [27,28].

Here, we test whether micro-scale environmental variation amplifies physiological variation among individual mussels, first by quantifying the degree of physiological variation among field-acclimatized animals, second by abolishing micro-scale environmental variation via acclimation to benign common garden conditions, and third by restoring this environmental variation using a reciprocal outplant approach to either a high, stressful site or a low, relatively benign site following common garden acclimation. If the magnitude of physiological variation decreases after common gardening, but is restored upon return to a variable environment, this would demonstrate a role for micro-scale environmental variation in generating physiological variation. The reciprocal outplant approach allows us to test whether micro-scale environmental variation alone is sufficient to magnify physiological variation among individuals, as opposed to specific combinations of environmental mean and variance. Lastly, we begin to test the generality of the responses by examining mussels from both a wave-protected (warm) site and a wave-exposed (cool) site.

We focus on the capacity to combat environmental stress, defined as any environmentally mediated disruption of macromolecular integrity that threatens or yields a shift away from homeostasis (*sensu* [29]). Environmental stress tolerance is thought to play a key role in setting biogeographic boundaries and driving evolutionary patterns on broad spatial and temporal scales [30,31]. Inter-individual variation in environmental stress tolerance, if heritable, would provide the substrate for selection over smaller scales as well. We specifically quantify the capacity to prevent oxidative stress, which results from an imbalance between the rate of production of reactive oxygen species (ROS) and the cell's antioxidant capacity. The rate of production of ROS is inherently linked to metabolism [32,33], and it tends to increase when organisms are pushed towards their thermal limits and/or when they are exposed to cycles of hypoxia and reoxygenation [32–35]. Although mussels' physiologies appear well suited to the oxidative stress potentially engendered by their intermittently warm and hypoxic lifestyle (coinciding when the shell valves are closed at low tide; [36,37]), available data support a role for variation in susceptibility to oxidative stress in setting different thermal tolerance limits among *Mytilus* congeners [38,39]. We quantify biochemical indices of variation among individuals in the antioxidant capacity to cope with sublethal thermal stress. Even though lethal temperature events are being documented at increasing frequency (e.g. [40]), these episodes are still rare in most intertidal systems [18,41]. On a much more frequent basis, intertidal animals experience sublethal body temperature fluctuations that can challenge cellular redox balance [32,42]. Lastly, we measure oxygen consumption rates on the same individuals to gain a complementary, organismal view of variation among individuals in oxygen utilization. Our results indicate context-dependent interactions between physiological and environmental variation that likely complicate prediction of the outcomes of global change.

2. Material and methods

(a) Exposed (cool) and protected (warm) collection sites

We collected adult *M. californianus* at Hopkins Marine Station in Pacific Grove, CA (36.6178° N, 121.9167° W) from two intertidal sites situated at similar heights and separated by 24 m. The first was a north-facing, wave-exposed site, which experienced more wave splash and reduced solar irradiance, resulting in generally cooler body temperatures and slightly longer feeding times ('exposed'). The southwest-facing, wave-protected site experienced less wave splash and more direct irradiance, contributing to warmer body temperatures ('protected'). See [18,43] for data characterizing the environment at each site. Differences exist between these sites in mean activities of antioxidant and aerobic enzymes [43]. All experiments were conducted on individuals of similar size (approx. 60–70 mm shell height) in order to minimize confounding effects of body size [43]. Nonetheless, we included body size as a covariate in our analyses.

(b) Field-acclimatized, common garden and outplant treatment groups

In order to adequately quantify the magnitude of variation among individuals, we used sample sizes of 10–21 individual mussels per treatment group for antioxidant capacity assays. A survey of more than 20 publications that evaluated inter-individual variation in physiological measures (including studies reviewed in [44]) indicated a median sample size of 22 individuals (range 6–190). The choice of sample size represented a trade-off between sufficiently

capturing this variation and the need to conduct nine biochemical assays per individual.

We compared both the mean physiological status and the magnitude of physiological variation among four treatment groups per site of origin (electronic supplementary material, figure S1). The first treatment group of mussels was collected directly from each site ('field-acclimatized'; $n = 62$ exposed, $n = 61$ protected), and immediately exposed to an acute thermal challenge, wherein one-third of the mussels were sampled at each time point (see below). An additional group of 210 mussels was collected on the same day and brought into the laboratory for common gardening for 28 days. Mussels were housed outdoors under natural photoperiod in acrylic aquaria supplied with flow-through seawater. The average temperature was $13.5 \pm 1^{\circ}$C. Mussels were fed every day with a mixed phytoplankton diet (Shellfish Diet 1800, Reed Mariculture). After 28 days of common gardening, mussels from each site were further subdivided into three additional treatment groups. The first group ('common garden'; $n = 41$ exposed, $n = 30$ protected) was sampled at the three time points of the acute thermal challenge. The remaining mussels were returned to the field at either a low intertidal site ('outplant-low'; $n = 33$ exposed, $n = 28$ protected) or an adjacent high intertidal site ('outplant-high'; $n = 37$ exposed, $n = 36$ protected). Mussels in the outplant groups were attached at a fixed orientation to acrylic plates. These plates were then affixed to the intertidal rock substrate with marine epoxy. This protocol abrogated any behavioural contributions to micro-scale environmental variation during the outplant period, such as changing orientation to solar irradiance. To each plate, we attached silicone-filled mussel shells containing dataloggers [18] to record estimates of body temperatures every 20 min ($n = 5$ outplant-high, $n = 3$ outplant-low). The outplant groups were left in the field for 28 days, after which they were immediately exposed to the acute thermal challenge. Owing to sample size considerations, outplant mussels were sampled only at the baseline and top of the heat ramp.

(c) Acute thermal challenge

In order to examine whether physiological variation among individuals changes in response to single episodes of acute stress, mussels were sampled at three time points on a heat ramp. 'Baseline' mussels were taken out of aquaria (common garden) or directly from the field as the tide was receding (field-acclimatized, outplant), and tissues were immediately harvested. The remaining mussels were placed in a humidity-controlled neonatal incubator (Ohio Care Plus) and the air temperature was increased from the ambient temperature of 20°C by 3°C every 30 min until reaching 33°C, where temperature was held for an additional 30 min. The peak temperature of 33°C is known to induce sublethal stress for this species [28,36]. A subset of the mussels was sampled at the peak temperature ('top'). The remaining mussels were returned to flow-through aquaria at 13–14°C and left to recover for 4 h before sampling ('recovery'). At each time point, gill, posterior adductor muscle and mantle (the non-gonadal component lining the distal edges of the shell valves) were harvested, frozen in liquid nitrogen and stored at −80°C until processing.

(d) Antioxidant capacity assays

Regulation of antioxidant mechanisms can vary among tissues [45]. Therefore, to better assess organism-wide antioxidant status, we measured catalase enzymatic activity against hydrogen peroxide (H_2O_2), antioxidant capacity against peroxyl radicals ($ROO^{\cdot}$) and antioxidant capacity against hydroxyl radicals ($OH^{\cdot}$) in three tissues with different metabolic frameworks and organismal functions: gill (aerobic, site of gas exchange), adductor muscle (anaerobic, contractile activity important for anti-predator behaviour) and mantle (primarily aerobic, responsible for deposition of new shell growth). Further details about these antioxidant capacity measurements are included in the electronic supplementary material.

(e) Multivariate comparisons of mean antioxidant status and the magnitude of antioxidant variation

The antioxidant capacity data were first analysed using univariate analysis of covariance, with body mass as the covariate, in SPSS (v. 20). Site of origin, treatment group and thermal challenge time point were included as factors in the model. Only catalase activities of muscle and mantle were found to be influenced by body mass (electronic supplementary material, table S1); these values were mass-corrected before conducting the multivariate analyses.

We then used principal components analysis (PCA) to transform z-scores of the nine measures of antioxidant capacity for each individual (3 tissues × 3 assays) into three orthogonal latent variables (the first three principal components) that could be visualized and interpreted more easily. These three axes accounted for approximately 52% of the overall variance in organismal antioxidant status (figure 1).

Using all nine dimensions of the coordinates generated by the PCA, the mean antioxidant status (i.e. the mean location in multidimensional space) then was compared among treatments using a non-parametric, permutation-based, multivariate analysis of variance with 10 000 permutations [46]. The magnitude of antioxidant variation among individuals was examined with an analogous, non-parametric method that compared each treatment group's mean Euclidean distance of individuals from that group's spatial centroid, a measure of multivariate dispersion [47]. This technique is analogous to comparing variances prior to conducting an ANOVA. For each type of test, all possible pairwise *post hoc* comparisons among the 20 sampling groups were generated. Given the large number of pairwise tests, the resulting p-values were adjusted using a false discovery rate (FDR) approach [48]. The FDR-adjusted, pairwise p-value equivalents (q-values in the FDR nomenclature; [48]) are presented below and in the electronic supplementary material. These analyses were conducted in MATLAB (v. R2011a) using the *Fathom* package [49] and the bioinformatics toolbox.

(f) Whole-animal metabolic rates

Mussels' aerobic metabolic rates ($n = 84$ exposed, $n = 42$ protected) were measured one week after collection (day 7), when we assumed that individuals retained similar physiological status to the field-acclimatized state, and again at the end of the common garden phase of the study (day 28). We used closed-chamber respirometry to estimate each individual's metabolic rate on both occasions. Individual mussels were placed in 125 ml glass jars within larger, flow-through aquaria and allowed to acclimate overnight prior to measurement. At the beginning of each metabolic trial, jars were sealed with a lid containing a pierceable rubber septum. A needle-shaped, fibre-optic oxygen probe (Hypotube with NeoFox spectrometer, Ocean Optics) was used to measure oxygen concentration in the seawater. Three to four measurements were taken for each individual over approximately 2 h. Total metabolic rates were estimated using the slope of oxygen decrease over time. Metabolic rate data were analysed using a general linear mixed-effects model in R [50], where site of origin, log of body mass and time were included as fixed effects. Mussel ID was included as a random effect to account for repeated measurements on the same individuals. Statistical significance of each term was assessed with likelihood ratio tests. After controlling for these variables in the statistical model, we used the predicted values for each individual to compare the magnitude of variation in individuals' metabolic rates between origin sites and between time points using a Levene's test.

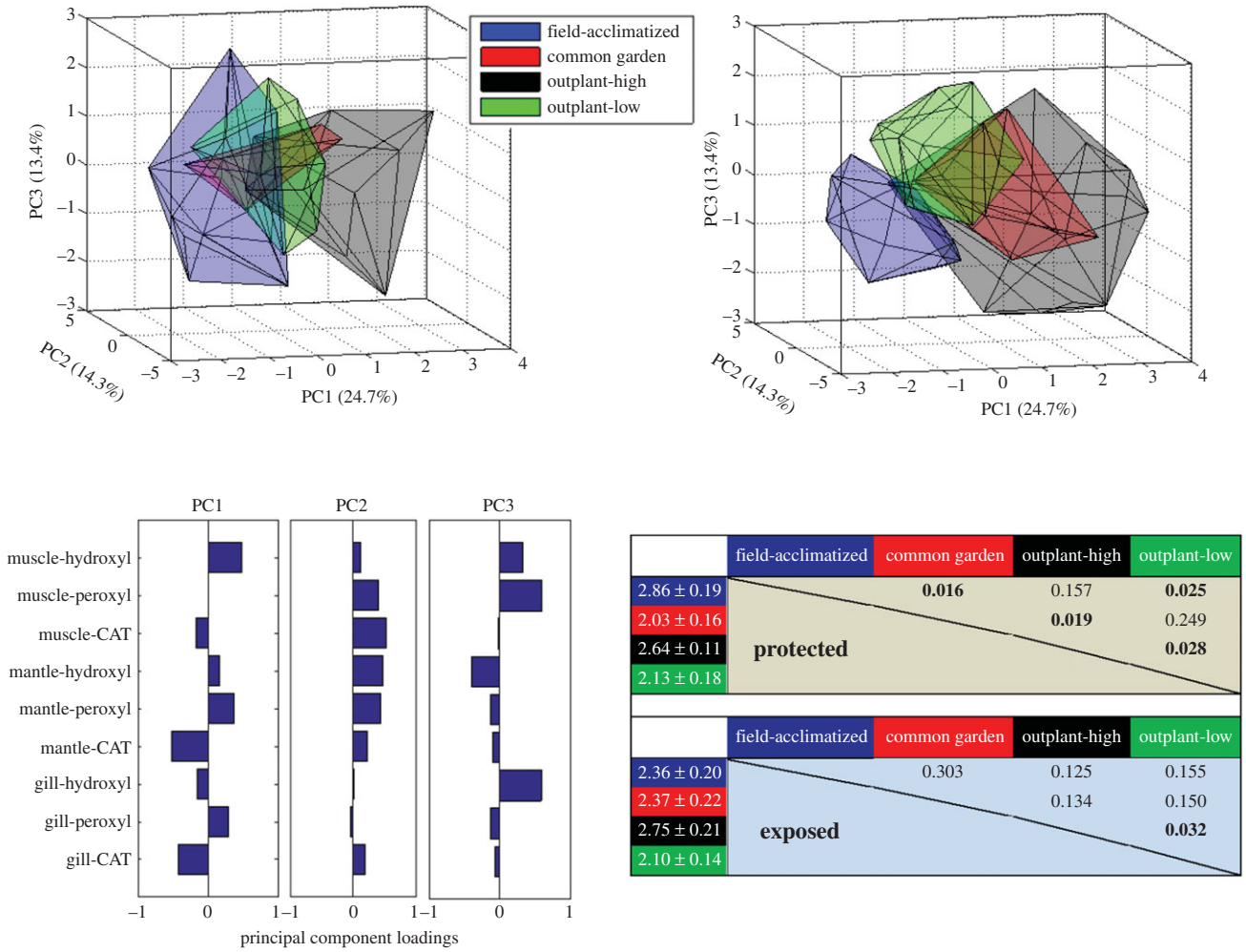

Figure 1. Mussels from both an exposed (cool) and a protected (warm) site were subjected to four treatments that manipulated micro-scale environmental variation. See electronic supplementary material, figure S1, for additional details of the experimental design. (*a*,*b*) Principal component plots illustrating variation among individual mussels in antioxidant status at the baseline of the heat ramp in the acute thermal challenge. Individuals are represented by vertices at the periphery of each multidimensional space. The three axes represent the first three principal component dimensions and their contribution to the overall variation among individuals. The volume of each multidimensional space is proportional to the amount of variation among individuals. (*a*) Antioxidant variation among mussels originally from the protected site decreased in the common-garden and outplant-low treatments relative to the field-acclimatized and outplant-high treatments. (*b*) For mussels originating from the exposed site, variation among mussels from the outplant-high and outplant-low treatments differed significantly ($p = 0.032$). This statistical difference is driven by a single individual in the outplant-high group. (*c*) PCA loadings for each of the nine measurements of antioxidant capacity on the first three principal component dimensions, which explained 52% of the overall variation in antioxidant status among individuals. CAT, catalase activity. (*d*) Statistical results (*p*-values) for pairwise comparisons of multivariate dispersion between treatment groups within each site. Numbers down the left side represent the mean distance ($\pm$s.e.m.) in principal component space to the group centroid for each colour-coded treatment. Bold font indicates statistical significance at $p < 0.05$.

3. Results

(a) Antioxidant status

Field-acclimatized mussels from the exposed and protected sites were physiologically indistinguishable from each other, exhibiting the same mean antioxidant status ($p = 0.096$) and comparable magnitudes of antioxidant variation among individuals ($p = 0.078$). However, groups of mussels from the two sites responded differently to the subsequent treatments.

In nearly every case, common garden acclimation and subsequent outplanting of mussels to either the high or low site generated changes in the mean antioxidant status (electronic supplementary material, figure S2 and table S2). We interpret such mean shifts as physiological plasticity at the population level. In general, these shifts followed a qualitatively consistent pattern, with the mean position shifting primarily along the first principal component axis from field-acclimatized to common-gardened groups. Outplanting to the high, stressful site tended to exaggerate the displacement from the original, field-acclimatized physiological mean, whereas outplanting to the low site tended to shift physiology back towards the field-acclimatized state, with the exception of protected-site mussels at the baseline of the heat ramp (electronic supplementary material, figure S2). Notably, even the outplant-low treatments failed to fully restore mean antioxidant status to the original, field-acclimatized levels (exposed and protected, baseline $p = 0.001$; exposed and protected, top $p < 0.001$; electronic supplementary material, figure S2). Displacements in mean antioxidant status were larger in magnitude among treatments than they were among timepoints of the acute thermal challenge within any given treatment (data not shown).

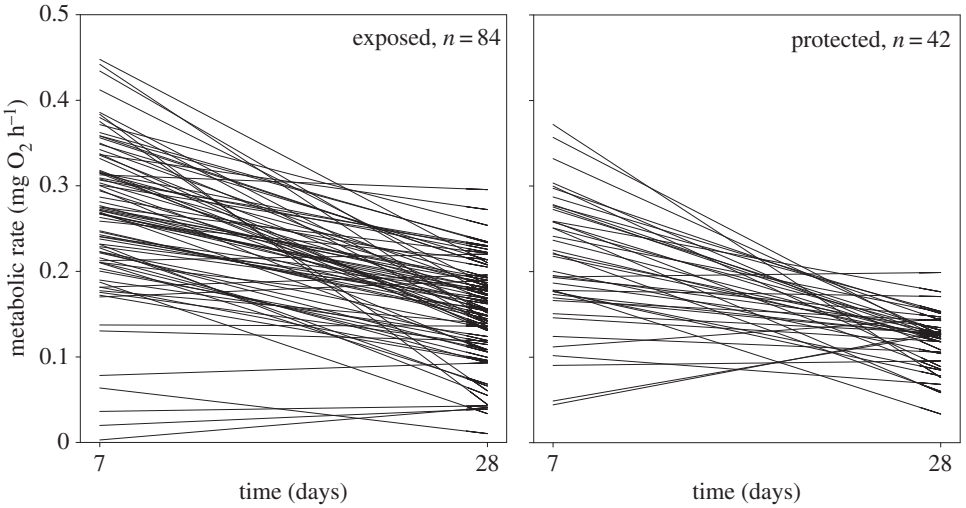

Figure 2. Whole-animal oxygen consumption rates of individual mussels measured at two time points, one week after collection from the field and after 28 days of common gardening. Repeated measurements for each individual are connected by solid lines. (*a*) Metabolic rates of mussels originating from a wave-exposed site, cool site. (*b*) Metabolic rates of mussels originating from a wave-protected, warm site, which showed a more dramatic decrease in metabolic variation among individuals after common gardening.

Antioxidant variation among individuals exhibited repeatable patterns at the baseline and top of the acute thermal challenge. Among mussels from the protected site, the magnitude of antioxidant variation decreased by approximately 30% from field-acclimatized to common garden at both the baseline ($p = 0.016$; figure 1*a*,*d*) and top of the heat ramp ($p = 0.019$; electronic supplementary material, figure S3*a*,*d*). The recovery time point data appeared to follow this trend (electronic supplementary material, figure S3*c*), though the differences were not significant (electronic supplementary material, table S3). In contrast, for mussels from the exposed site, there was no reduction in physiological variation following common gardening (baseline $p = 0.303$, figure 1*b*,*d*; top $p = 0.124$; electronic supplementary material, figure S3*b*,*d*). For protected-site mussels, the initial magnitude of field-acclimatized antioxidant variation was restored only when mussels were returned to the high, stressful site (outplant-high > outplant-low, baseline $p = 0.028$, figure 1*a*; top $p = 0.009$; electronic supplementary material, figure S3*a*). As for protected-site mussels, physiological variation was greater among exposed mussels in the outplant-high treatment than among those in outplant-low (baseline $p = 0.032$, figure 1*b*; top $p = 0.049$; electronic supplementary material, figure S3*b*). Notably, although the magnitude of variation among individuals changed among treatment groups in the instances just described, in no case did the magnitude of antioxidant variation shift rapidly within any given treatment among the three time points of the acute thermal challenge (electronic supplementary material, table S3 [grey boxes]).

(b) Whole-animal metabolic rate

Mean metabolic rates varied between sites of origin (protected < exposed, $p = 0.003$) and decreased with common gardening (day 7 > day 28, $p < 0.001$; figure 2). The random effect (individual) was significant in the general linear mixed-effects model (likelihood ratio test; $p < 0.001$); individuals have consistent differences in metabolic rate. As expected, total metabolic rate increased with log of body mass ($p < 0.0001$). There was no time by site interaction ($p = 0.823$).

The shifts in antioxidant variation among individuals were reflected qualitatively in the patterns of metabolic rate variation. Using the predicted values from the fitted GLMM for each individual at both time points, the magnitude of metabolic variation among individuals decreased after common gardening for both sites of origin (Levene's test $p < 0.001$; figure 2). Although there was no difference in the magnitude of metabolic variation between sites at day 7 ($p = 0.160$), by day 28 the protected site metabolic variation was substantially reduced relative to that of the exposed site ($p = 0.002$; figure 2). Thus, individuals from the protected site were more similar in both their oxygen consumption rates and their antioxidant capacities after common gardening.

4. Discussion

Future climate projections include shifts in both the mean and the variability of environmental conditions, yet understanding of the interactions between environmental variation and physiological variation remains relatively poorly developed [4,11]. Individuals within populations possess a range of genotypic and phenotypic repertoires [51]. Interacting with this functional variation is an often substantial degree of temporal and micro-scale spatial variation in how individuals experience environmental conditions. Global change will likely expose ectothermic intertidal organisms—and inhabitants of many other habitats—to changes in the frequency, magnitude and spatial variability of body temperature fluctuations [2,3]. The present study of antioxidant capacities and whole-animal metabolic rates confirms that mussels from nearby sites exhibit a substantial, and relatively similar, mean degree of physiological plasticity in response to shifts in the environmental context. More importantly, our results suggest that micro-scale environmental variation can amplify physiological variation among adjacent individuals. Surprisingly, acclimation to benign common garden conditions induced a decrease in the magnitude of antioxidant variation only among mussels from the protected site, matched by a dramatic reduction in the magnitude of metabolic variation among mussels from the same location. Moreover, the field-acclimatized level of antioxidant variation among mussels from the protected site was restored only after outplanting to the high, stressful site. Thus, the contribution of micro-scale

environmental variation to physiological variation among individuals appears to depend on the site in which the individuals settled and developed. Given that physiological plasticity, such as an increased reliance on antioxidant capacity to defend cellular homeostasis [52], is one of the first lines of defense against environmental change, it is imperative to understand how physiological mean–variance relationships respond to changes in environmental variability. We discuss some of the plausible underlying mechanisms driving these divergent, context-specific patterns of physiological variation among individuals from different sites. However, further data, including genomic profiles of individuals from different sites, are required to substantiate these mechanisms.

(a) Context-dependent interactions between genotypic and environmental variation

Groups of mussels originating from field sites only 24 m apart exhibited disparate responses to manipulation of micro-scale environmental variation. Without further replication at the level of site, we cannot draw conclusive inferences about *all* exposed and protected sites. Nonetheless, these seemingly idiosyncratic findings, within what many ecologists would consider a single population, likely complicate prediction of population-level responses to shifts in thermal variability, such as those anticipated with global change.

A likely mechanism for these divergent responses to manipulation of micro-scale environmental variation between groups of mussels from adjacent field sites involves context-specific, genotype by environment interactions [11,19]. [These 'genotypes' might instead be developmentally constrained phenotypic states, but we refer to all such 'fixed' differences as 'genotypes' hereafter.] Specifically, we hypothesize that the exposed site harbours an elevated degree of adult genotypic diversity, which is reflected in greater physiological variation among individuals after common gardening. Such differences could arise from patterns of settlement (larval habitat selection) and/or via post-settlement selection [53]. We would predict the latter to be strongest at the protected, warm site, where fewer genotypes would be capable of survival and adequate performance over the long term. In contrast, selective pressures—at least those owing to temperature—might be relaxed at the exposed, cooler site, conferring greater accommodation of genotypic and phenotypic/physiological variants [54].

Interactions between genotypic and environmental variation might also account for the lack of site differences in the field-acclimatized treatment. Under benign environmental conditions, individuals tend to exhibit low variance around trait means. In contrast, stressful environmental conditions often cause individuals to diverge in their responses, thereby increasing phenotypic variation (reviewed in [19]). This stress-induced amplification of individual differences occasionally exposes 'cryptic genetic variation' (CGV), which itself may act as the substrate for subsequent selection [55]. However, in this case, the mechanism is likely distinct from the classical descriptions of CGV. Whereas CGV tends to refer to rarely expressed variation elicited by infrequent, extreme events [55], in this case exposure of mussels to mundane, day-to-day micro-scale environmental variation at the protected site and in the outplant-high site appears to amplify the magnitude of physiological variation. Although the magnitude and direction of shifts in mean antioxidant status (i.e. plasticity) among treatments is comparable between exposed and protected sites, only mussels from the protected site exhibited decreases in the magnitude of physiological variation in the benign common garden treatment. Furthermore, mussels from both origin sites exhibited less physiological variation when outplanted to the low, less stressful site relative to the outplant-high site. These results agree with predictions of stress-induced phenotypic variation.

Interestingly, the magnitude of antioxidant variation among individuals did not change over the time course of the acute thermal challenge in any treatment group. Thus, at least in mussels, the magnitude of physiological variation appears to be determined by experience of temporal and spatial environmental variation over a period of days to weeks, rather than it responding abruptly to single, stressful events. The mean temperature threshold for induction of heat shock protein synthesis in *Mytilus* species is labile over comparable temporal scales [28,56], and in every case the mean antioxidant status shifted among treatment groups over weeks in our experiments. Further examination of physiological mean–variance relationships under novel, stressful conditions will greatly inform predictions of the outcomes of global change [57,58], particularly if these relationships are not fixed as our data suggest.

Repeated measurements of whole-animal metabolic rates on the same individuals offered complementary evidence for context-specific responses of physiological variation to manipulation of micro-scale environmental variation. Protected-site mussels converged on a similarly slow metabolic pace of life after common gardening, whereas exposed-site mussels maintained a greater degree of metabolic variation among individuals after the same treatment. Reduced variation in oxygen consumption rate after common gardening corresponded with reduced variation in the capacity to cope with metabolic by-products of oxygen utilization in mussels from the protected site, which is not surprising given the tight relationship between metabolic rate and ROS production [32]. Future progress in this arena will demand the ability to repeatedly sample the same individuals' biochemical status under different conditions (as we have done for metabolic rate). It then will be informative to explore physiological variation in a more robust fashion by correlating repeated measurements of individuals' biochemical status with individuals' unique thermal histories *in situ*, a feat which to our knowledge has not yet been achieved.

(b) Antioxidant capacities of all tissues respond to environmental change

The means of each of the nine antioxidant measures were affected by manipulation of micro-scale environmental variation ('treatment,' electronic supplementary material, table S1), but gill catalase activity, mantle catalase activity and anti-hydroxyl radical capacity of muscle contributed the most to overall variation among individuals in the PCA analysis (figure 1*c*). High levels of antioxidant plasticity in aerobic gill and mantle tissues are perhaps to be expected. Gills are the principal site of gas exchange and, thus, are likely poised to cope with fluctuating environmental oxygen concentrations and/or with variation in ROS production driven by changes in body temperature. Mantle tissue may assume some of this gas-exchange function when the animal is emersed [59]; mantle had the highest levels of antioxidant capacity against peroxyl radicals. Muscle, on the other hand, is generally considered to function largely via anaerobic pathways [59]. While muscle

exhibited the lowest overall antioxidant capacities, plasticity in hydroxyl radical scavenging and other antioxidant functions does suggest that oxidative, radical-generating processes play a part in thermal tolerance of this tissue. Indeed, muscle tissue in marine bivalves may be more susceptible to oxidative damage than mantle [60], in part because of muscle's low constitutive expression of antioxidant defences. Macromolecular damage in adductor muscle could lead to significant organismal consequences, either by limiting an individual's capacity to perform valve gaping behaviours that might contribute to evaporative cooling or by increasing susceptibility to predation.

Adjustments in antioxidant capacity likely stem from changes in the rate of ROS formation, potentially mediated by shifts in mitochondrial volume (plastic over weeks to months; [61]) or by adjustments in the level of unsaturation of fatty acids in the inner mitochondrial membrane [27,33]. These physiological changes would correspond with changes in overall oxygen utilization or in the leakiness of the inner mitochondrial membrane to protons, respectively.

(c) Variation in environmental factors other than temperature

The consequences of exposure to thermal stress may depend upon interactions with one or more other environmental parameters that might covary with temperature in the intertidal zone, such as oxygen levels, food availability, risk of desiccation or species interactions [62,63]. In particular, variation in food availability and food quality may contribute to physiological variation among individuals [64]. Within our two study sites, previous work offered strong evidence that changing food availability can contribute as much or more than temporal temperature variation to shifts in the mean activities of both ATP-generating and antioxidant enzymes [43]. Differences in the risk of desiccation owing to differences in emersion durations between the outplant-high and outplant-low sites could also contribute to the observed increase in physiological variation at the outplant-high site (e.g. see fig. 2 in [65]). We cannot rule out roles for these other parameters in driving physiological variation, nor can we yet determine at which life-history stage(s) (e.g. pre- versus post-settlement) selective factors exert the most influence.

Nonetheless, it is very likely that micro-scale variation in body temperature was a contributing factor in our experiments, in part because individual mussels do vary dramatically in their body temperatures in the field [18]. We substantiated those previous results, recorded in silicon-filled mussel shells, using thermocouples to measure body temperatures of live mussels at two additional sites. Adjacent individuals varied by up to 10°C in their body temperatures within less than 1 m^2 of rock (electronic supplementary material, figure S4). At the two outplant sites, a substantial degree of variation in body temperature persisted among the mussel mimics, despite the fact that all mussels were affixed to the rock at the same orientation. Micro-scale environmental variation in both outplant groups reached 8°C ranges of body temperature (electronic supplementary material, figure S5*b*). Adjacent individuals exposed to identical climate conditions can experience very different levels of physiological stress, owing to chance environmental factors such as wave splashing or shading [6,18]. Notably, the maximum and mean datalogger temperatures were higher at the outplant-high site (Mann–Whitney $U = 520805$, $p < 0.001$; electronic supplementary material, figure S5). Again, it appears to be the presence of micro-scale environmental variation around a sufficiently stressful mean, rather than the mere existence of such variation, that amplifies physiological variation among individuals.

5. Conclusion

Our results offer evidence that the presence of micro-scale environmental variation modulates patterns of physiological variation among individuals. Common garden acclimation reversibly reduced the magnitude of physiological variation only among mussels from a wave-protected field site. We propose that chronic or, in this case, episodic exposure to micro-scale environmental variation around a sufficiently stressful mean, rather than single extreme episodes, is necessary to increase the magnitude of physiological variation among individuals. These context-dependent interactions between physiological and environmental variation likely complicate prediction of the outcomes of global change. Physiologists often view variation as a nuisance that should be controlled by more rigorous experimental designs, rather than emphasizing variation's crucial role in ecological and evolutionary processes [15,66]. In the context of a more variable global climate, further studies are urgently needed to critically examine the heritable and plastic components of inter-individual variation at the physiological level [11,15].

Ethics. Animal collections were conducted under California Department of Fish and Game permit SC-7955.

Data accessibility. Data are deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.qc6tf.

Authors' contributions. A.G.J. designed the study, collected and analysed data, and drafted the manuscript. S.J. contributed to field studies and collected metabolic rate and antioxidant data. S.A. and J.D. collected antioxidant data. W.W.D. conceived of and coordinated the study, analysed data and drafted the manuscript. All authors gave final approval for publication.

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