Comparative Biochemistry and Physiology, Part A 238 (2019) 110577

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Inter-individual physiological variation in responses to environmental variation and environmental change: Integrating across traits and time☆

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A B S T R A C T

Greater understanding of physiological responses to climate change demands deeper comprehension of the causes and consequences of physiological variation. Increasingly, population trait means are being deconstructed into variable signals at the level of individuals. We advocate for greater consideration of such inter-individual physiological variation and how it both depends on and interacts with environmental variability. First, we review several studies on the intertidal mussel *Mytilus californianus* to illustrate how the magnitude of inter-individual variation may depend on the environmental context analyzed (i.e., is the mean condition benign or stressful?) and/or on the specific physiological metric investigated. Stressful conditions may reveal or mask variation in disparate ways at different levels of analysis (e.g., transcriptome vs. proteome), but we often lack crucial information regarding the relationships among these different physiological metrics and their consequences for fitness. We then reanalyze several published datasets to ask whether individuals employ divergent strategies over time in response to acute heat stress; such time-dependence would further complicate interpretation of physiological variation. However, definitive conclusions are precluded by limited sample sizes and short timescales in extant datasets. A key remaining challenge is to extend these analytical frameworks to longer periods over which individuals in a population experience repeated, but spatially variable, episodic stress events. We conclude that variation at multiple levels of analysis should be investigated over longer periods and, where possible, within individuals (or genotypes) experiencing repeated environmental challenges. Although difficult in practice, such studies will facilitate improved understanding of potential population-level physiological responses to climate change.

1. Introduction

Environmental and ecological physiologists have long focused on population and/or species means as the units of interest for investigating biological responses to climate change. Such studies have generated a wealth of data from which to estimate the macro-scale physiological consequences of environmental changes. This approach is rooted among assumptions that both selection and underlying complex biochemical interaction networks act to canalize phenotype (Flatt, 2005; Siegal and Bergman, 2002; Takahashi, 2018), which has validity when conducting the inter-specific comparisons that have long dominated these fields. However, this approach does not address the importance of within-population variation for selection to act upon, and it implicitly overlooks multiple environmental and biological influences on variation within populations. For example, biological sources of variation may interact with variation in space and time within the local environment, in some cases accentuating the degree of physiological variation. In the context of climate change, these sources of genetic and phenotypic standing variation – likely acting in concert with different forms of phenotypic plasticity (Gomez-Mestre and Jovani, 2013) – are essential for adaptive responses when populations are confronted with environmental shifts (Crawford and Oleksiak, 2007; Krebs and Feder, 1997; Nikinmaa and Waser, 2007; Shama, 2017; Williams, 2007).

With this more micro-evolutionary perspective in mind, we renew the argument for greater attention to the causes and consequences of inter-individual variation in physiological traits (Bennett, 1987; Crawford and Oleksiak, 2007; Dowd et al., 2015; Gunderson et al., 2016; Nikinmaa and Waser, 2007), here in the specific context of marine climate change physiology. We reiterate the need for studies that examine physiological responses to realistic patterns of spatial and temporal environmental variation, using data from studies of the intertidal mussel *Mytilus californianus* as a heuristic example. These data reveal not only the role of small-scale environmental variation in eliciting inter-individual physiological variation, but also opposing shifts in patterns of variation between metrics of physiological state measured on the same groups of mussels at the same time. Unfortunately, the mussel data are limited in some cases to single “snapshots” in time. We next explore the possibility that inter-individual variation in the time-course of responses to episodic stress complicates the interpretation of such snapshots. We present a mini-review of studies that investigated physiological state during recovery from acute heat stress, exploring whether the magnitude of inter-individual variation changes through

☆ This article is part of a special issue entitled: Mechanisms of biological sensitivity and resistance to a rapidly changing ocean, edited by: Dr. Logan Cheryl, Dr. Evans Tyler and Dr. Mike Hedrick.

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https://doi.org/10.1016/j.cbpa.2019.110577
Received 19 April 2019; Received in revised form 5 September 2019; Accepted 10 September 2019
Available online 12 September 2019
1095-6433/

time. We then briefly conceptualize how episodic, but spatially variable, environmental stress can influence population-level variation in life-history and related physiological strategies for energy allocation over longer periods than a single stressful event. These disparate allocation strategies might manifest in different ways across tissues or stages of information flow from genes to transcripts to proteins to physiological performance. Finally, we highlight some of the remaining challenges for incorporating inter-individual physiological variation into our projections of population-level patterns under future climate regimes.

2. Episodic environmental stress in present and future climates

A growing body of recent work has highlighted the role of environmental variation in physiological responses to climate change (Dowd et al., 2015; Vasseur et al., 2014). Indeed, while they are easiest to study in laboratory settings, increases in mean temperature and chronic temperature exposures are not the most biologically relevant aspects of global change. Instead, an increase in the frequency, duration, and severity of extreme events is most likely to have fitness consequences in the future ocean, especially in nearshore habitats and the intertidal zone that already experience extremely dynamic regimes (Dowd et al., 2015). These habitats harbor much of marine biodiversity (e.g., coral reef ecosystems), and they are susceptible to other anthropogenic disturbances. As climate change induces increases in mean temperature, the probability of thermally stressful episodes will increase dramatically (Harris et al., 2018; Kharin et al., 2018; National Academies of Sciences, 2016). Given this episodic signature of anticipated climate changes (Jentsch et al., 2007), one reasonable approach to studying the physiological effects of climate change is to focus on variation within a population under conditions that approach or exceed thresholds of organismal physiology, such as those for induction of stress response mechanisms or for thermal tolerance (Helmuth and Hofmann, 2001; Tomanek, 2010).

There are competing ideas in the literature regarding the influence of stressful conditions, which we define as those capable of inflicting macromolecular damage (Kültz, 2003), on functional variation. On one hand, the existence of numerous highly conserved elements of the inducible, cellular stress response (Kültz, 2005) suggests that stressful episodes could “mask” inter-individual variation by inducing a consistent physiological response (Oleksiak and Crawford, 2012), at least temporarily. However, it has also been proposed that stressful environmental conditions can “unmask” cryptic phenotypic variation, causing individuals to diverge in their phenotypic responses and, ultimately, in their fitness (Ghalambor et al., 2007). If this unmasking mechanism is acting within a population, we should expect physiological phenotypes to diverge under novel environmental conditions or those of greater intensity (both of which are hallmarks of projected climatic changes). Much remains to be done to reconcile these opposing theoretical considerations with actual measures of inter-individual variation in organismal function. We show in the next section that the amount of physiological variation observed in experiments on intertidal mussels in some cases may differ depending not only on differences in the mean condition but also on the physiological metric being investigated (e.g., protein vs. gene expression, see Liu et al., 2016).

Interactions between episodic stress and physiological variation within a population may be further complicated by the sometimes heteroscedastic pattern of environmental variation (i.e., the variance changes as the mean changes). For example, in the mussel system described below, long-term observations illustrate that the amount of variation in body temperature among neighboring individuals increases significantly as the mean body temperature increases (Miller and Dowd, this issue). Consequently, episodic extreme temperature events vary in frequency and duration among individuals even over a small spatial scale (Miller and Dowd, 2019, this issue). It is very likely that this spatial environmental heterogeneity further contributes to variation in individual stress responses across physiological traits and over time (Denny et al., 2011; Dowd et al., 2015). Therefore, it is difficult in field experiments to distinguish between the dual influences of changes in the environmental mean and changes in the environmental variance on physiological variation. Careful, manipulative studies could disentangle the relative influence of stress per se from that of spatial variation. However, in the next section we adopt the perspective that documenting patterns and consequences of physiological variation in realistically varying natural environments is inherently worthwhile, for the precise reason that this approach captures the important environmental reality of heteroscedasticity.

Finally, environmental variation and biological variation also interact, via genotype × environment processes such as acclimatization and other forms of plasticity, to produce a variety of physiological phenotypes. Thus, context-dependent changes in phenotypic variation exposed by temporal and spatial environmental heterogeneity may be further influenced by underlying, heritable genetic differences and/or by cryptic genetic variation (Ghalambor et al., 2015; Paaby and Rockman, 2014).

3. Investigation of multi-level physiological variation in the intertidal mussel *Mytilus californianus*

A series of datasets collected on the intertidal mussel *Mytilus californianus* offers an opportunity to illustrate potential context-dependent relationships between environmental conditions and physiological variation. Notably, these datasets span much of the biological hierarchy from tissue-level to whole-organism; different metrics capture either instantaneous physiological state (e.g., antioxidant capacity) or integrative responses to past environmental conditions (e.g., growth rate). Thus, the trends presented illustrate some of the complexities inherent in evaluating variance within and among traits in a single study system. As noted in the previous section, these data simultaneously capture the influences of both temporal and spatial environmental variation on patterns of physiological variation.

3.1. Study system and methods

The California mussel, *Mytilus californianus*, is a foundation species in rocky intertidal communities along the west coast of North America, where it experiences spatial and temporal environmental heterogeneity on a variety of scales (Denny et al., 2011; Helmuth et al., 2006). This sessile, tractable study system allows us to investigate how inter-individual variation is exposed or masked under different environmental scenarios (e.g., benign vs. stressful), and whether patterns of variation differ based on population origin. Using adult *M. californianus* in different field and laboratory contexts, our group has collected data that allow for investigation of inter-individual variation in antioxidant status, gene expression, protein expression, survival after acute thermal stress (i.e., thermal tolerance), growth, and whole-animal metabolic rate (Gleason et al., 2018; Jimenez et al., 2015). Treatment sample sizes equal eight or more for nearly every metric (see Appendix 1, Table S1). In addition, juveniles have been examined for variation in growth and thermal tolerance. We focus our discussion on patterns of inter-individual variation in response to heat stress. However, we acknowledge that a variety of other environmental variables might influence variation in intertidal mussel beds, such as food availability, wave exposure, and predation risk (Beadman et al., 2003; Connor and Robles, 2015; Fitzgerald-Dehoog et al., 2012).

Each dataset references a single set of adult individuals or a separate set of juvenile individuals from two origin field sites representing warm (wave-protected) and cool (wave-exposed) temperatures; juveniles were collected from high and low-intertidal beds at these two sites, yielding a total of four origins. In most cases, mussels from each site were sampled from four treatments: field-acclimatized, common gardened in the laboratory for one month, and outplanted in a fully crossed

design for one month to two intertidal sites that vary in thermal stress levels (high and low-intertidal zone for adults; wave-protected and wave-exposed micro-sites for juveniles). All individuals in the field treatments were sampled as the tide was receding in the morning. Thus, the metrics of inter-individual variation are best interpreted as cumulative responses to recent environmental variability, rather than indicating variation in response to an acute stress. Only in the case of adult antioxidant capacities were measurements repeated at multiple time points over an acute thermal ramp: before the exposure (baseline), after reaching the peak temperature of 33 °C, and after 24 h of recovery (see Jimenez et al., 2015). Unfortunately, the destructive sampling approach required using different individuals at these different time points. For all other metrics, only baseline data were collected.

From the same groups of adults in each treatment at the baseline time point, we quantified three metrics of antioxidant capacity in each of three different tissues (gill, muscle, and mantle tissues; Jimenez et al., 2015), global patterns of gene expression in gill (using Illumina paired-end 50 bp RNA-seq), and corresponding global patterns of protein expression in gill (using a non-targeted LC-MSn approach as in Kültz et al., 2015). Gill was chosen because of its prominent organismal roles in feeding and gas exchange. Of the data presented, gene and protein expression summaries are the only new findings; other metrics represent re-analyses of data contained in Gleason et al. (2018) and Jimenez et al. (2015). A brief description of the gene and protein expression dataset is available in Appendix 2; only the mussels from the protected origin site were assayed in all 4 treatment conditions for these omics approaches. The original intent of these studies was not to incorporate all metrics into one framework, so not all treatments and individuals match identically across traits and life stages. Yet, when considered holistically, these data offer several valuable insights on context-dependent patterns of inter-individual variation.

First, we assessed variation within each physiological metric using unbiased estimators. For univariate traits such as growth rate, we calculated the median absolute deviation (MAD) for each treatment group. MAD is a robust estimate of variance calculated using the absolute deviations of each sampled value (X_i) from the median ($\widetilde{X}$) of the respective treatment group (Hampel, 1974):

$$MAD = median(|X_i - \widetilde{X}|) \quad (1)$$

MAD is increasingly used in outlier tests and in analyses of differential variability as an alternative to differential expression (Ho et al., 2008; Leys et al., 2013). For multivariate datasets including gene and protein expression and antioxidant status, multivariate dispersion was calculated in an analogous fashion. Specifically, scaled data (using z-scores within each measurement variable) were used to estimate Euclidean dissimilarity indices. Using this scaling method, transcripts and proteins held equal weight across the calculation of all eigenvalues. MAD values for each multivariate dataset were then calculated by using the distance from the respective treatment median in multi-dimensional space as the absolute deviation in the MAD formula above (see Appendix 1, Fig. S1 for visual description). These multivariate analyses were run with a modified version of the multivariate homogeneity of group dispersions function in the "vegan" package in R (Oksanen et al., 2018; R Team Core, 2017). For the binary trait of thermal tolerance/survival, variance was estimated from the number of survivors in a treatment (Cox, 1989):

$$var(D) = \frac{k(n-k)}{n^2} \quad (2)$$

for $D = < X_1, \ldots, X_n >$, *where* k *denotes the number of* 1s/*survivors in* D

To statistically compare the magnitude of variation among treatments for univariate traits, an ANOVA was conducted on the deviations from the median used to calculate MAD (this is functionally equivalent to a Brown-Forsythe test for equality of variances). For multivariate traits, a similar pairwise comparison of deviations from the multivariate median was performed within the multivariate dispersion calculation (using the "permutest" function in the "vegan" package in R) (Oksanen et al., 2018). For the binary trait of thermal tolerance/survival, bootstrapping with 100,000 iterations was performed on variance values using the "boot" package in R. Statistically homogeneous groups are represented by letters (generated using the "multcompView" package in R) in the relevant figures; statistical summaries are provided in Appendix 3 (Graves et al., 2015).

Second, in order to facilitate comparison of the magnitudes of inter-individual variation across physiological traits, we calculated a modified version of the coefficient of variation (CV), a unit-less estimate of variation, for each treatment group. Presented here are CV values as percentages. This method used the above estimates of MAD substituted for standard deviation in the CV calculation:

$$CV = \left(\frac{MAD}{\overline{X}}\right) * 100 \quad (3)$$

The standard CV equation applies to univariate data only. Therefore, we calculated a multivariate coefficient of variation (CV_m) for the gene and protein expression and antioxidant status datasets using the "covMCD" function in the "robustbase" package in R (Albert and Zhang, 2010; Maechler et al., 2019; Reyment, 1960):

$$CV_m = \left[\frac{\mu^T \Sigma \mu}{(\mu^T \mu)^2}\right]^{\frac{1}{2}} \quad (4)$$

where μ *is a matrix estimated by the minimum covariance determinant method and* μ^T *is transposed*

Coefficients of variation for all traits and life stages can be found in Table 1, along with a graphical representation of two traits in Fig. 1; a graphical representation of juvenile metrics can be found in Fig. S2. Variation in all adult traits (quantified as CV and CVm) was different depending on trait, with growth having the largest CV and protein expression having the lowest CV_m.

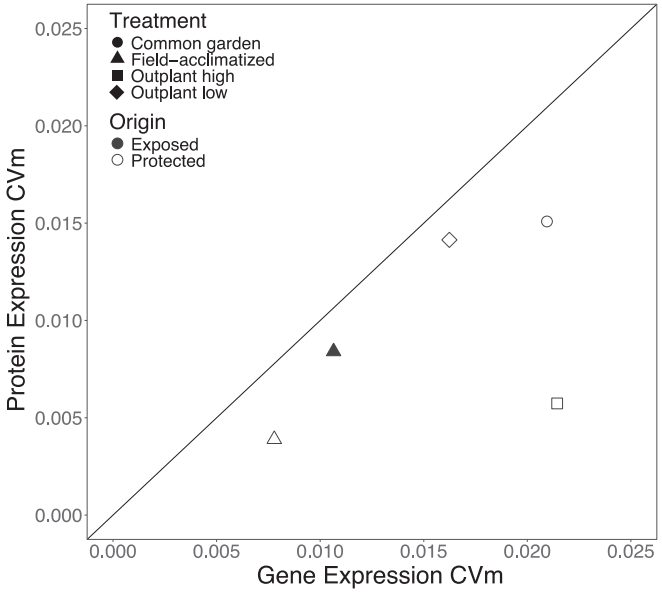


Fig. 1. Gene expression of adult *Mytilus californianus* exhibits a greater degree of inter-individual variation than protein expression. CV_m values for each metric are plotted for treatments as defined in the text. All points fall below the reference line (slope = 1). Note also that the magnitude of variation in gene expression changes more across treatments.

Table 1A
Coefficients of variation for all *M. californianus* adult metrics (data from Jimenez et al., 2015 and current study).

Treatment	Adult growth	Adult MR	Adult AO	Adult GE	Adult PE
or_PL//tr_field	NA	29.23	23.07	0.011	0.004
or_EL//tr_field	NA	24.64	7.47	0.008	0.008
or_PL//tr_CG	167.88	27.34	NA[a]	0.021	0.015
or_EL//tr_CG	72.93	31.16	14.47	NA	NA
or_PL//tr_outL	245.23	NA	NA[a]	0.016	0.014
or_EL//tr_outL	800.00	NA	4.84	NA	NA
or_PL//tr_outH	136.54	NA	10.75	0.021	0.006
or_EL//tr_outH	96.28	NA	5.25	NA	NA

Coefficient of variation expressed as a percentage. Treatment codes are as following: or = origin, tr = treatment; P = protected, E = exposed, L = low intertidal, H = high intertidal, CG = common garden, field = field-acclimatized
[a] Too many missing values to calculate CVm.

3.2. Physiological metrics differ in magnitude of variation depending on mussel origin and treatment condition, but not in a consistent fashion

Across multiple metrics of mussels' physiological state, inter-individual variation is prevalent and context-dependent (Figs. 2 & 3; Appendix 3). However, the magnitude and direction of changes in this variation across treatments depend on which metric is considered. Consistent with a previous analysis employing slightly different methods (Jimenez et al., 2015), we observe a reduction in the magnitude of variation between the field-acclimatized state and common garden conditions for metabolic rate (individuals from protected and exposed origins) and for antioxidant status (only individuals from protected origin) (Fig. 2B, C, & D). Under the presumption that common garden conditions are relatively benign, these results agree with the "unmasking" hypothesis. Meanwhile, protein expression (Fig. 2E) shows increased variation in the outplant high and outplant low treatment groups relative to field-acclimatized animals sampled directly from the protected site. The outplant high treatment, in which we expect the greatest mean intensity of thermal stress as well as the greatest magnitude of inter-individual variation in body temperature (see Miller & Dowd 2019, this issue), was marginally less variable in protein expression than the outplant low treatment ($p = .063$). Taken alone, this result comports with the predictions of the "masking" hypothesis under stressful conditions. Interestingly, gene expression exhibits the opposite (albeit statistically insignificant) trend between variation in the outplant high and outplant low treatments (Fig. 2D). Variation in gene expression was significantly higher in the outplant high treatment only when compared with variation in the field-collected individuals from the protected site, another example of a pattern consistent with the "unmasking" hypothesis in the adult *Mytilus* data.

Perhaps the most notable outcome of this adult mussel analysis is the lack of correspondence between patterns of inter-individual variation in gene and protein expression, specifically under stressful conditions (Figs. 1, 2D & E), despite the fact that both datasets were derived from the same tissues and individuals. While some portion of this discrepancy may be attributable to time lags between transcription and translation (see below), it is also increasingly apparent that variable gene expression profiles can converge on similar physiological outcomes (Nikinmaa and Waser, 2007; Oleksiak et al., 2005). Rather than just being noise, this variation in gene expression may be adaptive, allowing differential regulation of mRNAs to play a role in physiologically plastic responses. If we assume that the proteome better reflects current physiological function than does the transcriptome (because extant proteins are performing a large percentage of cellular functions), then flexibility in gene expression patterns among individuals is perhaps not surprising (particularly given recent discoveries regarding mRNA processing and turnover, expanded roles of micro-RNAs, etc.) (Baek et al., 2008; Greenbaum et al., 2003; Liu et al., 2016; Mata et al., 2005).

However, it is surprising that high outplant treatment (i.e., higher stress) did not increase overall variation in the proteome, particularly because the other, more direct metrics of function (metabolic rate and antioxidant capacity) did display patterns of variation consistent with the unmasking hypothesis. Ongoing work in our laboratory is examining the specific gene/protein entities and larger network components that contribute to these patterns of variation in the transcriptome and proteome. The working hypothesis is that considerable functional variation is itself hidden when omics data are analyzed at such a global level; functional co-expression "modules" are perhaps better units of analysis (e.g., as demonstrated in Kenkel and Matz, 2017).

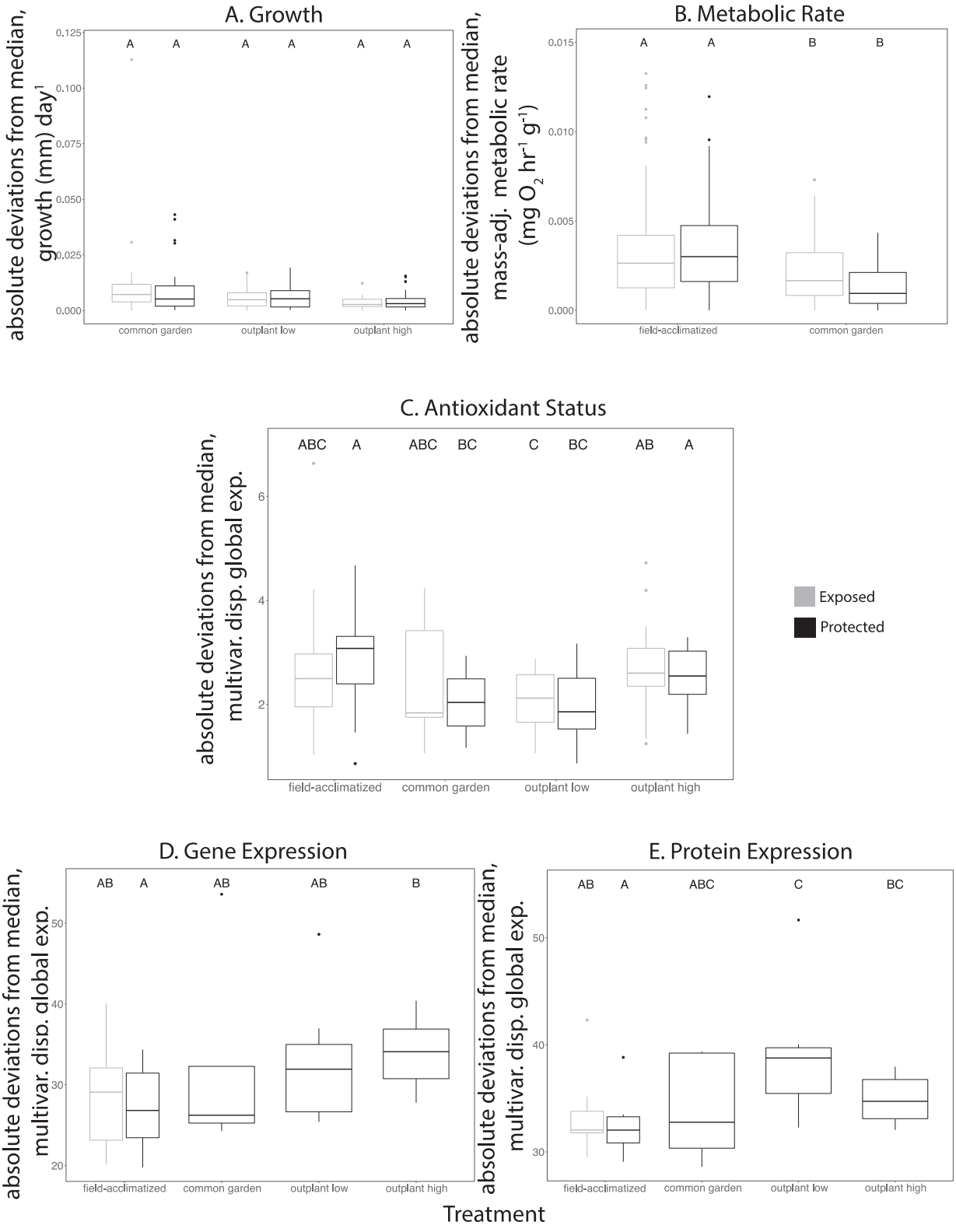
The repeated and tissue-specific measures of antioxidant capacity on adult mussels illustrate two additional points. First, the magnitude of inter-individual variation for any given treatment (e.g., outplant high) changes depending on when measurements are made relative to an acute stress event (Fig. S3), an observation that foreshadows the next section of this review. Time-series characterizing the dynamic changes in variation at the transcriptomic and proteomic levels should provide analogous, novel insights. Second, physiological (or gene/protein expression) measurements made on whole organisms potentially mask another, intra-individual level of variation, because patterns of variation at any given time point respond to different treatments in a tissue-specific manner (Fig. S3). This result is not surprising given the differing roles of tissues in physiological maintenance and corresponding differences in energetic allocation, but it has important implications for experimental designs. Specifically, studies of inter-individual physiological variation should include, at a minimum, careful justification of the choice of tissue, or, when possible, analyses of multiple tissues simultaneously. Future work may well see the further differentiation of responses among different cell types, as gene and protein expression can vary within a tissue (Elowitz et al., 2002).

Similar to adults, inter-individual variation among juvenile mussels exhibited contrasting patterns between metrics. Variation in growth was lower in the protected (warm) outplant treatment than in the exposed (cool) treatment, while the reverse was true for variation in thermal tolerance (Fig. 3). Common garden conditions greatly reduced variation in growth, but only reduced variation among individuals from the protected site in thermal tolerance, showing some evidence for our "unmasking" hypothesis under environmental stress. Otherwise, there were no systematic patterns based on origin in juveniles (Fig. 3). This could be due to the relatively large influence of developmental plasticity in juvenile thermal tolerance physiology. Given their broadcast-spawner life history strategy and the associated uncertainty in an offspring's future environmental conditions, juvenile mussels should benefit from flexibility in phenotype early in life (Beaman et al., 2016). More data are needed on patterns of inter-individual physiological variation in other metrics for juveniles to draw any further conclusions. However, it is tempting to speculate that because juveniles allocate little or no energy to reproduction they may face fewer and/or less pronounced allocation tradeoffs (Lika and Kooijman, 2003).

Patterns in our mussel datasets illustrate that inter-individual variation is context-specific – whether that be in a temporal, tissue-specific, or trait-specific manner. It remains to be determined whether these relationships are generalizable across environmental conditions or taxa, but our hope is that a broader synthesis will soon be possible as more studies explicitly address patterns of inter-individual physiological variation.

4. Inter-individual variation in the recovery response after one stressful event

Perhaps the greatest weakness of the preceding mussel analysis is the lack of temporal replication of measures of inter-individual physiological variation. If individuals differ in their dynamics of regulation, the magnitude of physiological variation would be expected to swell and shrink over time. For example, following imposition of an acute



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heat stress, we might expect low variance among individuals initially when all individuals mount a similar stress response, followed by increasing inter-individual variation later in recovery as some individuals terminate the response while others sustain it, and ultimately a return to low, baseline levels of variation once all individuals have terminated the response. If prevalent, such temporally shifting patterns of variation would severely confound our interpretation of the types of physiological "snapshots" presented above for mussels. Here, we reanalyzed published time-series data to search for evidence of such time-dependent patterns of physiological variation.

A very small subset of the marine heat stress physiology literature focuses on the dynamics of recovery responses, rather than presenting

Fig. 2. Inter-individual variation among adult mussels (*Mytilus californianus*) in several metrics of physiological state. Physiological variation was quantified using MAD in multiple experimental treatments using mussels from two origin sites (wave-exposed [grey]; wave-protected [black]). MAD is represented by the median line in the quartile boxes, which describe all absolute deviations from the median. Treatments along the x-axis are described in the text. Y-axis scales are dependent on trait, and therefore not consistent across traits. Significance by a Brown-Forsythe Test with a Tukey's HSD post hoc (or an analogous permutation-based pairwise comparison for multivariate traits in C-E) are denoted above each bar. A) Growth rate over one-month (Jimenez et al., 2015). B) Metabolic rate, quantified as oxygen consumption rate in seawater (Jimenez et al., 2015). C) A multivariate assessment of organismal antioxidant capacity, combining the abilities of three tissues (gill, muscle, and mantle) to detoxify hydrogen peroxide (primarily peroxidase activity of catalase), hydroxyl radical, and peroxyl radical (Jimenez et al., 2015). D) Gene expression. E) Protein expression. Data for B-E were collected on the same individuals; growth data in A were derived from mussels from the same sites in a different year. Note that the gene expression dataset used here includes only the subset of transcripts that were also represented and quantified in the proteomics analysis, enabling direct comparison (n = 1521 transcripts). Expression values for each gene/protein were first converted to z-scores before analysis. See Table 1A for normalized CV values of these measures; see Appendix 1, Table S1A for sample sizes.

snapshots in time. Using data from several of these studies, we quantified variance among individuals using a normalized MAD at post-heat stress time points (see MAD method in *Mytilus* section above). Variation was compared among time-points using a Brown-Forsythe Test on the same absolute deviations used to calculate MAD (as above), followed by a Tukey's HSD post hoc test for pairwise comparisons.

We found limited support for the predicted "fall-rise-fall" pattern of variation in studies of global gene expression, metabolic rate, glutathione content, redox potential, and photosynthetic efficiency (Fig. 4) (De Salvo et al., 2010; Heise et al., 2006; Jayasundara and Somero, 2013; Teranishi and Stillman, 2007). [Note that the temporal scale varies across the reviewed studies; we do not expect all physiological or organismal traits to follow the same time-course of response, particularly when comparing across species.] Although the estimated MAD values did fall and rise after heat stress as predicted in a few datasets, only temporal changes in variation of global gene expression patterns were statistically significant.

It has also been hypothesized that the magnitude of inter-individual variation is context-dependent; that is, more extreme conditions might separate individuals physiologically more than relatively benign conditions would (Ghalambor et al., 2015; Jimenez et al., 2016). Of the studies we examined, Jayasundara and Somero (2013) came closest to assessing the dynamic pattern of recovery following different magnitudes of thermal stress. However, in that study acclimation temperature and acute heat stress temperature co-varied and, therefore, confound interpretation. These caveats notwithstanding, we partitioned out variance at each time point for each of four acclimation treatments, observing that all temperature treatments except the highest acclimation/heat stress combination resulted in a similar trend of constant or decreasing variance with time post-heat stress. Importantly, despite these trends only the lowest temperature treatment had any statistically

Table 1B
Coefficients of variation for all *M. californianus* juvenile metrics (data from Gleason et al., 2018).

Treatment	Juvenile thermal tolerance	Juvenile growth
or_PL//tr_field	63.64	NA
or_PH//tr_field	78.57	NA
or_EL//tr_field	82.61	NA
or_EH//tr_field	71.43	NA
or_PL//tr_CG	69.57	22.38
or_PH//tr_CG	71.43	50.00
or_EL//tr_CG	65.22	51.13
or_EH//tr_CG	65.22	50.00
or_PL//tr_exp	69.57	40.75
or_PH//tr_exp	69.57	27.05
or_EL//tr_exp	73.91	33.00
or_EH//tr_exp	69.57	19.40
or_PL//tr_pro	43.48	41.67
or_PH//tr_pro	34.78	35.82
or_EL//tr_pro	60.87	34.75
or_EH//tr_pro	47.83	35.08

See Appendix 1, Fig. S2 for graphical comparison. Coefficient of variation expressed as a percentage. Treatment codes are as following: or = origin, tr = treatment; P & pro. = protected, E & exp. = exposed, L = low intertidal, H = high intertidal, CG = common garden, field = field-acclimatized.

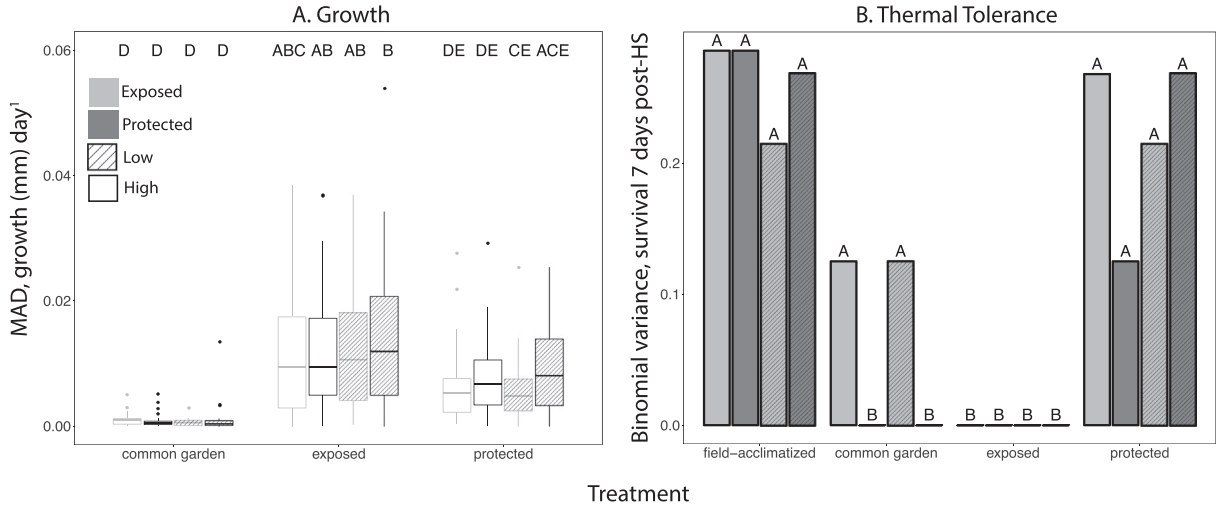

Fig. 3. Inter-individual variation among juvenile mussels (*Mytilus californianus*) in growth rate and thermal tolerance. Physiological variation was quantified using MAD and binomial variance for mussels from four different origin sites (high- and low-intertidal at a wave-exposed site [grey] and a wave-protected site [black]). Treatments along the x-axis are described in the text. Y-axis scales are dependent on trait, and therefore not consistent across traits. Significance by a Brown-Forsythe Test with a Tukey's HSD post hoc (growth) or by bootstrapping (survival) are denoted above each bar. A) Growth rate MAD over one month; note that overall growth rates were very low in the common garden treatment. MAD is represented by the median line in the quartile boxes, which describe all absolute deviations from the median. B) Survival variance of an acute heat ramp peaking at 39 °C. Binomial variance is shown using a bar plot. See Table 1B and Appendix 1, Fig. S2 for normalized CV values of these measures; see Appendix 1, Table S1B for sample sizes. Data from Gleason et al. (2018).

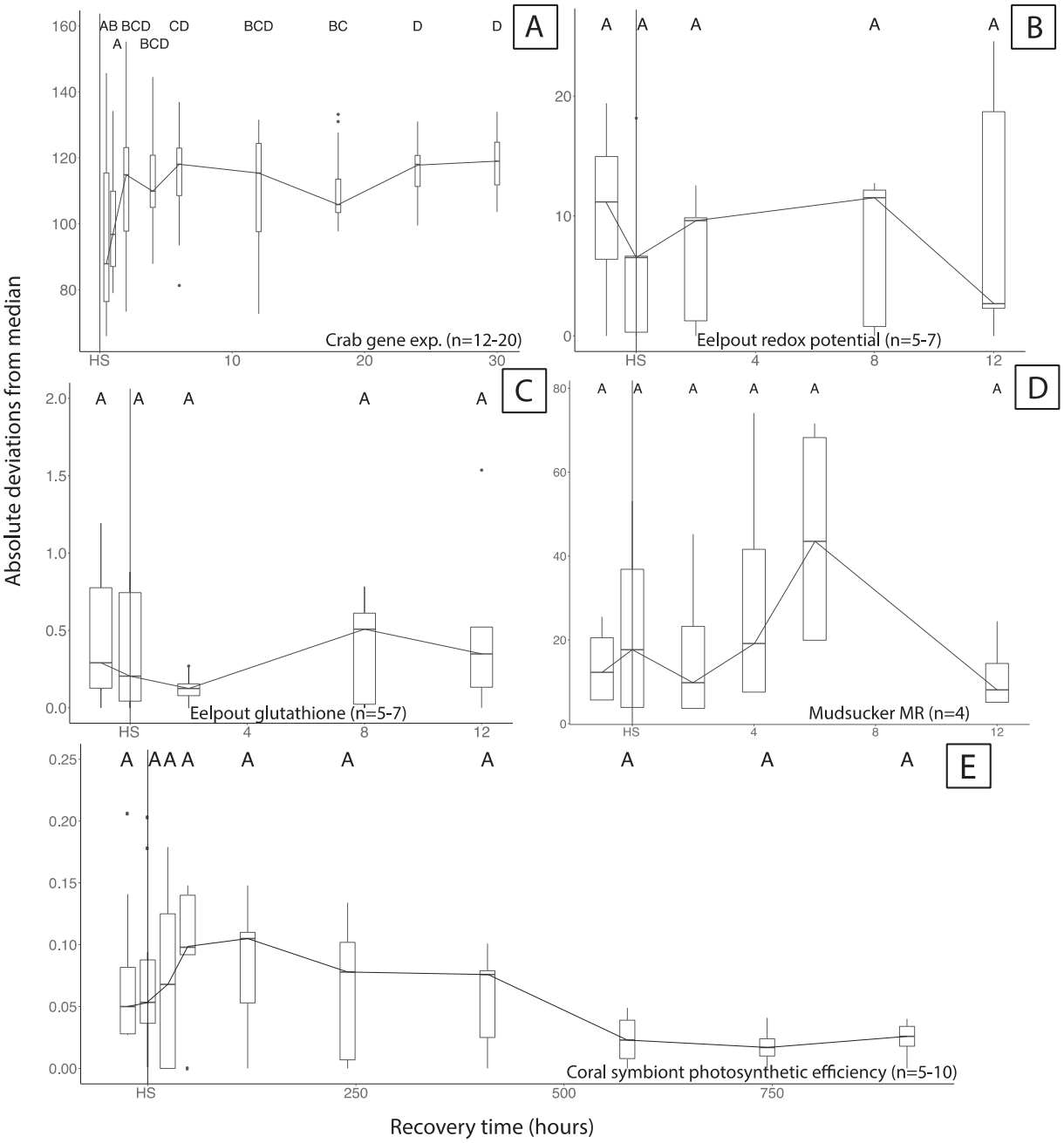

Fig. 4. Temporal changes in the magnitude of inter-individual variation during recovery from a heat stress episode. Five separate datasets from four studies are reanalyzed here for scaled univariate or multivariate median absolute deviation (MAD). MAD is represented by the median line in the quartile boxes, which describe all absolute deviations from the median. Statistically homogeneous groups generated from a Brown-Forsythe Test with a Tukey's HSD post hoc (or an analogous permutation-based pairwise comparison for A) are denoted above each bar. A) Crab gene expression multivariate dispersion (Teranishi and Stillman, 2007), B) Eelpout redox potential, C) Eelpout glutathione (Heise et al., 2006), D) Mudsucker metabolic rate for the 26 °C acclimation condition (Jayasundara and Somero, 2013), and E) Coral symbiont photosynthetic efficiency (De Salvo et al., 2010).

significant differences in variation through time; variation was highest very early during the acute heat stress in this treatment (Fig. S4). Only the 26 °C temperature treatment is shown in Fig. 4; the other treatments are compared in Fig. S4.

Although few statistically significant patterns emerge in these analyses, any conclusions regarding temporal fluctuations in the magnitude of inter-individual physiological variation within marine populations following heat stress are tentative at best. Unfortunately, sample size is relatively low in these studies, which decreased the statistical power to detect changes in variance by increasing the likelihood of variance being influenced by a few outliers. In presenting MAD as the measure of variance, we attempted to control more stringently for such outlier effects by calculating deviations from the median rather than from the mean (Leys et al., 2013). Mature conclusions await experiments with sufficient sample sizes to enable more robust statistical comparisons (see, for example, studies reviewed in Williams, 2007).

As in the previous section, this mini-review highlights the need for increased attention to the types of experimental designs that might prove fruitful in future physiological work. In our opinion, the field stands to benefit enormously from temporally repeated experimental designs, similar to those possible and used more frequently in the environmental endocrinology literature, that repeatedly quantify both

variation in underlying physiological traits and variation in organismal performance. Such studies are particularly informative when they can connect patterns of short-term physiological variation to long-term metrics of functional performance. For example, rapid negative feedback of the corticosterone stress response after imposition of an acute stress was associated with increased resilience in some tree swallows (Zimmer et al., 2019). The ability to have fast-acting negative feedback on corticosterone production differs among individuals in several vertebrates, and this characteristic has consistently been correlated with positive organismal outcomes (Liberzon et al., 1997; Mizoguchi et al., 2001; Rich and Romero, 2005). However, the added power available to endocrinologists who study the same animals through time will be impossible to replicate using current biochemical and omics approaches that require destructive sampling.

5. Connecting short-term physiological variation to long-term consequences in different environmental scenarios

Heterogeneous and extremely dynamic habitats such as the intertidal zone result in considerable variation among individual in how they experience the environment. Some individuals experience very frequent episodic stress, while their neighbors rarely encounter damaging conditions (Fig. 5). The consequences of these patterns of variation have been explored most thoroughly for single, extreme events that might induce substantial mortality. In certain scenarios, interactions between physiological variation and spatial environmental variation can have profound effects on the likelihood of a population's persistence (Denny, 2018; Denny et al., 2011).

A key remaining challenge is to extend the analytical approaches we have applied above to longer time-scales over which organisms experience multiple stressful episodes of spatially varying magnitude. One of the first steps toward such a synthesis is to determine whether physiological variants (or variants in underlying gene/protein network structure) align with individual-level patterns of environmental variation, and to then ascertain whether such patterns arise from fixed genetic differences (e.g., by habitat sorting) or via acclimatization (i.e., from plasticity). In other words, do individual physiologies match or mismatch with environmental conditions?

Intuitively, we might anticipate plasticity allowing individuals exposed to high thermal variability to express a generalist strategy with a broader performance breadth (Fig. 6A, dashed line). In contrast, individuals experiencing a relatively narrow window of temperatures would be expected to exhibit a specialist strategy (Fig. 6A, solid line). Both strategies might be viable within a small spatial scale that is heterogeneous in environmental forcings (Denny et al., 2011). There is some empirical support for these predictions, such as a widening thermal breadth for metabolic rate in mealworm beetles acclimated to thermally variable conditions (Bozinovic et al., 2013). This change is accompanied by a generalist-specialist tradeoff in maximum performance (note difference in height of the curves in Fig. 6A). Similar results have been observed for swimming performance in fish, with the added observation that individuals vary considerably in their acclimation capacity and performance breadth (Seebacher et al., 2015). Thus, variable constraints exist on individuals' abilities to optimize physiology in relation to their specific environment.

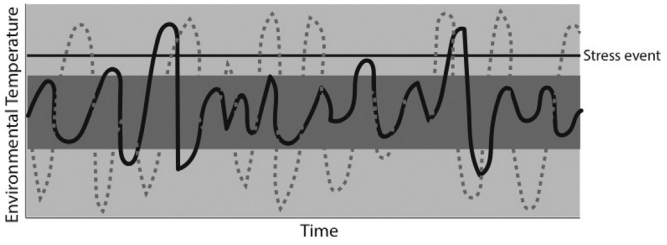

Fig. 5. The frequency and magnitude of environmental events capable of inducing a stress response differ among individuals. Dark, narrow band corresponds to black time-series and grey, wide band corresponds to dashed, grey time-series, representing two potential environmental profiles characterized by narrow or wide thermal variability. A threshold stress event is defined, and the two profiles differ in the number of times they cross that threshold. For simplicity, this threshold is presented here as being fixed among individuals; however, we expect variation in the threshold in natural populations. This figure is referenced in Fig. 6, which incorporates the same colour scheme.

The potential long-term manifestations of these constraints are conceptualized in Fig. 6B & C, in which the energy budgets of the specialist and generalist physiologies are illustrated in both a wide and narrow thermal fluctuation regime. The long-term performance of each strategy depends critically on the “match” or “mismatch” to the thermal regime it experiences. For example, the generalist is expected to sustain sufficient ATP production to fuel substantial growth and reproduction in the variable thermal regime (Fig. 6B, dashed box), but it performs relatively poorly in the less-variable thermal regime because of the generalist-specialist tradeoff (Fig. 6C, dashed box). The opposite is true for the specialist strategy, assuming the thermal optimum aligns with the mean temperature in the less-variable regime. However, if individuals are imperfect at adjusting physiology to environment, as empirical data clearly suggest, then everyone's performance suffers except in the unlikely scenario of perfect matching.

Variation in these energetic strategies could be particularly important in future environments characterized by more frequent and/or more unpredictable stress events, such that these strategies may become further mismatched to the pattern of thermal variability. For example, thermal specialists in a future, highly variable ocean would have reduced energy availability, and perhaps allocate very little energy to growth and reproduction, thus impacting fitness. Positive evolutionary outcomes will depend on maintenance of physiological diversity – by mechanisms such as gene flow and spatially varying selection – on which selection can act (Munday et al., 2013).

6. Conclusions

Gaining a better understanding of variation in environmental stress responses requires attention to dynamic patterns across time and space within a population. Context-dependent patterns of phenotypic/physiological variation will continue to be hidden whenever data are presented as mean responses. The observations enabled by experiments that explicitly assess inter-individual physiological variation address at least three major issues.

1. Climate change does not impact individuals equally, with thermal refugia and spatial heterogeneity in habitat conditions such as temperature likely playing a major role in population-level responses (Denny et al., 2011). The resulting biological outcomes also depend critically on how functionally variable individuals are distributed within this heterogeneous environmental space (Porlier et al., 2009).
2. It remains uncertain under what scenarios environmental stress and environmental heterogeneity mask or unmask functional, physiological variation. The answer in our analyses depends on the metric of choice, but some metrics such as growth and reproductive success better reflect integrative organismal function and fitness. Expression patterns in omics data, when combined with such organismal metrics, have the potential to expose the underlying mechanistic variation, but in themselves tell us little about organismal outcomes. We also suspect, but have yet to experimentally confirm, that patterns of inter-individual variation depend on the intensity of environmental stress imposed. Additional work clearly is needed to resolve these uncertainties. For example, a cohesive framework for assessing different metrics of physiological variation should account for the fact that different traits may be prone to more or less variation because of varying strengths of selection, the (in)flexibility of

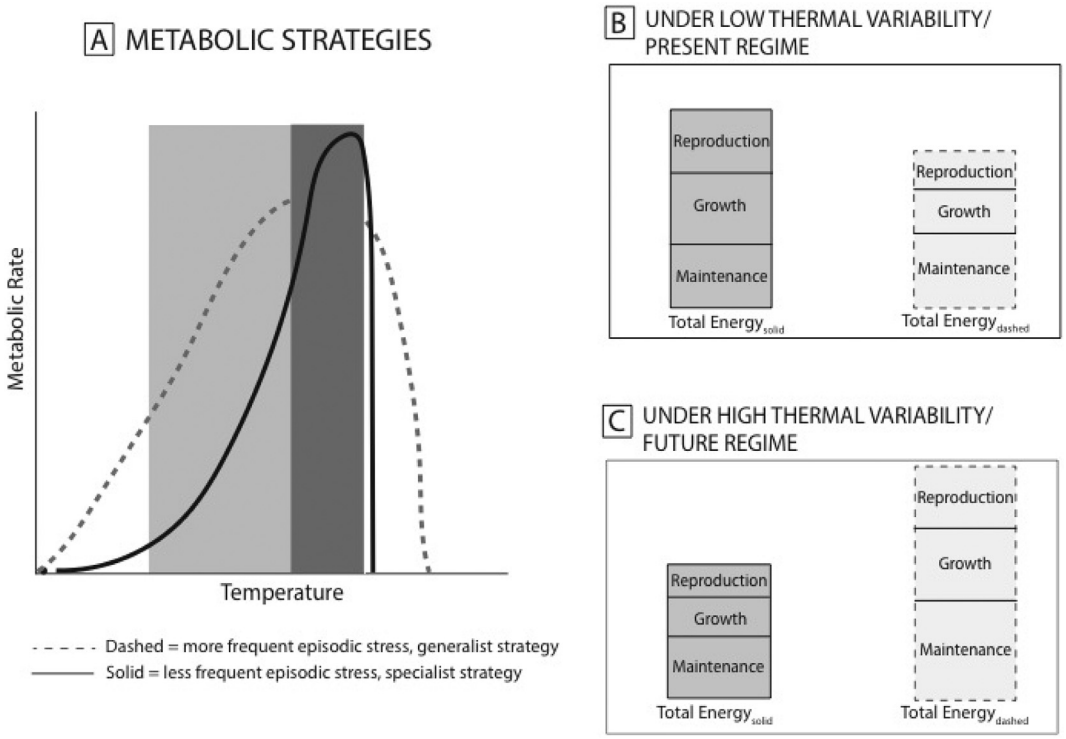

Fig. 6. Variable physiological energy allocation strategies interact with different environmental variability scenarios over longer time periods. A) Dashed vs. solid environmental variability scenarios depicted in Fig. 5 may correspond with a specialist or generalist strategy, respectively, in shape of the thermal performance curve for metabolic rate. We cannot assume the area under these curves are constant across all scenarios. B & C) When these specialist and generalist strategies are matched to corresponding environmental conditions, metabolic performance is maximized. However, physiological-environmental mismatch, due either to imperfect acclimatization when an individual finds itself in a sub-optimal environmental regime or to future climatic shifts, can reduce performance for both strategies. The relative sizes of these boxes illustrate only that a specialist strategy may maximize available energy under narrow thermal variability regimes in comparison with a generalist strategy that devotes more energy maintaining a wider range of available responses, and vice versa.

regulatory pathways, and/or inherent energetic trade-offs. In the mussel system, the lack of genetic information also hampers our ability to distinguish between the possible causes of variation (genetic variation, whether cryptic or not; environmentally induced variation, arising via developmental or reversible plasticity) and poses a major challenge.

These caveats notwithstanding, in the mussel example there seem to be very few origin-specific trends in variation across exposure treatments, suggesting that recent acclimatization is driving much of the shifts in patterns of physiological variation among mussels, across traits and irrespective of underlying genetic variation. This apparent primacy of recent experience may be particularly relevant to life-histories involving little predictability between parental and offspring environments. Such life histories are prominent in the sea (Caley et al., 1996; Gaines and Bertness, 1992; Grantham et al., 2003; Suryan et al., 2009), opening the possibility for future environmental shifts to dramatically alter patterns of inter-individual physiological variation in a variety of marine organisms. Understanding the sources of phenotypic variation within a population is critical under climate change scenarios in which genetic change alone is unlikely to match the pace of environmental change (Pespeni et al., 2013; Reusch, 2014). Partitioning variation among individuals with different genotypes and across traits also will allow for mechanistic connections among physiology, population genetic structure, and possibly higher-order ecological patterns (Harvey et al., 2013, 2014).

3. Lastly, physiological responses to environmental stress and physiological variation within a population are both poorly represented by snapshots. Future experiments should address temporal dynamics of variation in multiple physiological metrics, especially under repeated episodic stress events. The destructive nature of many physiological approaches poses another challenge to our ability to track individuals through time, but technological advances will soon overcome this constraint. It is our hope that marine environmental physiologists will place increasing emphasis on longer-term studies linking physiological and environmental variation.

Funding

This work was supported by National Science Foundation (USA) grants IOS-1256186 and IOS-1655822.

Ethics

Animals were collected under California Department of Fish and Wildlife permit SC-7955.

Data accessibility

Data for gene and protein expression are available in the supplement.

The authors, Richelle L. Tanner and W. Wesley Dowd, declare no conflict of interest with regard to manuscript submission, “Inter-individual physiological variation in responses to environmental variation and environmental change: integrating across traits and time”.

Acknowledgements

Dietmar Kültz and Lani Gleason provided invaluable support for the proteomics and transcriptomics analyses, respectively. Bhavya Papudeshi at the National Center for Genomic Analysis and Support

provided valuable support in the processing of transcriptomics data. We wish to thank Doris Abele, Nishad Jayasundara, Monica Medina, and Jonathon Stillman for sharing primary data. We also wish to thank two anonymous reviewers for their constructive feedback on the manuscript. Lastly, we thank Cheryl Logan and Tyler Evans for the invitation to contribute to this timely discussion.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.110577.

References

Albert, A., Zhang, L., 2010. A novel definition of the multivariate coefficient of variation. Biom. J. 52, 667–675.
Baek, D., Villén, J., Shin, C., Camargo, F.D., Gygi, S.P., Bartel, D.P., 2008. The impact of microRNAs on protein output. Nature 455, 64.
Beadman, H., Caldow, R., Kaiser, M., Willows, R., 2003. How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. Mar. Biol. 142, 487–494.
Beaman, J.E., White, C.R., Seebacher, F., 2016. Evolution of plasticity: mechanistic link between development and reversible acclimation. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2016.01.004.
Bennett, A.F., 1987. Interindividual variability: an underutilized resource. New Dir. Ecol. Physiol. 15, 147–169.
Bozinovic, F., Catalan, T.P., Estay, S.A., Sabat, P., 2013. Acclimation to daily thermal variability drives the metabolic performance curve. Evol. Ecol. Res. 15, 579–587.
Caley, M., Carr, M., Hixon, M., Hughes, T., Jones, G., Menge, B., 1996. Recruitment and the local dynamics of open marine populations. Annu. Rev. Ecol. Syst. 27, 477–500.
Connor, K.M., Robles, C.D., 2015. Within-site variation of growth rates and terminal sizes in *Mytilus californianus* along wave exposure and tidal gradients. Biol. Bull. 228, 39–51.
Cox, D.R., 1989. Analysis of Binary Data, 2nd ed. Routledgehttps://doi.org/10.1201/9781315137391.
Crawford, D.L., Oleksiak, M.F., 2007. The biological importance of measuring individual variation. J. Exp. Biol. 210, 1613–1621.
DeSalvo, M.K., Sunagawa, S., Fisher, P.L., Voolstra, C.R., Iglesias-Prieto, R., Medina, M., 2010. Coral host transcriptomic states are correlated with symbiodinium genotypes. Mol. Ecol. 19, 1174–1186.
Denny, M.W., 2018. Survival in spatially variable thermal environments: consequences of induced thermal defense. Integr. Zool. 13, 392–410.
Denny, M.W., Dowd, W.W., Bilir, L., Mach, K.J., 2011. Spreading the risk: small-scale body temperature variation among intertidal organisms and its implications for species persistence. J. Exp. Mar. Biol. Ecol. 400, 175–190. https://doi.org/10.1016/j.jembe.2011.02.006.
Dowd, W.W., King, F.A., Denny, M.W., 2015. Thermal variation, thermal extremes and the physiological performance of individuals. J. Exp. Biol. 218, 1956–1967. https://doi.org/10.1242/jeb.114926.
Elowitz, M.B., Levine, A.J., Siggia, E.D., Swain, P.S., 2002. Stochastic gene expression in a single cell. Science 297, 1183–1186.
Fitzgerald-Dehoog, L., Browning, J., Allen, B.J., 2012. Food and heat stress in the California mussel: evidence for an energetic trade-off between survival and growth. Biol. Bull. 223, 205–216.
Flatt, T., 2005. The evolutionary genetics of canalization. Q. Rev. Biol. 80, 287–316.
Gaines, S.D., Bertness, M.D., 1992. Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360, 579–580.
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.
Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N., Hughes, K.A., 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature 525, 372–375.
Gleason, L.U., Strand, E.L., Hizon, B.J., Dowd, W.W., 2018. Plasticity of thermal tolerance and its relationship with growth rate in juvenile mussels (*Mytilus californianus*). Proc. R. Soc. B Biol. Sci. 285, 20172617.
Gomez-Mestre, I., Jovani, R., 2013. A heuristic model on the role of plasticity in adaptive evolution: plasticity increases adaptation, population viability and genetic variation. Proc. R. Soc. B Biol. Sci. 280, 20131869.
Grantham, B.A., Eckert, G.L., Shanks, A.L., 2003. Dispersal potential of marine invertebrates in diverse habitats. Ecol. Appl. 13, 108–116.
Graves, S., Piepho, H.-P., Selzer, L., 2015. multcompView.
Greenbaum, D., Colangelo, C., Williams, K., Gerstein, M., 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. 4, 117. https://doi.org/10.1186/gb-2003-4-9-117.
Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. Annu. Rev. Mar. Sci. 8, 357.
Hampel, F.R., 1974. The influence curve and its role in robust estimation. J. Am. Stat. Assoc. 69, 383–393.
Harris, R.M., Beaumont, L.J., Vance, T.R., Tozer, C.R., Remenyi, T.A., Perkins-Kirkpatrick, S.E., Mitchell, P.J., Nicotra, A., McGregor, S., Andrew, N., 2018. Biological responses to the press and pulse of climate trends and extreme events. Nat. Clim. Chang. 8, 579.
Harvey, B.P., Gwynn-Jones, D., Moore, P.J., 2013. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. Ecol. Evol. 3, 1016–1030.
Harvey, B., Al-Janabi, B., Broszeit, S., Cioffi, R., Kumar, A., Aranguren-Gassis, M., Bailey, A., Green, L., Gsottbauer, C., Hall, E., 2014. Evolution of marine organisms under climate change at different levels of biological organisation. Water 6, 3545–3574.
Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., Pörtner, H.-O., 2006. Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. J. Exp. Biol. 209, 353–363.
Helmuth, B.S., Hofmann, G.E., 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biol. Bull. 201, 374–384.
Helmuth, B., Broitman, B.R., Blanchette, C.A., Gilman, S., Halpin, P., Harley, C.D.G., O'Donnell, M.J., Hofmann, G.E., Menge, B., Strickland, D., 2006. Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. Ecol. Monogr. 76, 461–479.
Ho, J.W., Stefani, M., Dos Remedios, C.G., Charleston, M.A., 2008. Differential variability analysis of gene expression and its application to human diseases. Bioinformatics 24, i390–i398.
Jayasundara, N., Somero, G.N., 2013. Physiological plasticity of cardiorespiratory function in a eurythermal marine teleost, the longjaw mudsucker, *Gillichthys mirabilis*. J. Exp. Biol. 216, 2111–2121.
Jentsch, A., Kreyling, J., Beierkuhnlein, C., 2007. A new generation of climate-change experiments: events, not trends. Front. Ecol. Environ. 5, 365–374.
Jimenez, A.G., Jayawardene, S., Alves, S., Dallmer, J., Dowd, W.W., 2015. Micro-scale environmental variation amplifies physiological variation among individual mussels. Proc. R. Soc. B Biol. Sci. 282, 20152273.
Jimenez, A.G., Alves, S., Dallmer, J., Njoo, E., Roa, S., Dowd, W.W., 2016. Acclimation to elevated emersion temperature has no effect on susceptibility to acute, heat-induced lipid peroxidation in an intertidal mussel (*Mytilus californianus*). Mar. Biol. 163, 55. https://doi.org/10.1007/s00227-016-2828-8.
Kenkel, C.D., Matz, M.V., 2017. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nat. Ecol. Evol. 1, 0014.
Kharin, V.V., Flato, G.M., Zhang, X., Gillett, N.P., Zwiers, F., Anderson, K.J., 2018. Risks from climate extremes change differently from 1.5°C to 2.0°C depending on rarity. Earths Future 6, 704–715. https://doi.org/10.1002/2018EF000813.
Krebs, R.A., Feder, M.E., 1997. Natural variation in the expression of the heat-shock protein HSP70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. Evolution 51, 173–179.
Kültz, D., 2003. Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. J. Exp. Biol. 206, 3119–3124.
Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67, 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635.
Kültz, D., Li, J., Zhang, X., Villarreal, F., Pham, T., Paguio, D., 2015. Population-specific plasma proteomes of marine and freshwater three-spined sticklebacks (*Gasterosteus aculeatus*). Proteomics 15, 3980–3992.
Leys, C., Ley, C., Klein, O., Bernard, P., Licata, L., 2013. Detecting outliers: do not use standard deviation around the mean, use absolute deviation around the median. J. Exp. Soc. Psychol. 49, 764–766. https://doi.org/10.1016/j.jesp.2013.03.013.
Liberzon, I., Krstov, M., Young, E.A., 1997. Stress-restress: effects on ACTH and fast feedback. Psychoneuroendocrinology 22, 443–453.
Lika, K., Kooijman, S.A., 2003. Life history implications of allocation to growth versus reproduction in dynamic energy budgets. Bull. Math. Biol. 65, 809–834.
Liu, Y., Beyer, A., Aebersold, R., 2016. On the dependency of cellular protein levels on mRNA abundance. Cell 165, 535–550. https://doi.org/10.1016/j.cell.2016.03.014.
Maechler, M., Rousseeuw, P., Croux, C., Todorov, V., Ruckstuhl, A., Salibian-Barrero, M., Verbeke, T., Koller, M., Conceicao, E.L., di Palmo, M.A., 2019. Robustbase: Basic Robust Statistics.
Mata, J., Marguerat, S., Bähler, J., 2005. Post-transcriptional control of gene expression: a genome-wide perspective. Trends Biochem. Sci. 30, 506–514. https://doi.org/10.1016/j.tibs.2005.07.005.
Miller, L.P., Dowd, W.W., 2019. Repeatable patterns of small-scale spatial variation in intertidal mussel beds and their implications for responses to climate change. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 236 110516.
Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D.-H., Tabira, T., 2001. Chronic stress differentially regulates glucocorticoid negative feedback response in rats. Psychoneuroendocrinology 26, 443–459.
Munday, P.L., Warner, R.R., Monro, K., Pandolfi, J.M., Marshall, D.J., 2013. Predicting evolutionary responses to climate change in the sea. Ecol. Lett. 16, 1488–1500. https://doi.org/10.1111/ele.12185.
National Academies of Sciences, E., and Medicine, 2016. Attribution of Extreme Weather Events in the Context of Climate Change. National Academies Press.
Nikinmaa, M., Waser, W., 2007. Molecular and cellular studies in evolutionary physiology of natural vertebrate populations: influences of individual variation and genetic components on sampling and measurements. J. Exp. Biol. 210, 1847–1857.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. Vegan: Community Ecology Package.
Oleksiak, M.F., Crawford, D.L., 2012. The relationship between phenotypic and environmental variation: do physiological responses reduce interindividual differences? Physiol. Biochem. Zool. 85, 572–584.
Oleksiak, M.F., Roach, J.L., Crawford, D.L., 2005. Natural variation in cardiac metabolism and gene expression in *Fundulus heteroclitus*. Nat. Genet. 37, 67.
Paaby, A.B., Rockman, M.V., 2014. Cryptic genetic variation: evolution's hidden

substrate. Nat. Rev. Genet. 15, 247.
Pespeni, M.H., Sanford, E., Gaylord, B., Hill, T.M., Hosfelt, J.D., Jaris, H.K., LaVigne, M., Lenz, E.A., Russell, A.D., Young, M.K., 2013. Evolutionary change during experimental ocean acidification. Proc. Natl. Acad. Sci. 110, 6937–6942.
Porlier, M., Bélisle, M., Garant, D., 2009. Non-random distribution of individual genetic diversity along an environmental gradient. Philos. Trans. R. Soc. B Biol. Sci. 364, 1543–1554.
Reusch, T.B., 2014. Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. Evol. Appl. 7, 104–122.
Reyment, R.A., 1960. Studies on Nigerian Upper Cretaceous and Lower Tertiary Ostracoda. University of Stockholm.
Rich, E.L., Romero, L.M., 2005. Exposure to chronic stress downregulates corticosterone responses to acute stressors. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 288, R1628–R1636.
Seebacher, F., Ducret, V., Little, A.G., Adriaenssens, B., 2015. Generalist–specialist trade-off during thermal acclimation. R. Soc. Open Sci. 2, 140251. https://doi.org/10.1098/rsos.140251.
Shama, L.N., 2017. The mean and variance of climate change in the oceans: hidden evolutionary potential under stochastic environmental variability in marine sticklebacks. Sci. Rep. 7, 8889.
Siegal, M.L., Bergman, A., 2002. Waddington's canalization revisited: developmental stability and evolution. Proc. Natl. Acad. Sci. 99, 10528–10532.
Suryan, R.M., Saba, V.S., Wallace, B.P., Hatch, S.A., Frederiksen, M., Wanless, S., 2009. Environmental forcing on life history strategies: evidence for multi-trophic level responses at ocean basin scales. Prog. Oceanogr., Comparat. Mar. Ecosyst. Struct. and Func. 81, 214–222. https://doi.org/10.1016/j.pocean.2009.04.012.
Takahashi, K.H., 2018. Multiple modes of canalization: links between genetic, environmental canalizations and developmental stability, and their trait-specificity. Semin. Cell Dev. Biol. https://doi.org/10.1016/j.semcdb.2018.05.018.
R Team Core, 2017. R: a Language and Environment for Statistical Computing. Vienna Austria R Found. Stat. Comput. R Foundation for Statistical Computing, Vienna, Austria.
Teranishi, K.S., Stillman, J.H., 2007. A cDNA microarray analysis of the response to heat stress in hepatopancreas tissue of the porcelain crab *Petrolisthes cinctipes*. Comp. Biochem. Physiol. Part D Genomics Proteomics 2, 53–62. https://doi.org/10.1016/j.cbd.2006.11.002.
Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. 213, 971–979.
Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. R. Soc. B Biol. Sci. 281. https://doi.org/10.1098/rspb.2013.2612.
Williams, T.D., 2007. Individual variation in endocrine systems: moving beyond the 'tyranny of the golden mean'. Philos. Trans. R. Soc. B Biol. Sci. 363, 1687–1698.
Zimmer, C., Taff, C.C., Ardia, D.R., Ryan, T.A., Winkler, D.W., Vitousek, M.N., 2019. On again, off again: acute stress response and negative feedback together predict resilience to experimental challenges. Funct. Ecol. 0. https://doi.org/10.1111/1365-2435.13281.