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Selection Experiments in the Sea: What Can Experimental Evolution Tell Us About How Marine Life Will Respond to Climate Change?

MORGAN W. KELLY[1,*] AND JOANNA S. GRIFFITHS[2]

[1]*Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803;*
and [2]*Department of Environmental Toxicology, University of California, Davis, California 95616*

Abstract. Rapid evolution may provide a buffer against extinction risk for some species threatened by climate change; however, the capacity to evolve rapidly enough to keep pace with changing environments is unknown for most taxa. The ecosystem-level consequences of climate adaptation are likely to be the largest in marine ecosystems, where short-lived phytoplankton with large effective population sizes make up the bulk of primary production. However, there are substantial challenges to predicting climate-driven evolution in marine systems, including multiple simultaneous axes of change and considerable heterogeneity in rates of change, as well as the biphasic life cycles of many marine metazoans, which expose different life stages to disparate sources of selection. A critical tool for addressing these challenges is experimental evolution, where populations of organisms are directly exposed to controlled sources of selection to test evolutionary responses. We review the use of experimental evolution to test the capacity to adapt to climate change stressors in marine species. The application of experimental evolution in this context has grown dramatically in the past decade, shedding light on the capacity for evolution, associated trade-offs, and the genetic architecture of stress-tolerance traits. Our goal is to highlight the utility of this approach for investigating potential responses to climate change and point a way forward for future studies.

Introduction

Climate change poses an unprecedented threat to the planet's biodiversity, and there is an urgent need to understand which taxa and ecosystems will be most vulnerable (Pacifici *et al.*, 2015; Urban, 2015). One factor that may ameliorate extinction risk in some species is the ability to evolve greater tolerance of climate change stressors; however, the capacity to evolve rapidly enough to keep pace with climate change is poorly understood in most taxa (Chevin *et al.*, 2010; Hoffmann and Sgrò, 2011; Munday *et al.*, 2013; Razgour *et al.*, 2019; Gaitán-Espitia and Hobday, 2021). In order to understand whether evolution will provide a buffer against climate change, we need to understand which species and which traits can evolve at a rate that allows them to keep pace with the velocity of environmental change.

Theory predicts that the rate of evolution depends on the strength of selection and the magnitude of genetic variation for the trait in question (Gomulkiewicz and Holt, 1995; Bell and Gonzalez, 2009). Adaptive genetic variation, in turn, tends to increase with population size but may also be either eroded or maintained by different forms of selection (Barton and Keightley, 2002; Turelli and Barton, 2004). The rate that new variation in a trait is added by mutations will also depend on a trait's architecture, with highly polygenic traits presenting a larger mutational target size (Houle, 1998). Taken together, these factors imply that there can be no one-size-fits-all prediction for the rates of evolution in changing environments, mostly because we know very little about the genetic basis or magnitude of adaptive genetic variation for most ecologically important traits.

One approach to measuring adaptive variation is to use quantitative genetic breeding experiments or trait data from individuals with a known pedigree to estimate additive variance for traits thought to be under selection (Sunday *et al.*, 2011; Kelly *et al.*, 2013; Chirgwin *et al.*, 2015). Estimates of genetic variation can then be incorporated into models predicting evolutionary change in altered environments and selection regimes. This approach is powerful, because genetic variance can, in theory, be measured for any trait for a species that can be bred

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* To whom correspondence should be addressed. Email: morgankelly@lsu.edu.

in captivity, or where pedigrees can be estimated by using genetic markers for individuals from wild populations. This approach is limited, however, in that traits that will influence fitness in a changing climate must be identified *a priori*. A second approach, used increasingly over the past decade, is to directly expose populations of organisms to controlled sources of selection, in either laboratory or field settings, and test their capacity to evolve (reviewed in Garland and Rose, 2009; Kawecki *et al.*, 2012). These types of experiments have the advantage of directly testing populations of organisms under future conditions, with no assumptions about which traits will be the targets of selection.

In this review, we will focus on how selection experiments and experimental evolution have been used to test the capacity of marine species to adapt to climate change stressors. The majority of these experiments have focused on microbes, but others have used metazoans with short generation times or have subjected the larvae of longer-lived organisms to a single episode of selection and used sequencing to test for genetic changes (Table 1). We will argue that there are several features of marine systems that make this approach particularly useful when it comes to understanding how marine species and ecosystems will respond to climate change.

The first important feature of marine systems is that, in contrast to terrestrial systems, nearly all primary productivity is accomplished by phytoplankton (Falkowski *et al.*, 2008). Because phytoplankton have large effective population sizes and short generation times, they are especially likely to exhibit at least some adaptation to climate change. This adaptation could have substantial effects on global carbon cycling, given the outsized role of phytoplankton in ocean food webs. Because phytoplankton have short generation times, and because many can be cultured in mesocosms, they are especially amenable to tests of adaptive capacity *via* experimental evolution (Reusch and Boyd, 2013). These experiments have yielded critical insights into the ways that ecological trajectories might be altered by evolutionary responses to climate change.

A second feature of marine systems is that many multicellular species have biphasic life cycles, with mobile larvae and sedentary adults. As a result, adults and larvae often experience different environments and selection pressures and may be differentially vulnerable to climate change (Przeslawski *et al.*, 2015; Marshall *et al.*, 2016). Selection experiments, when combined with sequencing, can help to tease apart the effects of these different sources of selection by testing whether selection acts on different loci in adults and larvae, a phenomenon demonstrated in wild populations by using molecular data (Hilbish, 1985; Schmidt and Rand, 2001).

The third feature of marine ecosystems is that anthropogenic change in the oceans is highly multivariate, with major axes of change, including temperature, pH, dissolved oxygen, nutrients, and removal of top predators from food webs (Crain *et al.*, 2008). Each of these changes is likely to impose a unique source of selection, with multivariate selection potentially producing different adaptive trajectories from any one source of selection acting alone (Brennan *et al.*, 2017). Experimental evolution allows investigators to impose multiple sources of selection in different combinations to examine these potentially synergistic effects.

Finally, marine species are especially likely to be living at the limits of their tolerance, because, with the exception of the intertidal zone, many marine environments lack the variation in thermal microhabitats often found in the terrestrial realm (Pinsky *et al.*, 2019). While mobile terrestrial species may be subject to the Bogert effect, where behavioral thermoregulation buffers them against direct selection on their thermal physiologies (Huey *et al.*, 2003), aquatic ectotherms cannot escape changes in water temperature. As a result, thermal tolerances of marine species are especially likely to have been shaped by selection imposed by historical temperature regimes, leading to local adaptation to temperature (Sanford and Kelly, 2011). Future changes in temperature will represent a direct change in the selection regimes experienced by aquatic species and may also more directly lead to population shifts and declines (Pinsky *et al.*, 2013).

Taken together, all of these features of marine systems imply that experimental evolution will yield especially important insights into the effects of climate change. In this review, we will focus on six questions about climate change impacts that are particularly well suited to this approach: (1) How much capacity do species have to adapt to climate change? (2) Can hybridization and introgression promote evolutionary rescue? (3) How is adaptation to climate change shaped by trait correlations and trade-offs? (4) What is the genetic architecture of traits involved in adaptation to climate change? (5) How is the genetic diversity of marine populations shaped by selection on different life stages? (6) How is adaptation to climate change influenced by different modes of selection?

We conclude with a discussion of how directed evolution may be applied to the conservation of threatened species. Our goal is to highlight the utility of experimental evolution approaches and to point a way forward in the use of these experiments to understand the effects of climate change in marine ecosystems.

1. How Much Capacity Do Species Have to Adapt to Climate Change?

The most straightforward application of experimental evolution is simply to measure the capacity for evolutionary change in a given population or species over a fixed period of time. Tests of adaptive capacity have revealed effects of adaptation on individual species' vulnerabilities and also a potential for ecosystem-level consequences of evolutionary change.

In the past, assessments of climate change vulnerability have tended to ignore the effects of evolution, especially in long-lived organisms, where evolution was thought to be too slow to have any measurable demographic effect on ecological timescales.

Table 1

List of studies that have used selection experiments to test the capacity of marine species to adapt to climate change stressors

Taxon	Source of selection	Number of generations	Traits measured	Reference
pH experiments				
Phytoplankton (*Emiliania huxleyi*)	High $p\mathrm{CO}_2$	~1700 (3 years)	Growth and survival	Bach *et al.*, 2018
Mussel (*Mytilus galloprovincialis*)	High $p\mathrm{CO}_2$	1	Growth and survival, allele frequency changes	Bitter *et al.*, 2019
Sea urchin (*Strongylocentrotus purpuratus*)	High $p\mathrm{CO}_2$	1	Allele frequency changes	Brennan *et al.*, 2019
Cyanobacterium (*Trichodesmium*)	High $p\mathrm{CO}_2$	570–850 (4.5 years)	Growth, nitrogen fixation, proteomics	Hutchins *et al.*, 2015
Coccolithophore (*Gephyrocapsa oceanica*)	High $p\mathrm{CO}_2$	670	Photosynthesis, growth, nitrogen	Jin *et al.*, 2013
Diatom (*Phaeodactylum tricornutum*)	High $p\mathrm{CO}_2$	1860	Growth, respiration	Li *et al.*, 2017
Green alga (*Chlamydomonas reinhardtii*)	High $p\mathrm{CO}_2$	900	Growth, cell size, photosynthesis, respiration	Lindberg *et al.*, 2020
Coccolithophore (*Emiliania huxleyi*)	High $p\mathrm{CO}_2$	1000	Growth rate, calcification, gene expression, cell size	Lohbeck *et al.*, 2012, 2013, 2014; Schlüter *et al.*, 2016
Sea urchin (*Strongylocentrotus purpuratus*)	High $p\mathrm{CO}_2$	11	Gene expression	Pespeni *et al.*, 2013
Phytoplankton (*Ostreococcus* sp.)	High $p\mathrm{CO}_2$, stable and fluctuating	4400	Growth, carbon and nitrogen production, sequence divergence	Schaum and Collins, 2014; Schaum *et al.*, 2016, 2018
Abalone (*Haliotis rufescens*)	High $p\mathrm{CO}_2$	1	Survival, lipids	Swezey *et al.*, 2020
Mussel (*Mytilus edulis*)	High $p\mathrm{CO}_2$	3	Survival, growth	Thomsen *et al.*, 2017
Coccolithophore (*Gephyrocapsa oceanica*)	High $p\mathrm{CO}_2$	1000	Growth, carbon, and nitrogen production	Tong *et al.*, 2018
Temperature experiments				
Phytoplankton strains	High temperature	300	Growth, photosynthesis, and respiration	Barton *et al.*, 2020
Symbiodinium strains	High temperature	41–69 asexual	Growth and photosynthesis	Chakravarti *et al.*, 2018
Copepod (*Tigriopus californicus*)	High temperature	15–21	Survival, allele frequency changes, sequence divergence, gene expression	Griffiths *et al.*, 2020
Copepod (*Tigriopus californicus*)	High temperature	10	Survival	Kelly *et al.*, 2012
Coccolithophore (*Emiliania huxleyi*)	High temperature	2.5 years	Thermal reaction norm, response to high $p\mathrm{CO}_2$	Listmann *et al.*, 2016
Green microalga (*Dunaliella tertiolecta*)	Size and high temperature	290	Population density, cell volume, biovolume, reactive oxygen species	Malerba and Marshall, 2020
Diatom (*Thalassiosira pseudonana*)	High temperature	350–450	Growth, nitrogen fixation	O'Donnell *et al.*, 2018
Coral (*Acropora spathulata*)	High temperature	1	Survival, bleaching and growth, genetic diversity	Quigley *et al.*, 2020
Green alga (*Chlamydomonas reinhardtii*)	High temperature	10 years	Growth	Schaum *et al.*, 2017
Salinity experiments				
Oyster (*Crassostrea virginica*)	Low salinity	1	Allele frequency changes, gene functions	JSG, MWK, and Kevin M. Johnson, California Polytechnic State University and California Sea Grant, unpubl. data
Multistressor experiments				
Copepod (*Acartia tonsa*)	High temperature, high $p\mathrm{CO}_2$	20	Survival, fecundity, allele frequency changes, nucleotide diversity, gene expression	Brennan *et al.*, 2021
Picoplankton (*Ostreococcus tauri*)	Light, phosphate, salinity, temperature stable and fluctuating	200	Growth, chlorophyll, viral resistance	Heath *et al.*, 2017

Table 1 (*Continued*)

Taxon	Source of selection	Number of generations	Traits measured	Reference
Copepod (*Tigriopus californicus*)	High temperature, low salinity, high salinity	5	Heat tolerance, fecundity	Kelly *et al.*, 2016a
Green alga (*Chlamydomonas reinhardtii*)	Salinity, pH, phosphate	200	Growth, single-nucleotide polymorphisms, methylation	Hutchins *et al.*, 2015; Heath *et al.*, 2017; Kronholm *et al.*, 2017; Bach *et al.*, 2018; O'Donnell *et al.*, 2018

However, many marine organisms, including many that are long lived, have large effective population sizes and are able to produce tremendous numbers of larvae at one time. For example, the red sea urchin (*Mesocentrotus franciscanus*) can live for more than a century, but a single female can produce 2×10^6 eggs in a single spawning event (Ebert and Southon, 2003). Consequently, if genetic variation for response to environmental change exists in this species, strong selection could produce substantial changes in allele frequencies in a single cohort of larvae without exerting a demographic cost strong enough to cause population declines.

As a result, the first goal of evolution experiments is often merely to demonstrate that adaptive variation for climate change responses exists, especially in the larvae of longer-lived metazoans. For example, an early selection experiment demonstrated genetic variation for the response to low pH in larvae of the purple sea urchin (*Strongylocentrotus purpuratus*) (Pespeni *et al.*, 2013). Similar experiments have also demonstrated a capacity to evolve in response to pH in mussels and abalone (Thomsen *et al.*, 2017; Swezey *et al.*, 2020), temperature in corals (Quigley *et al.*, 2020), and low salinity in oysters (JSG, MWK, and Kevin M. Johnson, California Polytechnic State University and California Sea Grant, unpubl. data).

In shorter-lived species, tests of adaptive capacity have moved beyond the simple question of whether species will evolve, to ask how evolutionary change might affect key ecological processes. In particular, evolutionary responses to climate change in phytoplankton are likely to have substantial cascading ecological effects, given that they are responsible for nearly all of the ocean's primary productivity (Falkowski *et al.*, 2008). A major ecosystem impact of climate change may be decreased rates of carbon sequestration, because respiration increases at a faster rate with warming than does photosynthesis (Regaudie-de-Gioux and Duarte, 2012). However, long-term selection experiments with three marine phytoplankton demonstrated that thermal adaptation counteracted the short-term effects of warming on metabolic rates, leading to the maintenance of, or even increases in, rates of carbon sequestration after accounting for the effects of adaptation (Barton *et al.*, 2020). This finding has global consequences, because marine phytoplankton fix 40% of all carbon (Field *et al.*, 1998).

Tests of evolutionary capacity have also yielded unexpected insights into which species or populations might be vulnerable to climate change. For example, geographic range size is often assumed to be inversely correlated with extinction risk from climate change, because large geographic ranges are assumed to stem from a broad environmental tolerance at the species level. But in some species, genetic subdivision may produce a collection of locally adapted populations whose environmental niches are narrower than the species as a whole. *Tigriopus californicus* copepods exhibit strong local adaptation to temperature, but less than 1% of the total quantitative variance for thermal tolerance is partitioned within populations (Kelly *et al.*, 2012). A 10-generation selection experiment revealed that heat-tolerant phenotypes observed in low-latitude populations could not be achieved in high-latitude populations, through either acclimation or selection; and many populations plateaued in their responses to selection, suggesting that standing variation had already been depleted. These experiments demonstrated that broad geographic ranges do not necessarily imply low extinction risk in every population of a widely distributed species.

Selection experiments have also revealed greater capacity for rapid evolutionary change in some traits than in others. For example, in *Tigriopus* copepods, hybrids between northern and southern populations have substantial capacity to evolve increased tolerance of warmer temperatures over just five generations of selection but little capacity to evolve increased tolerance of hyper- or hypo-osmotic stress (Kelly *et al.*, 2016a). This may be evidence for the importance of environmental gradients in maintaining genetic variation at the species level: *Tigriopus* populations experience a strong latitudinal gradient in temperature, with a concomitantly strong differentiation among populations in their temperature responses (Willet, 2010; Kelly *et al.*, 2012; Pereira *et al.*, 2017). By contrast, variation in salinity regimes follows more of a patchwork geographic pattern, and associated variation in salinity responses among populations also appears to be lower and less predictable (Leong *et al.*, 2017; Debiasse *et al.*, 2018).

2. Can Hybridization and Introgression Promote Evolutionary Rescue?

Experimental evolution can also be used to test the effects that gene flow among populations has on the likelihood of evolutionary rescue. For some species, the genetic variation needed for resiliency may be found at the scale of the species rather than at the population level, as is the case for the copepod *Tigriopus californicus* (Kelly *et al.*, 2012). Populations that are locally adapted to current environmental gradients may lack the genetic variants needed to further increase their tolerance to changing stressors and will eventually suffer from population decline. However, evolutionary rescue facilitated by gene flow or managed translocations may be a possible mechanism for population persistence (Davis and Shaw, 2001; Hamilton and Miller, 2016). Hybridization between populations and closely related species is common in the wild and has been documented as a mechanism of adapting to changing environments in fish and corals (Willis *et al.*, 2006; Bay *et al.*, 2019; Oziolor *et al.*, 2019). Adaptive introgression through assisted evolution may be a viable method to artificially increase population resiliency when gene flow between tolerant and intolerant populations is minimal (*i.e.*, limited dispersal distances). Experiments that aim to test the feasibility of hybridization and adaptation to climate stressors will provide important insight into the potential trade-offs and possibility of success.

The potential for adaptive introgression in the copepod *T. californicus* was investigated by hybridizing two populations with divergent heat tolerance limits, followed by subsequent selection for increased thermal tolerance (Griffiths *et al.*, 2020). *Tigriopus* is a prime example of the challenges that hybridized populations will face. The first generation of hybrids may contain beneficial and maladapted alleles on the same linkage block, and the rate of recombination will determine the capacity of the beneficial adapted alleles to escape before being removed by selection against maladaptive alleles (Sachdeva and Barton, 2018b). The relative strength of selection on beneficial and maladapted alleles will also determine the speed of introgression. However, if environmental tolerance (such as heat tolerance) is polygenic, the strength of selection will be distributed across many loci, potentially weakening the efficiency of integrating beneficial alleles (Sachdeva and Barton, 2018a). Maladaptation of foreign genotypes may also stem from intralocus selection, where negative epistatic interactions cause migrant alleles to be disfavored in the predominant genetic background of the new population. In *T. californicus*, strong mito-nuclear incompatibilities among interpopulation crosses and low recombination rates are predicted to make adaptive introgression difficult. Despite these barriers, 15 generations of heat selection successfully increased hybrid individuals' heat tolerance; this was coupled with higher frequencies of alleles from the heat-tolerant population.

Adaptive introgression is receiving more attention as a viable method for increasing resiliency to stressors as more examples are discovered in nature (Bay *et al.*, 2019; Oziolor *et al.*, 2019). These experiments have been tested in other non-marine species, but there is now an increasing number of studies that are experimentally testing the use of adaptive introgression for promoting evolutionary rescue in marine systems. For example, Quigley *et al.* (2020) hybridized *Acropora* individuals sourced from warm and cold reefs on the Great Barrier Reef and subjected families to heat selection. They found that interpopulation breeding maintained genetic variation for key genomic variants involved in heat tolerance that will be critical for future adaptation to heat stress.

3. How Is Adaptation to Climate Change Shaped by Trait Correlations and Trade-Offs?

One of the most important insights that can be gained through experimental evolution approaches is the potential effect of trait correlations on the outcomes of adaptation. While positive and negative phenotypic correlations between traits may provide some evidence for genetic correlations, these correlations may also be produced by other factors, including correlated sources of selection across environments. Selection experiments provide a powerful tool to directly test for genetic correlations, by testing whether selection on one trait produces correlated responses in others.

Genetic correlations between traits play an important role in shaping evolutionary responses to changing environments in several ways. First, evolution of increased stress tolerance may incur life-history costs. For example, *Tigriopus californicus* copepods selected for increased tolerance of heat stress also had a 30% reduction in fecundity relative to control lines (Fig. 1B; Kelly *et al.*, 2016a). By measuring costs of adaptation, experimental evolution can help to improve predictions about climate change effects: even in the cases where populations are able to evolve greater stress tolerance, it may come at a cost of decreased growth rates.

Second, when multiple environmental variables are changing simultaneously, there may be trade-offs among stress-tolerance traits, so that evolution of increased tolerance of one stressor incurs a loss of tolerance for others. More generally, correlations between stress traits may impede adaptation if the correlation opposes the multivariate direction of selection on those traits (Lande and Arnold, 1983; Blows and Hoffmann, 2005). One important example of a trait correlation that might hinder the response to multivariate selection is the opposing adaptive responses to elevated CO_2 and temperature in algae. Green algae (*Chlamydomonas reinhardtii*) that were experimentally adapted to warm temperatures had higher growth rates and were better competitors than algae exposed to ambient conditions (Schaum *et al.*, 2017). Higher growth rates were attributed to increased photosynthetic capacity, which

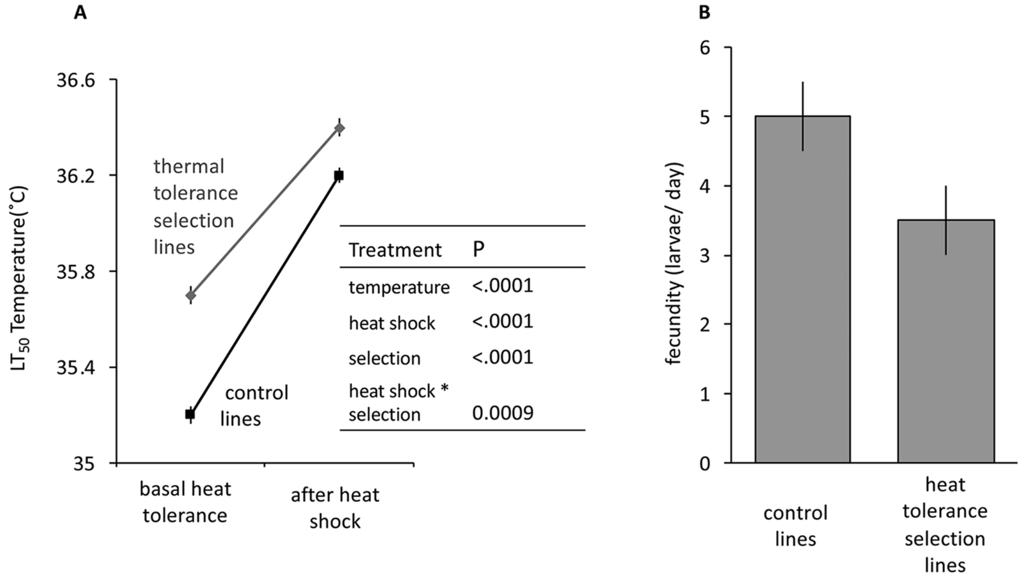

Figure 1. Costs and trade-offs incurred through the evolution of increased heat tolerance in *Tigriopus californicus* copepods. (A) Reaction norms showing basal heat tolerance (LT_{50}, ±SE) for adult male *T. californicus* reared at 19 °C and heat tolerance after 30 °C heat shock for control lines and lines selected for increased thermal tolerance. (Inset) Results of a logistic regression testing for effects of temperature, heat shock treatment, and selection treatment on probability of mortality. (B) Mean fecundity (larvae per day, ±SE) for female *Tigriopus californicus* from hybrid lines selected for five generations for increased tolerance of heat stress and control lines maintained under laboratory conditions.

was correlated with reduced susceptibility to photoinhibition when cultures were exposed to high irradiance. However, other studies have observed markedly different phenotypic responses in phytoplankton when evolved to elevated CO_2 conditions (Li *et al.*, 2017). *Chlamydomonas reinhardtii* exhibited reduced growth and respiration to remedy the reduction in photosynthetic capacity (Lindberg and Collins, 2020). These two studies (Li *et al.*, 2017; Lindberg and Collins, 2020) suggest that the combined stressors of warming and elevated CO_2 may impose a selection pressure in opposite directions on the same cellular machinery.

Finally, even when selection is univariate, genetic covariances will produce correlated responses in other traits besides the one directly under selection. If these correlated traits are ecologically important, the consequences of adaptation to a changing environment will extend beyond the direct demographic effects of evolutionary rescue. Several studies targeting stress tolerance have observed that as populations evolved increased mean stress tolerance, the plasticity of that tolerance declined (Fig. 1A; Kelly *et al.*, 2016b; Brennan *et al.*, 2021). Because plasticity may also contribute to resilience, a loss of plasticity implies a reduction in future resilience to environmental stress. Other correlated responses will have ecological consequences that extend beyond the resilience of a single species. For example, adaptation to increased temperatures is negatively correlated with cell size in phytoplankton, with implications for both carbon cycling and food webs (Malerba and Marshall, 2020).

4. What Is the Genetic Architecture of Adaptation to Climate Change?

Experimental evolution has become a valuable tool in the effort to describe the genetic basis of ecologically important traits. This is because selection experiments can be combined with sequencing to identify the targets of selection, a technique termed "evolve and re-sequence" (Turner and Miller, 2012). When comparing control and experimental lines or experimental lines pre- and post-selection for increased stress tolerance, loci that experience changes in allele frequencies are expected to be involved in tolerance to that stressor. Tolerance to climate change-related stressors is expected to be controlled by polygenic traits, because there are many possible avenues to achieve tolerance. Emerging evidence confirms the polygenic nature of heat tolerance in copepods and corals (Dixon *et al.*, 2015; Griffiths *et al.*, 2020; Quigley *et al.*, 2020), salinity tolerance in oysters (Fig. 2; JSG, MWK, and Kevin M. Johnson, California Polytechnic State University and California Sea Grant, unpubl. data), and acidification tolerance in mussels and sea urchins (Bitter *et al.*, 2019; Brennan *et al.*, 2019). These studies demonstrated repeatable changes in allele frequencies found on multiple chromosomes. Evolve and re-sequencing techniques offer a valuable solution for identifying loci under selection for polygenic traits involved in adaptation to environmental gradients; these can often be difficult to identify *via* population genomic approaches (Yeaman, 2015).

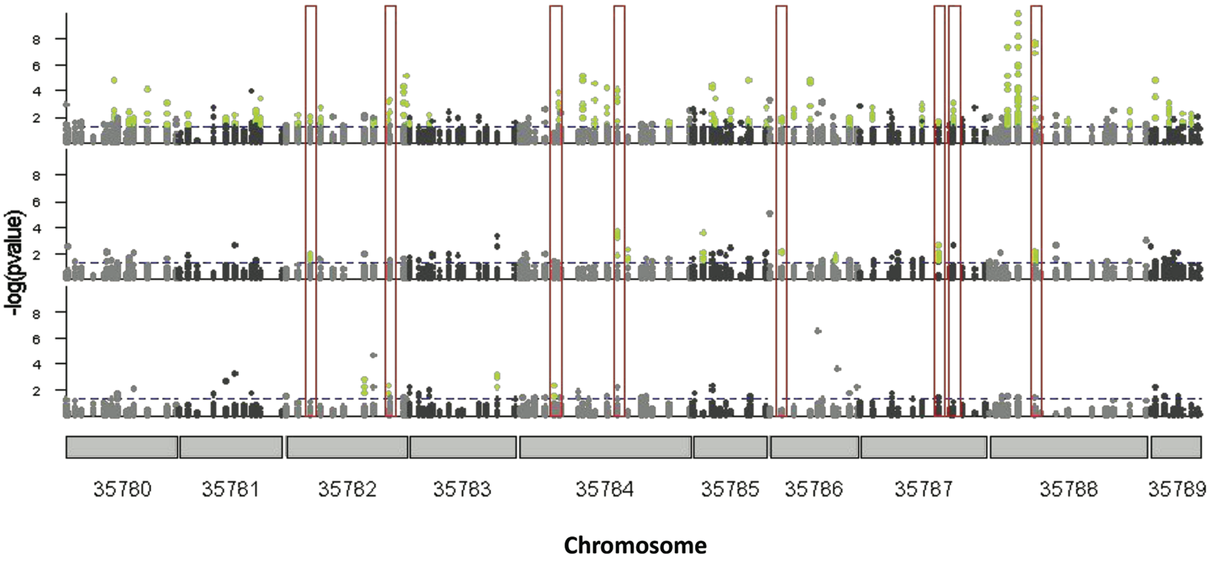

Figure 2. Manhattan plot showing allele frequency changes for single-nucleotide polymorphisms (SNPs) from three single-pair crosses exposed to low-salinity selection. *P*-values are displayed as –log from Fisher's exact test, and the blue horizontal dashed line represents the significance threshold after Bonferroni correction. The SNPs colored green pass our threshold for considering the gene to be under selection (three significant SNPs per gene). Red boxes highlight genes that are under selection in more than one cross. Chromosome names originate from the *Crassostrea virginica* reference genome.

Characterizing loci that contribute to adaptation (*i.e.*, gene function and expression, nucleotide diversity, epigenetic patterns) will allow us to better model adaptive processes. Experimental evolution studies often find that genes under selection are enriched in functions related to stress responses: calcification and metabolism in response to ocean acidification (Bitter *et al.*, 2019; Brennan *et al.*, 2019), and immunity, calcification, and metabolism in response to temperature (Quigley *et al.*, 2020). While these studies illuminate cellular and physiological mechanisms of tolerance, exploring the genetic architecture can inform the processes and rates of adaptation. Griffiths *et al.* (2020) found that genes under selection for heat tolerance were highly conserved among source populations (low protein sequence divergence). Polygenic traits are predicted to be under stabilizing selection in natural populations to maintain trait optima along environmental gradients; this results in reduced genetic variation at each locus maintained by purifying selection on protein coding sequences. Experimental evolution studies have also revealed complicated roles for evolutionary changes in gene expression. Brennan *et al.* (2021) discovered that after three generations of selection to greenhouse conditions, the response was explained by transcriptional changes. However, after 30 generations, gene expression was no longer correlated with genes that changed in allele frequencies, suggesting that the transcriptional response was canalized. Epigenetic changes have also been found to be important for adapting to environmental stressors. For example, blocking methylation and histone acetylation decreased rates of adaptation to stress in *Chlamydomonas reinhardtii* (Kronholm *et al.*, 2017), suggesting a need to include epigenetic variation in models of adaptation (Kronholm *et al.*, 2012).

5. How Is the Genetic Diversity of Marine Populations Shaped by Selection on Different Life Stages?

Many marine organisms have biphasic life cycles, with mobile larvae and sedentary adults. This means that their selective environments are often quite different. Adults may produce millions of larvae, with a tiny fraction actually metamorphosing into juveniles. As a result, selection during the larval phase (Gaffney *et al.*, 1992), chance (Hedgecock and Pudovkin, 2011), and oceanographic currents (Sotka *et al.*, 2004) are likely to provide a substantial filter on which genotypes arrive in a particular adult habitat. If the genotypes that have high fitness as larvae are not the same as those that have high fitness as adults, decoupled selection during the larval phase, plus displacement of larvae *via* oceanographic currents, may lead to maladaptation in benthic adults; and post-settlement selection on mismatched genotypes may effectively block gene flow between habitats that would otherwise be connected by dispersal (Burgess *et al.*, 2012). Empirical evidence for decoupled selection on larvae and adults comes from measurements of allele frequencies through time in cohorts of benthic invertebrates, with mortality-driven changes in allele frequencies as newly settled juveniles mature into adults (Hilbish, 1985; Schmidt and Rand, 2001).

Selection experiments can be used to explore the potential for decoupled selection on larvae and adults in several ways. The first and most simple contribution that selection experiments can make is simply to demonstrate that genetic variation for performance across ecologically relevant environmental gradients exists in marine larvae. This has now been demonstrated across a range of environmental gradients and taxa, including pH in sea urchins (Brennan *et al.*, 2019) and mussels (Bitter *et al.*, 2019) and low salinity in oysters (Fig. 2; JSG, MWK, and Kevin M. Johnson, California Polytechnic State University and California Sea Grant, unpubl. data). Selection experiments can also test more directly for decoupled selection between larvae and adults by performing selection at the larval phase and then asking whether the resulting juveniles and adults are better or worse adapted to that stressor than controls. Interestingly, adult mussels from low-pH habitats produce larvae that fare better under low pH, suggesting that filtering at either the larval stage or the adult stage has improved the match between larvae and their environment (Thomsen *et al.*, 2017). However, three generations of selection in the lab failed to improve survival under low pH in these same populations, suggesting that pH-driven selection in the field was different or more multifaceted than selection imposed in the lab (Thomsen *et al.*, 2017).

6. How Is Adaptation Influenced by Different Modes of Selection?

A final important feature of experimental evolution is the capacity to test the effects of different modes of selection on adaptation. Anthropogenic change in the oceans will be highly multivariate, with major axes of change, including temperature, pH, dissolved oxygen, nutrients, and removal of top predators from food webs (Crain *et al.*, 2008). Each of these changes is likely to impose a unique source of selection, with combined multivariate selection potentially acting in synergistic ways. Rates of climate change are also likely to vary across latitude, and levels of temporal and spatial heterogeneity will vary across different marine habitats. All of these factors will produce a much more complicated selection environment than a monotonic change in a single environmental variable. Selection experiments present an opportunity to test the effects of all of these and other facets of the selective environment on rates of adaptation.

Experiments that have varied selection in complex ways have yielded important and sometimes unexpected insights. For example, marine phytoplankton evolved in stable *versus* fluctuating high-CO_2 environments exhibit markedly different phenotypic responses, with differences in C:N ratio, cell size, and lipid content between fluctuating and stable selection lines (Schaum *et al.*, 2016). Responses to multivariate selection also often appear to depart from expectations based on single-stressor studies. In the most comprehensive examination of multidriver responses to date, Brennan *et al.* (2017) evolved a green alga in 96 unique combinations of 8 sources of selection. While the algae responded primarily to a few dominant drivers, they exhibited a stronger response to these drivers in a multidriver context than in isolation, likely because selection was stronger in the multidriver environments. Finally, the timescale of selection often appears to have substantial effects on the observed response, indicating caution when extrapolating long-term responses from short-term studies (Schlüter *et al.*, 2016; Li *et al.*, 2017; Tong *et al.*, 2018).

Coda: Applying Directed Evolution and Selective Breeding to Conservation of Threatened Species

Directed selection has become an increasingly common experimental approach in climate change studies, but it is also increasingly being applied directly to the conservation of threatened species, including those that are threatened by climate change. The capacity for evolutionary adaptation to environmental stressors is dependent on the amount of genetic variation present in natural populations. The presence of heritable genetic variation for traits that improve fitness in the novel environment provides a glimmer of hope for population persistence, but human intervention may still be necessary. For example, at low population densities individuals may be unable to find each other to reproduce, as in the white abalone (Rogers-Bennett *et al.*, 2016); or the variation needed for tolerance may exist at spatial scales larger than dispersal capabilities (Griffiths, 2020). Assisted evolution is a possible method to promote resistance and to supplement rapidly declining populations.

Supportive breeding programs often protect sensitive early life stages from stress through management practices that include seawater buffering against low aragonite saturation, temperature controls, antibiotic additions to reduce disease, and high food availability (Barton *et al.*, 2015; Rogers-Bennett *et al.*, 2016). While these practices increase survival in the hatchery, the resulting organisms may be ill prepared for stressful conditions in natural settings upon release. Artificial selection may be a viable method to improve tolerance of climate change-related stressors. For example, genetic variation for low pH tolerance was found in a hatchery population of red abalone (Swezey *et al.*, 2020), suggesting that this trait can be selectively bred in the hatchery to increase resiliency to ocean acidification. These techniques can also be applied to non-aquaculture species, such as corals. Chakravarti and van Oppen (2018) were able to increase the thermal tolerance in experimentally evolved *Symbiodium* lines. By inoculating corals with a variety of heat-tolerant *Symbiodium*, researchers can potentially increase coral fitness under warming temperatures.

Integrating hatchery-reared individuals into the wild population has been successful with small population sizes, such as in Pacific abalone (Sekino *et al.*, 2019). However, the genetic and demographic impact of releasing selectively bred individuals may be minimal if the natural population size is large relative to introduced individuals; this would increase the time needed for beneficial alleles to spread throughout the population. Population genetics theory predicts that genetic rescue through gene flow of introduced individuals will be slow (Schiffers *et al.*, 2012); therefore, introduction of selectively bred individuals will require an intermediate supplementation rate to increase the likelihood of genetic rescue while limiting genetic swamping (Bell *et al.*, 2019).

Trait correlations will be an important consideration when determining the effort needed for resiliency breeding. For example, in some managed populations, further selection may not be required. Traits that are favored in captive breeding settings, such as fast growth and disease resistance, have been correlated with increased resistance to ecologically relevant stressors, such as ocean acidification resistance in oysters (Durland *et al.*, 2019; Fitzer *et al.*, 2019). However, in other systems, rapid growth has been negatively associated with fitness in acidified conditions, as in red abalone (Swezey *et al.*, 2020), so that ecological resiliency runs antagonistic to traits that increase production yield.

Trait correlations will also be important when considering the fitness of individuals in both captive and natural environments. Captive rearing can cause individuals to be maladapted to the wild upon release, through either domestication selection or selective breeding for ecologically relevant traits (Christie *et al.*, 2012). For example, domestication selection for rapid growth in brown trout hatcheries is associated with higher metabolic rates and risk-taking behaviors; these behaviors were found to be unfavorable in the natural environment (Álvarez and Nicieza, 2005), as they increased vulnerability to predators (Johnsson and Abrahams, 1991). Intentional selective breeding in captive individuals may also inadvertently result in reduced fitness in the wild, partly because there are multiple stressors and potential sources of selection acting in the wild that are difficult to replicate in a captive setting (Thomsen *et al.*, 2017). In addition, strong selection has been shown to result in reduced plasticity to ambient conditions or other ecologically important stressors (Parker *et al.*, 2017; Brennan *et al.*, 2021). As a result, selectively bred individuals in controlled lab settings may have reduced fitness in naturally fluctuating environments.

Finally, another unintentional consequence of selective breeding in hatcheries is a reduction in mean genetic diversity, on which resiliency relies (Chen *et al.*, 2017). Genetic diversity may already be limited in hatchery settings as a result of small population sizes, inbreeding, and genetic drift; and rare maladaptive alleles may be swept to high frequencies during selective breeding. Genetic diversity can be bolstered by harnessing the natural variation that already exists in the wild and by breeding captive and wild individuals, if available. Hybridization between wild populations or closely related species can also be used to increase genetic diversity before selective breeding efforts and maintain genetic diversity post-selection (Quigley *et al.*, 2020). Balancing the need for increased tolerance to stressors without losing genetic diversity will be a difficult but necessary task moving forward.

Guidelines for Experimental Evolution Studies

The work we have reviewed here and our own experience conducting artificial evolution experiments suggest several important considerations for the design of future studies aimed at assessing a species' adaptive capacity.

Effective population size (N_e) *of experimental lines*. Theory predicts that populations will lose genetic diversity at a rate proportional to $1/(2N_e)$ per generation, where N_e is the effective population size. In cases where experimenters are testing the capacity of a population to adapt based on standing genetic variation, experimental populations will necessarily contain only a subset of the genetic variation present in wild populations. As a result, it is important to remember that the evolutionary responses in experimental populations will tend to be smaller than those in natural populations and may not include responses based on rare variants. However, data from evolution experiments can still be used to produce estimates of realized heritability and additive genetic variance for environmental tolerance traits, necessary to parameterize evolutionary rescue models (Gomulkiewicz and Holt, 1995).

Effective population size is also an important consideration for experiments testing the capacity for adaptation based on new mutations, because the number of new mutations entering a population each generation is proportional to its size. We recommend maximizing effective population size within the limits of experimental constraints but also including a minimum of three experimental replicates to examine potential effects of drift on evolutionary trajectories. We also recommend including information about experimental population sizes in the interpretation of results, by estimating how rare a natural adaptive variant would need to be to be missed by a particular experimental design.

Source population(s) for experimental lines. Genetic variation for response to future environments may be maintained through local adaptation to current environmental gradients. As a result, it is important to consider source populations sampled to create experimental lines. For example, equatorward populations might have greater thermal tolerance but might also have depleted variation for thermal tolerance due to the effects of both drift and selection in trailing-edge populations. Where possible, multiple populations should be included as sources for laboratory selection lines. Hybridization of multiple populations can also limit the loss of genetic diversity post-selection (Quigley *et al.*, 2020), but it can also lead to outbreeding depression driven by epistatic effects (Griffiths *et al.*, 2020).

Strength of selection. Strong selection can erode variation by decreasing effective population size and can alter the genetic architecture of adaptation by fixing more large-effect mutations with negative pleiotropic effects. We recommend that experimenters should consider how laboratory selection regimes compare to natural rates of change and (where possible) test multiple rates of change to examine how the strength of selection affects evolutionary outcomes. In conjunction with the strength of selection, the number of generations over which selection is applied also needs to be considered. For long-lived species, single-generation selection experiments may be the only viable option. However, with shorter-lived species, weaker selection coefficients may be applied over multiple generations, which may slow the loss of genetic diversity. In addition, multiple generations of selection will allow more time for recombination to break up large linkage blocks that may contain maladaptive alleles. Ultimately, the chosen strength of selection may depend on the fate of selectively bred individuals, whether they will be replacing a nearly extant population or introducing beneficial alleles into a larger natural population through low rates of supplementation and gene flow.

Method of selection. The effective population size of laboratory selection lines is often determined by logistical constraints, including the efficiency with which adaptive genotypes can be sorted from non-adaptive genotypes. One approach is to gradually change experimental conditions and allow genotypes to compete with one another in experimental culture. This will typically be much more efficient and allow for larger effective population sizes than when genotypes must be sorted by hand, but it carries the disadvantage that the strength of selection typically cannot be measured directly. Experimenters will also want to consider the use of fluctuating *versus* directional selection. While directional selection can increase tolerance to a specific stressor, it may come at a cost of reduced plasticity. Fluctuating experiments will more closely mimic conditions found in natural environments, and they often lead to increased tolerance to further stressful conditions (Schaum *et al.*, 2016). In addition, fluctuating selection can also accelerate adaptation if relaxed selection allows for a restoration of larger population sizes (Schaum *et al.*, 2018). Experimenters may also want to consider selection to multiple stressors if the ultimate goal is to understand adaptation to conditions experienced by wild populations.

Interactions between acclimation and adaptation. For many stress-tolerance traits, the capacity for acclimation will affect observed stress tolerance and possibly the realized additive variance for that trait. As a result, it is important to consider the rate of change in environmental variables, how this rate relates to natural temporal variation, and how it might affect capacities for both acclimation and adaptation. Both acclimation and adaptation will likely play an important role for species persisting in novel environments, and the interactions can be investigated in experimental evolution studies. For example, prior exposure to a non-lethal stressor before selection can increase an individual's tolerance more than with selection alone. This method may be selecting for alleles with higher plastic responses to stress that provide an added benefit to novel stressors.

Finally, and more generally, we recommend that marine researchers consult the wealth of experimental evolution literature on terrestrial organisms when designing experiments, because there is a longer history of theory and experimentation in these systems and they are often more experimentally tractable (*e.g.*, see review by Garland and Rose, 2009).

Conclusion

Until recently, it was rare to consider the capacity for rapid adaptation to climate change as a potential buffer against extinction risk. This was probably due to the assumption that evolution would occur too slowly to have any impact on a species' vulnerability to climate change. We now understand that evolution may help to ameliorate extinction risk in some cases, but we still know little about the circumstances where evolution can be expected to play a significant role in ecological responses to climate change. Experimental evolution is a critical tool in the continued effort to fill this knowledge gap. A better understanding of the factors that promote adaptation will provide critical support to efforts aimed at promoting resilience to climate change in sensitive species.

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