

UNIVERSITY OF CALIFORNIA

Santa Barbara

The Evolution of Plastic Responses to Global Change:
Studies in Two Species of Coastal Marine Invertebrates

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Ecology, Evolution and Marine Biology

by

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September 2023

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Vita of Samuel Neill Bogan

Education

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Publications

- Bogan SN**, Johns JW, Griffiths JS, Davenport DL, Smith SJS, Schaal SM, Downey-Wall AM, Lou RN, Lotterhos K, Guidry M, McGirr JA, Rivera HE, Roberts SB, Puritz JB, and Silliman KE. A Dynamic Web Resource for Robust and Reproducible Genomics in Marine and Non-model Species: marineomics.io (in press).
- Bogan SN**, Strader ME, and Hofmann GE. 2023. Associations between DNA methylation and gene regulation depend on chromatin accessibility during transgenerational plasticity. *BMC Biology* 21: 149.
- Bogan SN**, Johnson KM, and Hofmann GE. 2020. Changes in Genome-wide Methylation and Gene Expression in Response to Future $p\text{CO}_2$ Extremes in the Antarctic Pteropod *Limacina helicina antarctica*. *Frontiers in Marine Science* 6: 788.
- Bogan SN** and Place SP. 2019. Accelerated Evolution at Chaperone Promoters among Antarctic Notothenioid Fishes. *BMC Evolutionary Biology* 19: 205.
- Bogan SN**, McMahon JB, Pechenik JA, and Pires A. 2019. Legacy of Multiple Stressors: Ocean Acidification and Nutrition in Larvae and Juveniles of a Marine Gastropod. *Biological Bulletin* 236(3): 159–173.
- Publications in revision or under review*
- Bogan SN**, Porat OI, Meneses MJ, and Hofmann GE. Thermal Plasticity Has Greater Fitness Costs among Thermally Tolerant Genotypes (in revision)
- Bogan SN**, and Yi SV. Potential Role of Epigenetics as Drivers of Plastic Responses to the Environment Across Cells, Organisms, and Populations (in revision).

Academic Honors & Awards

- 2023 **Graduate Division Dissertation Fellowship,**
UC Santa Barbara
- 2023 **Charles A. Storke Award,**
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- 2023 **Finalist: Mary Rice Award for Best Student Oral Presentation,**
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- 2022 **Nomination for President’s Dissertation Year Fellowship,**
UC Santa Barbara Dept. of Ecology, Evolution and Marine
Biology 2021 **Worster Award,**
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Biology 2020 **Nomination for Outstanding Teaching Assistant Award,**
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- 2021 **Grant in Aid of Research** (\$769),
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- 2021 **Worster Summer Research Grant** (\$6,580),
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- 2020 **Faculty Research Grant** (\$10,654; co-written with Dr. Gretchen Hofmann), UC Santa Barbara Academic Senate
- 2019 **Mildred E. Mathias Graduate Student Research Grant** (\$1,400), University of California Natural Reserve System
- 2018 **NSF Student Travel Grant** (\$1,800), National Science Foundation
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Invited Talks & Panels

- 2023 Bogan SN, Porat OI, and Hofmann GE. Thermal Plasticity has Greater Fitness Costs among Tolerant Genotypes. **Mary Rice Award Best Student Presentation Session, Society of Integrative and Comparative Biology Annual Meeting**, Austin, TX (invited talk).
- 2022 Bogan SN, Strader ME, and Hofmann GE. One Tool in a Toolkit: Integrative, Genewise Tests of Associations Between the Environment, DNA Methylation, and Gene Expression. **Congress of Marine Epigenetics**, Marine Biological Laboratory, Woods Hole, MA (featured speaker).
- 2022 Bogan SN, Therkildsen NO, and Wares JP. Panel: Synthesis across Genomic Data Sources. **Research Coordination Network for Evolution in Changing Seas Training and Integration Workshop**, Shoals Marine Lab, NH (invited panelist).
- 2021 Bogan SN, Leach TS, Sugano CS, Kozal LC, Hoshijima U, and Hofmann GE. *In situ* Environments Induce Maternal Effects on Larval Performance in the Purple Sea Urchin. **Santa Barbara Channel LTER Seminar**, (invited talk).
- 2018 Bogan SN, Ingraham M, and Place SP. New Turns and ‘U-Turns’ in *Cis- Trans*-regulation of Molecular Chaperones Among Antarctic Fishes. **UC Davis Graduate Group in Population Biology**, Davis, CA (invited talk).

Conference Presentations

- 2023 Bogan SN, Strader ME, and Hofmann GE. The phenome wide heritability of adaptive plasticity. **Society of Molecular Biology and Evolution**, Ferrara, Italy (poster).
- 2021 Bogan SN, Strader ME, and Hofmann GE. Gene Regulatory Roles of DNA Methylation During Transgenerational Plasticity in the Sea Urchin *Strongylocentrotus purpuratus*. **Society of Integrative and Comparative Biology Annual Meeting**, virtual meeting (talk).

- 2020 Bogan SN, Meneses MJ, and Kozal LC, Albrecht AM, and Hofmann GE. Sex-dependent Effects of Local Adaptation on the Thermal Physiology of *Tigriopus californicus* Across a Latitudinal Cline. **Western Society of Naturalists**, virtual meeting (talk).
- 2020 Bogan SN, Johnson KJ and Hofmann GE. Changes in Genome-wide Methylation in Response to Ocean Acidification in the Pteropod *Limacina helicina antarctica* and Evolutionary Differences in DNA Methylation Across Pteropoda. **AGU Ocean Sciences Meeting**, San Diego, CA (poster).
- 2019 Bogan SN, Kozal LC, Meneses MJ, Albrecht A.M., and Hofmann GE. Eco-evolutionary and Molecular Underpinnings of Plasticity in Thermal Tolerance Among Natural Populations of *Tigriopus californicus*. **Western Society of Naturalists**, Ensenada, Mexico (poster).
- 2018 Bogan SN, Ingraham M, and Place SP. Regulation of a Lost Inducible Heat Shock Response in Antarctic Fishes. **POLAR2018**, Davos, Switzerland (poster).
- 2018 Bogan SN, McMahon JB, Pechenik JA, and Pires A. Latent and Interactive Effects of Ocean Acidification and Nutrition Across the Larva to Juvenile Transition in an Intertidal Gastropod. **Society of Integrative and Comparative Biology Annual Meeting**, San Francisco, CA (talk).
- 2018 Bogan SN, M Ingraham, and SP Place. Regulatory Origins of a Lost Inducible Heat Shock Response in Antarctic Fishes. **Society of Integrative and Comparative Biology Annual Meeting**, San Francisco, CA (poster).
- 2016 Bogan SN, Pechenik JA, Burns R, and Mei M. Influence of Reduced pH on Growth and Development of the Marine Gastropod *Crepidula fornicata*. **Society of Integrative and Comparative Biology Annual Meeting**, Portland, OR (poster).

Research Appointments

- 2018 – pres. **Graduate Student Researcher**, University of California, Santa Barbara, Dept. of Ecology, Evolution and Marine Biology, CA
- 2020 – 2021 **Graduate Student Researcher**, Santa Barbara Coastal LTER
- 2017 **Graduate Student Researcher**, NSF Office of Polar Programs, McMurdo Station, Antarctica
- 2016 – 2018 **Graduate Student Researcher**, Sonoma State University, Dept. of Biology, CA
- 2016 **Visiting Student Researcher**, KAUST, Red Sea Research Center, Saudi Arabia
- 2015 **Undergraduate Researcher**, University of Washington, Friday Harbor Labs, WA

Teaching Appointments

- 2018 – pres. **Graduate Student Researcher**, University of California, Santa Barbara, Dept. of Ecology, Evolution and Marine Biology, CA

- 2020 – 2021 **Graduate Student Researcher**, Santa Barbara Coastal LTER
- 2017 **Graduate Student Researcher**, NSF Office of Polar Programs, McMurdo Station, Antarctica
- 2016 – 2018 **Graduate Student Researcher**, Sonoma State University, Dept. of Biology, CA 2016 **Visiting Student Researcher**, KAUST, Red Sea Research Center, Saudi Arabia
- 2015 **Undergraduate Researcher**, University of Washington, Friday Harbor Labs, WA
- 2022 **Teaching Assistant**, Invertebrate Zoology, UCSB, Dept. of Ecology, Evolution and Marine Biology
 - I taught undergraduate laboratory sections for a predominantly marine invertebrate zoology curriculum focused on the macroevolution, taxonomy, and anatomy of major animal phyla accompanied by a diversity of live specimens collected from the Santa Barbara Channel.
- 2019 – 2021 **Teaching Assistant**, Global Change Biology, UCSB, Dept. of Ecology, Evolution and Marine Biology
 - I developed curricula for and led undergraduate discussion sections focused on evaluation of scientific literature on advances in the field of global change biology.
- 2019, '21, '23 **Teaching Assistant**, Introductory Biology: The Diversity of Life, UCSB, Dept. of Ecology, Evolution and Marine Biology
 - I led laboratory activities relating to taxonomic and experimental investigations of microbial, protozoan, invertebrate, and vertebrate biodiversity at the undergraduate level.
- 2018 **Teaching Assistant**, Physiological Marine Molecular Ecology, Northeastern University, Three Seas MSc in Marine Biology
 - I led lectures and laboratory activities relating to physiology and environmental stress responses in marine metazoans for a two-week, graduate-level field course at University of Washington's Friday Harbor Labs, WA in addition to optimizing western blot and PCR protocols.
- 2016 – 2018 **Teaching Associate**, SSU, Dept. of Biology
 - I led introductory lab sections for two courses in molecular and cellular biology or ecology and evolution and was responsible for generating short lectures and contributing to lesson plans.
- 2017 **Instructor**, EXCEL for Youth Summer Learning Program
 - I developed and taught a curriculum in experimental marine biology for ages 9 – 12. Classes focused on making the scientific process interactive, accessible, and memorable for younger ages as well as socioeconomic and ethnic groups that have been disenfranchised in science.

Professional Development

- 2020– pres. **Working Group Co-leader**
- I currently serve as the co-leader of the MarineOmics Working Group funded by NSF’s Research Coordination Network for Evolution in Changing Seas. Our working group runs marineomics.github.io. Information about our aims and mission can be found on this site.
- 2022 **RCN-ECS Training and Integration Workshop**
- I attended a workshop at Shoals Marine Lab, NH, hosted by the Research Coordination Network for Evolution in Changing Seas that aimed to train students in skills and tools to promote collaboration and synthesis in marine science and evolution.
- 2020 **Virtual Lab Meeting Training Program**
- I participated in a program hosted by the Research Coordination Network for Evolution in Changing Seas where I attended the meetings for the lab of Dr. Steven Roberts at the University of Washington where I received feedback on my PhD research and hosted discussions on diversity, equity, and inclusion.
- 2019 – 2021 **RCN-ECS Coordinated Readings**
- For three years I participated in the Research Coordination Network for Evolution in Changing Seas’ group readings related to marine population biology, phenotypic plasticity, and conservation genomics in addition to discussing and synthesizing readings with the network.

Outreach, Service & Leadership

- 2022 – pres. **Editorial Reviewer**, *Integrative and Comparative Biology*
- I currently serve as an editorial reviewer for *Integrative and Comparative Biology*’s upcoming symposium “Genomics of marine larval evolution and development”.
- 2020 – pres. **Peer Reviewer**
- I have served as a peer reviewer for the journals *Molecular Ecology*, *Molecular Ecology Resources*, *Evolutionary Applications*, *Journal of Animal Ecology*, *Marine Ecology Progress Series*, *Integrative and Comparative Biology*, and *Frontiers in Physiology*.
- 2022 **Graduate Mentor**, CAMP Summer Research Internship
- In the summer of 2022, I mentored an undergraduate research fellow with the California Alliance for Minority Participation’s Summer Research Internship.
- 2022 – 2023 **Mentor**, Society of Integrative and Comparative Biology
- I have volunteered to mentor an attendee of the upcoming 2023 annual meeting of the Society of Integrative and Comparative Biology in Austin, TX.

- 2021 – 2022 **Graduate Mentor**, Worster Award, UCSB Dept. of Ecology, Evolution, and Marine Biology
- With funding from UCSB EEMB’s Worster Award, I mentored an undergraduate researcher during 10 weeks of field and laboratory work in the summer of 2021 before working with this student on an honors research project during the 2021 – 2022 academic year.
- 2021 **Internship Coordinator**, MarineOmics Working Group, NSF RCN for Evolution in Changing Seas
- In 2021 I funded, constructed, and led an internship for three undergraduate researchers, three graduate student mentors, and three faculty advisors representing seven institutions that provided remote networking opportunities and professional development panel discussions and taught interns foundational computational methods in genomics.
- 2021 **Mentor**, Western Society of Naturalists Mentorship Program
- I have volunteered as a mentor for a first-time attendee of the Western Society of Naturalists’ virtual annual meeting, helping this student prepare for their first poster presentation and connecting them with researchers that this student later interviewed with for post- baccalaureate positions.
- 2020 – 2021 **President** (elected), Graduate Student Advisory Committee, UCSB Dept. of Ecology, Evolution, and Marine Biology
- I served as the chair and leader of my department’s graduate student governance group where we oversaw relations between students and the department, organized professional development programs, and served as an interface between UCSB’s graduate division and our graduate student body.
- 2019 – 2020 **Department Representative**, UCSB Graduate Student Association
- I have served as a representative to the university-wide graduate student association on behalf of students within my department.
- 2019 – 2020 **Graduate Mentor**, UCSB Gorman Scholars Program
- I mentored an undergraduate researcher to aid in their professional development and independent research through a competitive scholarship that aims to broaden participation in STEM.
- 2019 – 2020 **Treasurer**, Graduate Student Advisory Committee, UCSB Dept. of Ecology, Evolution, and Marine Biology
- I oversaw financial transactions for functions organized by graduate students related to scientific training, symposia organization, and social events.
- 2019 – 2020 **Volunteer**, World Oceans Day, Sea Center, Santa Barbara
- I have volunteered as a scientific educator for a World Oceans Day event hosted by the Santa Barbara Natural History Museum’s Sea Center where I engaged the public on issues facing the world’s oceans and shared my experience as a scientist.

- 2018 – 2022 **Volunteer**, Family Ultimate Science Experience, UCSB
- I led monthly experiential learning activities for students and their families at public middle schools in Santa Bara County that cover topics in biology, chemistry, engineering, and physics.
- 2019 **Student Representative**, Faculty Hiring Committee, UCSB
- I served as the student representative in the Dept. of Ecology, Evolution, and Marine Biology for a hiring committee aiming to recruit a tenure-track faculty member specializing in genomics.
- 2017 **Collaborator**, Seeker: Group Nine Media
- I reached out to and collaborated with Seeker, a science education network, to develop an episode for the series “Science at the Extremes” focusing on my research group’s fieldwork at McMurdo Station, Antarctica, resulting in over 780,000 online views.
- 2015 – 2016 **Student Representative** (elected), Dickinson College Dept. of Biology
- Planned and organized events for the Dickinson College Dept. of Biology, including symposia, seminars, and dinners. I also played an active role in recruitment and tenure selection for biology faculty.

Undergraduate Student Mentees

- 2022 **Kelly Castellon**, California Alliance for Minority Participation Summer Research Fellow, UC Santa Barbara
- 2021 – 2022 **Olivia Porat**, Worster Fellow, UC Santa Barbara
- Graduated with distinction and honors
 - Scientific educator with the San Francisco Bay Marine Science Institute
- 2020 – 2022 **Kaitlin Macaranas**, RCN-ECS MarineOmics Intern, CSU Bakersfield
- NSF Graduate Research Fellow
 - MSc student at Western Washington University
- 2019 – 2020 **Michael Meneses**, Gorman Research Scholar, UC Santa Barbara
- Graduated with honors
 - PhD student at Woods Hole Oceanographic Institution
- 2017 – 2018 **Madison Ingraham**, Undergraduate Researcher, Sonoma State University
- MSc student at Sonoma State University

Abstract

The Evolution of Plastic Responses to Global Change:

Studies in Two Species of Coastal Marine Invertebrates

by

Samuel Neill Bogan

Global climate change will likely impose selective pressures on biodiversity to not only increase limits for environmental stress, but the phenotypic plasticity of those limits. While we know a great deal about natural selection on performance traits such as upper thermal tolerance, our knowledge of the evolution of plastic responses to the environment is limited. Whether and when the plasticity of performance traits like thermal tolerance and body size incur fitness costs and benefits is unclear. Opposing theories and a lack of empirical research have resulted in a poor understanding of genetic variation underpinning adaptive phenotypic plasticity. Here I report the results of my dissertation research, which aimed to study ecological and evolutionary processes shaping (i) fitness effects of plasticity in thermal tolerance, (ii) intraspecific variation in plastic and evolved responses to warming, and (iii) the heritability of plastic responses to the environment. Studying populations and families of two coastal marine invertebrates, the intertidal copepod *Tigriopus californicus* and the purple sea urchin *Strongylocentrotus purpuratus*, I demonstrated potential for plastic responses to global change to evolve via natural selection evidenced by fitness effects and heritability of phenotypic plasticity. However, this evolution may be constrained by factors including fitness tradeoffs between performance and its plasticity, low genetic variation for maladaptive plasticity, and countergradient variation in performance reducing genetic variation for plasticity.

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Chapter I: Introduction

The evolution of adaptive phenotypic plasticity is predicted to contribute to biodiversity's persistence under global climate change (Matesanz et al., 2010; Usui et al., 2023). The evolution of plastic responses to global change via natural selection depends on eco-evolutionary process affecting its fitness costs or benefits and its genetic variation, which both remain poorly understood (Arnold et al., 2019; Hendry, 2016; M. Kelly, 2019; Van Buskirk & Steiner, 2009). Furthermore, studies on the evolution and function of plasticity in traits such as thermal performance have primarily been conducted in laboratory settings, and the relative contribution of plasticity to phenotypic variance has rarely been partitioned from evolved variation in natural populations (Acker et al., 2023; Schilthuizen & Kellermann, 2014). If plasticity and its evolution are to be incorporated in the management of species threatened by climate change (Donelson et al., 2023) or models predicting adaptive responses by biodiversity to environmental change (Buckley & Kingsolver, 2021), we need to develop comprehensive understandings of the function, fitness effects, and genetic variance of plastic responses to global change drivers.

1.1. Studying the evolution of thermal plasticity: history and contemporary need under climate change – Phenotypic plasticity is defined as change in a trait's expression between distinct environments independent of genetic variation in that trait (Pigliucci, 2001; Via et al., 1995). Plasticity affects nearly all measurable traits (DeWitt & Scheiner, 2004) and has been argued to be a property of genotypes (Pigliucci, 2006), a property of traits (Parsons et al., 2020), and a property of genotypes specific to different traits (Scheiner, 1993), but it is ubiquitously considered to be a heritable trait itself (Richards et al., 2006). In this dissertation, I consider plasticity to be a property of genotypes specific

to different traits. Physiological mechanisms that promote acclimatization to variable or novel environments are driven by the plasticity of molecular, cellular, and organismal processes (Beldade et al., 2011) and frequently evolve across environmental gradients (Conover et al., 2009). Many habitats are predicted to increase their mean temperature and thermal variance as a result of warming (Lewis & King, 2017; Oliver et al., 2021), possibly imposing positive selective pressures on ectotherms to increase their potential for thermal acclimation and adaptive plasticity of thermal performance traits (*AR5 Synthesis Report*, n.d.; Fox et al., 2019).

The extent to which adaptive thermal plasticity evolves via natural selection is unclear (Arnold et al., 2019). Theoretical predictions that genetic variation for adaptive phenotypic plasticity should be limited are starting to emerge (Walter et al., 2023), but studies quantifying genetic variation in thermal plasticity have not accounted for whether plasticity provides fitness costs or benefits (Campbell-Staton et al., 2021; Oostra et al., 2018a). Additionally, our understanding of thermal plasticity as a functional trait, which informs its evolution, requires field studies that go beyond the laboratory to observe and test how thermal plasticity is induced and varies in nature (Clusella-Trullas et al., 2021; Terblanche & Hoffmann, 2020). These limits to our knowledge of thermal plasticity's evolution are linked to historical obstacles in research on phenotypic plasticity in ecology and evolution (Sommer, 2020). These obstacles now exist as frontiers in global change biology that must be overcome to better predict how biodiversity will adapt to global change and manage threatened species (Fox et al., 2019).

The first concept of plasticity was introduced as the Baldwin Effect in 1896, which provided a foundation for future studies on the evolution of plasticity by stating that

‘learned characters’ can affect fitness and influence evolution (Baldwin, 1896). As phenotypic plasticity became more widely studied, its ability to evolve or affect evolution was largely ignored and considered to be inconsequential due to several semantic and conceptual problems introduced by influential papers (Sommer, 2020). For example, Waddington first described the process of genetic assimilation in 1957 whereby alternative phenotypes are exposed to selection by phenotypic plasticity and become canalized for an alternative phenotype under that selection pressure (Waddington, 1957). He proceeded to frequently avoid the term genetic assimilation and was inconsistent in his terminology in future papers (Waddington, 1977). This fueled skepticism about genetic assimilation, the evolution of phenotypic plasticity, and its evolutionary role (Amundson, 2005). In 1965, Bradshaw observed that plasticity in response to a common environmental variable could vary significantly between species of the same genus and described the first concept of a genotype-by-environment interaction, whereby the plasticity of a character is a genetically encoded, independent quality of that character (Bradshaw, 1965). While this laid the foundation for evolutionary research on phenotypic plasticity, it did not influence the acceptance of plasticity’s evolutionary significance in the field (Sommer, 2020).

The publication of Mary Jane West Eberhard’s 1989 review ‘Phenotypic plasticity and the origin of diversity’ marked a fundamental shift in evolution that spurred interest in plasticity as an evolutionary process by addressing how pervasive environmentally-responsive, alternative phenotypes are in nature and introducing several theoretical predictions that remain of interest today (West-Eberhard, 1989). Many of these predictions related to the evolution of plasticity via natural selection, conditions selecting for the canalization of plastic traits, and conditions that give rise to genetic accommodation (a

genetic change in the environmental regulation of a phenotype) or genetic assimilation (Ehrenreich & Pfennig, 2016). While there is empirical evidence supporting the existence of these processes in nature, it remains difficult to predict when and how plastic traits may evolve via natural selection. For example, directional selection on phenotypic plasticity has been historically difficult to measure, potentially due to (i) theoretical issues regarding when costs of plasticity should arise in nature and (ii) subsequent impacts of these theoretical issues on experimental design (Hendry, 2016; Van Buskirk & Steiner, 2009). These challenges undoubtedly stem from plasticity's slow burn adoption in evolutionary biology and must be overcome to improve predictions of biodiversity's adaptation to changing climates (Buckley & Kingsolver, 2021; M. Kelly, 2019; Kingsolver & Buckley, 2017).

1.2. Frontiers in the study of thermal plasticity and its evolution – The evolution of thermal acclimation and the plasticity of thermal performance traits will likely affect how biodiversity persists under future thermal stressors such as atmospheric and marine heat waves (Buckley & Kingsolver, 2021; Pazzaglia et al., 2021). Numerous open questions exist about factors that influence thermal plasticity's evolution including (i) what promotes canalization versus evolutionary increases in plasticity (van Heerwaarden & Kellermann, 2020), (ii) to what extent does plasticity buffer against or promote evolved variation in thermal performance (M. Kelly, 2019; Stevens et al., 2023), and (iii) what eco-evolutionary processes promote and sustain genetic variation for thermal plasticity (Bodensteiner et al., 2021; M. Kelly, 2019).

The potential for canalization of upper thermal tolerance to be driven by tradeoffs between thermal tolerance and its plasticity is becoming increasingly discussed, motivated by observations that these two traits often negatively correlate in ectotherms (Barley et al., 2021; Gunderson, 2023; M. C. Sasaki & Dam, 2021; Svensson et al., 2020; van Heerwaarden & Kellermann, 2020). However, fitness tradeoffs between thermal tolerance and thermal plasticity have never been reported in empirical studies and (van Heerwaarden & Kellermann, 2020). Doing so requires an expansion of canonical quantitative techniques for modeling the fitness costs of phenotypic plasticity so that they are fitted as a contingency of basal thermal tolerance (Lande & Arnold, 1983). Modeling context dependence in thermal plasticity's fitness costs would address a hypothesized explanation for why phenotypic plasticity frequently exhibits neutral costs such as those observed for thermal plasticity (Arnold et al., 2019) – neutral costs of plasticity may be the net result of positive and negative fitness effects that are contingent on unmeasured evo-evolutionary variables such as tradeoffs with other phenotypes or differential costs between morphotypes (Hendry, 2016; Van Buskirk & Steiner, 2009). Experimentally testing for a fitness tradeoff between thermal tolerance and its plasticity would require the development of expanded quantitative methods, contribute to evolutionary literature on the canalization of plastic traits, and mark a novel test of a process that is may affect the evolution of thermal physiology (van Heerwaarden & Kellermann, 2020).

Whether and how fitness tradeoffs between thermal tolerance and its plasticity affect adaptation to future climate change depends on how thermal plasticity evolves in natural populations in response to contemporary spatiotemporal variation in temperature (Swaegers et al., 2023). It is easy to fall into the assumption that the plasticity of thermal

tolerance is obligately positive with respect to temperature and inherently adaptive. While this can hold true in experimental systems exposing organisms to static treatments of increased temperature, natural abiotic environments are highly variable and exhibit multivariate abiotic changes that coincide with increased temperature (Dowd et al., 2015). As a result, thermal plasticity can be maladaptive and reduced in a population or exhibit evolutionary changes across space and time that are not consistent with an adaptive, acclimatory response. Thermal plasticity may also account for a small proportion of phenotypic variance in thermal tolerance across space or time, but plastic and genetic contributions to intraspecific variation in thermal performance are rarely partitioned in natural populations (Acker et al., 2023; Schilthuizen & Kellermann, 2014).

Alternative evolutionary processes affecting thermal plasticity include co- and counter-gradient variation whereby genetic effects either align with or oppose the directional effect of the environment in which a genotype exists. Interpreting studies of thermal plasticity's evolutionary constraints ultimately hinges on its function in natural populations and how that function interacts with the evolution of thermal tolerance. Here, genotype-environment covariance is a critical part of that function – does plasticity further alter phenotype in the same direction as evolution or does a phenotype's evolution counteract plastic effects? Studies of covariation between genetic and environmental effects during 'evolution in action' (e.g., seasonal or temporal environmental variation) are scarce (Schaum et al., 2022) but necessary to determine how biodiversity will or will not adapt to climate change (Albecker et al., 2022).

Adaptive changes in phenotypic plasticity depend on genetic variation underpinning either adaptive or maladaptive plasticity (Pfennig, 2021). If insufficient genetic diversity exists within a population for alleles underpinning the plasticity of a phenotype, it cannot evolve under natural selection (Grether, 2005). While numerous studies have measured genetic variance for the plasticity of singular phenotypes or the fitness effect of plasticity in a trait, there is a need for measuring its joint heritability and fitness effects, particularly in a global change context (M. Kelly, 2019). Progress toward a general understanding of genetic variance for (mal)adaptive plasticity can be made by measuring additive genetic variation underpinning the plasticity of one phenotype across diverse taxa or genetic variance for the plasticity of a diverse array of phenotypes in one species. While achieving the former is made difficult by a lack of power across studies measuring the plasticity of the same traits in response to the same cues, the latter is made possible by high throughput measures of organismal traits and/or molecular phenotypes (Blows et al., 2015; Blows & McGuigan, 2016; Pavlyshyn et al., 2022; Verma & Ritchie, 2017).

Field and laboratory studies addressing eco-evolutionary processes shaping natural selection on the plasticity of environmental traits, genetic variation for plasticity, and how plasticity affects or interacts with the evolution of trait means each contribute a portion of results to the joint set of information necessary for understanding how environmental acclimation under environmental change evolves. To this end, I have aimed to study natural selection on thermal plasticity, its genetic variation, and its interactions with genetic effects on thermal tolerance in populations and families of two coastal, marine ectotherms.

1.3. *Tigriopus californicus* and *Strongylocentrotus purpuratus*: tractability and value for the study of thermal plasticity's evolution – Marine populations and species exhibit stronger gradients in thermal limits across latitude relative to terrestrial systems (M. Sasaki et al., 2022; Sunday et al., 2011) and are faced with increased severity and frequency of unprecedented temperature anomalies such as marine heat waves (Oliver et al., 2021) and changes in abiotic variables that correlate with temperature. Coastal populations of invertebrates affected by both temperature and $p\text{CO}_2$ inhabiting environments such as the California Current also face future increases in the severity and frequency of coastal upwelling where strong winds induce turnover of seawater that transports deep, cold, acidic seawater to surface oceans (Huyer, 1983; Xiu et al., 2018). The diversity of thermal physiologies, life histories, and population dynamics that exist among coastal marine invertebrates provide valuable systems in which to study the evolution of phenotypic plasticity in thermal acclimation (Albecker et al., 2021; D. J. Marshall et al., 2012; Sanford & Kelly, 2011; M. Sasaki et al., 2022). I conducted thesis research on two coastal marine invertebrates with starkly different life history strategies, population dynamics, ecological niches, and utilities for studying the evolution of plastic responses to global change. These species are the intertidal copepod *Tigriopus californicus* and the purple sea urchin *Strongylocentrotus purpuratus*, both native to California's Pacific Coast, where my dissertation research was conducted (Pearse, 2006; Powlik, 1999).

The intertidal copepod *Tigriopus californicus* is a tractable, valuable study species with which to test the tradeoff and limit hypotheses. *T. californicus* is a harpacticoid copepod inhabiting supralittoral splash pools distributed along the Pacific Coast of North America between Alaska and Baja California (Edmands, 2001). These pools experience

dramatic diurnal and seasonal variation in temperature (Powlik, 1999), underscoring the importance of thermal plasticity for this species. Populations of *T. californicus* also exhibit strong genetic differentiation and local adaptation across their biogeographic distribution, likely aided by short dispersal distances, such that genetically fixed thermal tolerance increases at lower latitudes (Barreto et al., 2018; M. W. Kelly et al., 2011; Sanford & Kelly, 2011). With a broad biogeographical distribution, populations possess genetically fixed differences in their plasticity of thermal tolerance, but it remains unclear whether and how this functional trait correlates with baseline thermal tolerance or latitude (M. W. Kelly et al., 2011; Pereira et al., 2017). This variation in thermal physiology provides phenotypic diversity that is necessary for evolutionary studies on thermal plasticity, which are made tractable by a generation time as short as 21 days (Powlik et al., 1997), easily measured fitness correlates, and the species' mating habits. For example, external clutches of eggs held by brooding females can be dissected, and averaged egg counts from one or several broods per female correlate with their lifetime reproductive success and survival (Powers et al., 2020). The multiple broods laid by a female in her lifetime are also unipaternal (Burton, 1985) enabling the breeding of full-sibling cohorts often used as replicate family-level genotypes by selection gradient study designs. In sum, the strong local adaptation of *T. californicus* across a large biogeographical temperature gradient and its life history make it ideally suited to explore fitness costs of thermal tolerance and its plasticity.

The purple sea urchin *S. purpuratus* has a longer generation time than *T. californicus* (Leahy, 1986), but it provides tractability for generating highly controlled experimental crosses and studying plasticity during early development (Cameron et al., 1999; M. W. Kelly et al., 2013). Populations inhabiting environmental gradients or mosaics

exhibit genetic evidence of local adaptation and interpopulation variation in performance and gene expression under ecologically relevant stress (Evans et al., 2017; M. W. Kelly et al., 2013; Pespeni et al., 2013; Pespeni & Palumbi, 2013). In response to experimental upwelling, *S. purpuratus* exhibits transgenerational and developmental plasticity of differential gene expression, DNA methylation, and several performance traits including larval growth rate, biomineralization, and lipid content (Bogan et al., 2023; Strader et al., 2019, 2020, 2022; Wong et al., 2018, 2019). Significant genetic variation for the plasticity of body size and biomineralization in response to upwelling exists in at least some populations (Strader et al., 2022). However, it is not yet understood whether any of the plastic responses described are adaptive, maladaptive, or neutral under the experimental conditions used, how heritable this plasticity is, and whether plasticity's fitness effects and heritability covary.

1.4. Objectives – To understand the potential for natural selection to drive the evolution of plastic responses to global change, I aimed to conduct the three following laboratory and field studies. With these objectives, I studied eco-evolutionary processes constraining thermal plasticity's fitness effects, genetic variation in the plasticity of environmental performance, and relative contributions of environmental versus genetic effects on variation in thermal physiology among wild populations:

Chapter II objective: Testing for fitness tradeoffs between thermal tolerance and its plasticity in Tigriopus californicus. In this chapter, I aimed to test the longstanding hypothesis that thermal tolerance and its plasticity negatively correlate in some ectotherms because of fitness tradeoffs between the two traits. Under the fitness trade off hypothesis,

thermally tolerant genotypes incur greater costs to reproduction and/or survival as the plasticity of their upper thermal limit increases (A. Agrawal et al., 2010; Roff & Fairbairn, 2007; van Heerwaarden & Kellermann, 2020). To date, no study has explicitly tested whether the costs of plasticity in thermal tolerance depend on genotypes' basal thermal tolerance.

I first determined that thermal tolerance and its plasticity indeed negatively correlate across both populations and family-level genotypes of *T. californicus* reared under common garden conditions for two generations before developmental conditioning to high and low temperature. Using a selection gradient model, I measured the fitness costs of (i) thermal tolerance in high and low temperature and (ii) the plasticity of thermal tolerance between developmental temperatures using fecundity as a fitness correlating character. Lastly, I modeled interactive costs of basal thermal tolerance and thermal plasticity to determine whether the costs of thermal plasticity were higher in thermally tolerant genotypes.

Chapter III objective: Quantifying evolved and plastic sources of variation in thermal tolerance across seasonal changes in temperature in natural populations of Tigriopus californicus. In this chapter, I combined natural history and hypothesis driven approaches to understand the role of plasticity in upper thermal tolerance as a functional trait in natural populations of *T. californicus* and to determine whether plastic variation in thermal limits covaries with genetically fixed evolution during seasonal changes in temperature. Such covariation can be representative of (i) co-gradient variation, whereby plastic effects on upper thermal tolerance align with the direction of genetic effects on a trait in response

seasonal increases in temperature, or (ii) countergradient variation, where thermal plasticity opposes the direction of evolutionary change in upper thermal tolerance during seasonal warming (Albecker et al., 2022; Stamp & Hadfield, 2020).

I achieved these aims by recording upper thermal limits of wild *T. californicus* from four latitudinally distributed populations in coastal California over a ten-month period and integrating these phenotypic measures with time series temperature data that I recorded in supralittoral pools at each collection site. Using common garden lines derived from animals collected during peak summer and low winter temperatures, I estimated evolved seasonal variation in upper thermal limits for each population and sex before deriving plastic, seasonal variation in thermal tolerance from field-collected and common garden phenotypic variation. By modelling the plastic and genetic contributions to total seasonal variance exhibited by natural populations, I was able to calculate covariance between these effects consistent with co-gradient or counter-gradient variation.

Chapter IV objective: Measuring the heritability of adaptive transcriptional responses to upwelling in Strongylocentrotus purpuratus. Predictions regarding whether adaptive plasticity should generally exhibit sufficient or constrained genetic variance necessary for its evolution is mixed (Chevin & Hoffmann, 2017; Sih et al., 2004). Evaluating whether adaptive and maladaptive plasticity generally possess higher or lower genetic variance, which requires large datasets of multiple phenotypes or taxa, has rarely been pursued but will further our understanding of adaptive plasticity's potential to evolve (M. Kelly, 2019). Studying *S. purpuratus*, I integrated phenotypic assays of organismal performance traits, fitness correlating traits, and RNA-seq derived from a quantitative genetic breeding design

to test whether the heritability of transcriptional responses to ecologically relevant environmental stress varies as a function its effects on phenotype and fitness.

Dams and sires were conditioned to simulated upwelling (low temperature + high $p\text{CO}_2$) or an ambient treatment during gametogenesis before 40 individual crosses were conducted between parents from the same treatment group. Resulting embryos were then reciprocally cultured under upwelling and ambient conditions through larval development, at which time sampling for phenotyping and RNA-seq was performed. I leveraged selection gradient models predicting fitness costs of phenotypic plasticity, structural equation models of transcriptional plasticity's phenotypic effects, and animal models estimating additive genetic variance for transcriptional plasticity. By associating transcriptional plasticity's heritability, phenotypic association, and fitness outcome, I was able to measure how heritable adaptive transcriptional plasticity is across the transcriptome and whether this heritability varies according to differential expression's fitness effects.

Chapter II: Thermal plasticity has higher fitness costs among thermally tolerant genotypes of *Tigriopus californicus*

Abstract

Under climate change scenarios, ectotherms will likely be faced with pressure to adapt to novel thermal environments by increasing upper thermal tolerance and its plasticity, a measure of thermal acclimation. Ectotherm populations with high thermal tolerance are often less thermally plastic, a tradeoff hypothesized to result from (i) a phenotypic limit on thermal tolerance above which plasticity cannot increase it any further or (ii) fitness tradeoffs between the two traits. We empirically tested the limit and tradeoff hypotheses by leveraging the tractability and thermal biology of the intertidal copepod *Tigriopus californicus*. Using populations from 4 latitudinally-distributed sites in coastal California, 6 lines per population were reared under a laboratory common garden for two generations before 96 full sibling replicates ($n = 4-5$ per line) from a third generation were developmentally conditioned to 21.5 and 16.5 °C until adulthood. We then measured the upper thermal tolerance and fecundity of each sibship at high and low temperatures. We detected a significant fitness tradeoff between baseline thermal tolerance and its plasticity. *T. californicus* populations and genotypes with higher thermal tolerance were less thermally plastic. Under our experimental conditions, we detected negative directional selection on thermal plasticity under ambient temperature evidenced by reduced fecundity. These fitness costs of plasticity were significantly higher among thermally tolerant genotypes, consistent with the tradeoff hypothesis. This tradeoff was strongest under ambient temperature. Thermal tolerance directly affected plasticity after controlling for

fitness effects, indicating support for the limit hypothesis. Observed thermal plasticity and fecundity were best explained by models incorporating both the limit and tradeoff hypotheses rather than those with parameters associated with one. Our study provides a novel empirical test of the fitness tradeoff and limit hypotheses. We discuss our results' insights into how natural selection acting on thermal tolerance and its plasticity may be constrained by limits and slowed by a fitness tradeoff.

1. Introduction

Increasing annual temperatures and frequent, severe heat waves may impose selective pressures on ectotherms to adapt via positive directional selection on upper thermal tolerance and its plasticity (Fig. 1A), which drives acclimation to novel temperatures (Arnold et al., 2019; Huey et al., 2012). However, thermally tolerant populations of ectotherms are often less thermally plastic (Barley et al., 2021; Stillman, 2003; van Heerwaarden et al., 2016; van Heerwaarden & Kellermann, 2020). Two hypotheses may explain negative correlations between tolerance and plasticity. We refer to these as the “limit” and “tradeoff” hypotheses (Fig. 1B). The limit hypothesis states that plasticity may be limited in tolerant genotypes due to a phenotypic threshold above which plasticity cannot increase tolerance any further (DeWitt et al., 1998; Sørensen et al., 2016). The tradeoff hypothesis states that thermal tolerance and its plasticity share an intrinsic fitness tradeoff: thermal plasticity is more costly in tolerant genotypes and, conversely, tolerance is more costly in plastic genotypes (A. Agrawal et al., 2010; Roff & Fairbairn, 2007). Measurements of thermal plasticity's fitness costs are scarce, particularly in ectotherms (Arnold et al., 2019), and, to our knowledge, no empirical tests of the fitness

tradeoff hypothesis have been conducted (van Heerwaarden & Kellermann, 2020). Studying populations of the intertidal copepod *Tigriopus californicus* that have evolved strong phenotypic divergence in thermal tolerance and its plasticity (M. W. Kelly et al., 2011; Pereira et al., 2017), we measured the fitness costs of genotypes' thermal plasticity conditional upon their thermal tolerance **in a laboratory experiment**. To evaluate the limit and tradeoff hypotheses using strong inference, we conducted quantitative hypothesis testing using our experimental results.

In a global warming context, the fitness tradeoff hypothesis is consequential to the evolution of thermal tolerance and its plasticity. If tolerance and plasticity possess a fitness tradeoff, adaptation to environments positively selecting for both traits may be hindered such that increases in one phenotype come at the cost of the other. The evolutionary rates of both traits are thus slowed. Under the limit hypothesis, simultaneous adaptation acting on tolerance and plasticity should remain unhindered until populations approach a phenotypic asymptote (Fig. 1 C). While these two models are not mutually exclusive and can co-occur, determining their proportional contributions to negative associations between tolerance and plasticity can improve predictions of how biodiversity will adapt to future climates (Roff & Fairbairn, 2007; van Heerwaarden & Kellermann, 2020).

Due to practical and biological reasons, detecting fitness effects of phenotypic plasticity has been historically challenging. The plasticity of thermal performance is no exception. One method of measuring plasticity's fitness costs is selection gradient estimation, where a Lande and Arnold-style regression is used to predict a fitness-correlating trait across genotypes and environments as a function of their phenotype within an environment and their plasticity in that phenotype across environments (Lande & Arnold, 1983). Van

Buskirk & Steiner quantitatively reviewed estimates of selection gradients acting on phenotypic plasticity and found neutral costs in most studies with high absolute fitness overrepresented among those with small sample sizes (Van Buskirk & Steiner, 2009). Arnold et al. reviewed selection gradient estimates on thermal plasticity and found that plasticity's fitness effects were insignificant in 4 out of 5 studies restricted to plants and birds (Arnold et al., 2019). Combining the results of two other studies in plants, fitness costs of thermal plasticity were significant in 3 out of 7 measured traits (Choi et al., 2019; De Lisle et al., 2022). It has been suggested that plasticity's neutral costs are the result of context-dependence that often goes unexplored. For example, plasticity may be more costly or beneficial depending on the environment that selection gradients are measured in or interactions between plasticity and other traits (Hendry, 2016). Fitness tradeoffs between upper thermal tolerance and its plasticity represent context-dependence that may further resolve thermal plasticity's costs. Measuring selection gradients acting on thermal plasticity in ectotherms is also an important step, as it is potentially more important in responses to thermal stress relative to endotherms for which selection gradient estimates do exist (Paaijmans et al., 2013).

We experimentally tested the fitness tradeoff and physiological limit hypotheses by leveraging the thermal ecology and tractability of *T. californicus*. We cultured lines from 4 populations distributed over 4 °N of latitude under a laboratory common garden for 2 generations before splitting a third generation of 96 full-sibling genotypic replicates (n = 192 cultures) across high and low temperature developmental conditions. We have observed that these populations exhibit a tolerance-plasticity tradeoff across latitude. Once matured, we measured the generation time, body size, upper thermal

tolerance, and fecundity of each sibship at high and low temperatures. Using a multivariate expansion of the traditional Lande & Arnold selection gradient model (Lande & Arnold, 1983), we measured the direct effect of baseline thermal tolerance on thermal plasticity, controlling for fitness (limit hypothesis), and fitness costs of thermal plasticity conditional upon baseline tolerance (tradeoff hypothesis). Finally, we used likelihood statistics to contrast support for these effects and their associated hypotheses given our experimental observations.

2. Methods

2.1. Collection and common garden culturing of Tigriopus californicus – *Tigriopus californicus* were collected during summer and winter from 4 latitudinally-distributed populations (Fig. 1A) in coastal California over a four day period between August 6th – 9th, 2021 and February 16th – 19th, 2022: the Bodega Marine Reserve in Bodega Bay, CA (38.316394 °N, -123.071980 °W), Four Mile Beach in Santa Cruz, CA (36.965262 °N, -122.125983 °W), the Kenneth S. Norris Rancho Marino Reserve in Cambria, CA (35.540090 °N, -121.092475 °W), and Point Dume State Beach in Malibu, CA (34.002035 °N, -118.805029 °W). Animals were collected from three splash pools at each site, stored in 500 mL cups, and transported in coolers under a 12:12 hr photoperiod to laboratory facilities at the University of California, Santa Barbara (UCSB). During 4 days of travel and one week of incubation in the lab per collection, transport coolers and incubation maintained an average temperature of 19.96 °C and 13.00 °C during August and February, respectively. These transport temperatures were -0.24 °C and -0.77 °C away

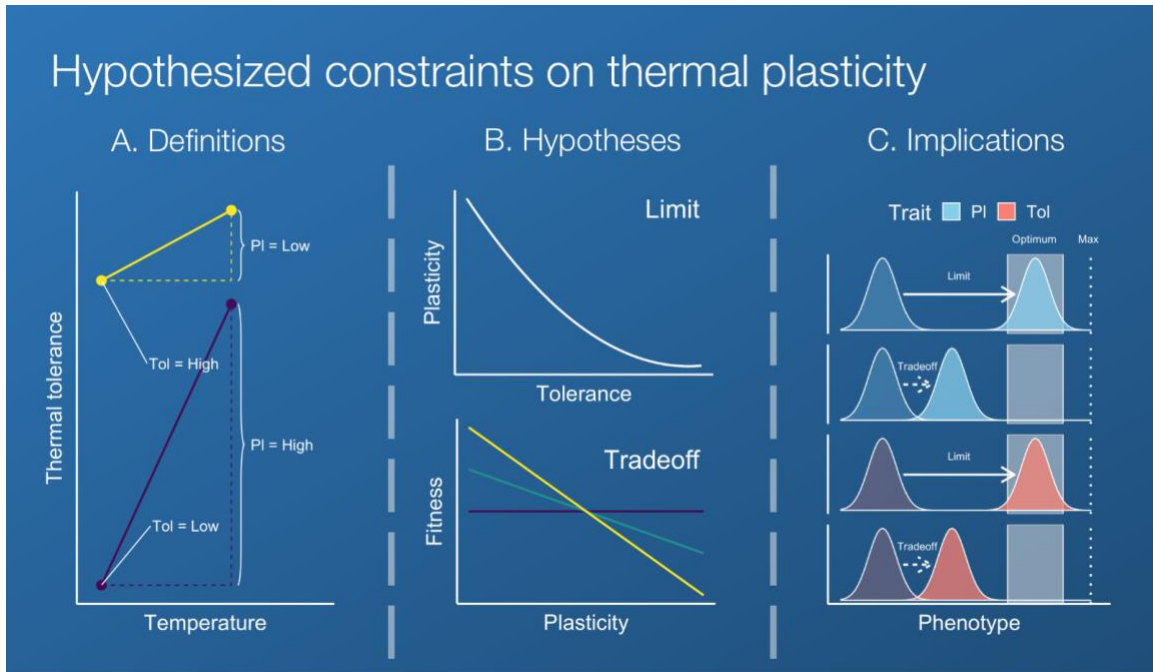


Figure 1 | *Hypothesized constraints on thermal plasticity.* (A) Parameters associated with baseline upper thermal tolerance (Tol) and its plasticity (PI) across a thermal reaction norm. (B) Visualizations of how thermal plasticity is constrained under the limit and tradeoff hypotheses. (C) A conceptual diagram of constraints on simultaneous adaptive evolution of thermal tolerance (‘Tol’; red) and its plasticity (‘PI’; blue) under the fitness tradeoff hypothesis. Under an environment positively selecting for increases in both traits, physiological limits (solid arrow) impose a maximum above which traits cannot increase (‘Max’; dotted line). Fitness tradeoffs (dashed arrow) may limit adaptation via reductions in fitness among tolerant and plastic genotypes, potentially preventing natural selection from achieving phenotypic optimums in either trait (grey rectangle). Distributions represent hypothetical variance in tolerance and plasticity before selection (transparent) and after (non-transparent).

from mean *in situ* temperatures recorded at all sites 21 days prior to August and February collections. During 4 days of transport, polystyrene collection cups received daily seawater changes using aerated, 0.5 μm filtered seawater and received an *ad libitum* diet of 20% spirulina fish food flakes (Handschumacher et al., 2010). Upon arrival at UCSB, collection cups continued to receive daily seawater changes, were fed *ad libitum*, and were held in an incubator set to 20.2 °C (August) or 13.8 °C (February) with a 12:12 hr photoperiod for 7 days before the initiation of common garden lines.

To remove environmental effects from phenotypic variation, 12 common garden lines ($n = 3$ per population) were reared in the laboratory for 2 generations before a third generation was split and conditioned to high and low temperatures until maturity. Phenotyping for upper thermal tolerance and fitness correlated traits, described below, was performed on temperature conditioned G3 cultures. These generations are referred to as G1 – G3 (laboratory-reared generations 1 – 3) from here forward. At initiation, $n = 100$ gravid females per pool were added into new 500 mL polystyrene culture cups and incubated at 16.5 °C, the mean annual temperature recorded *in situ* across all four collection sites between 2020 – 2021 (Fig. 1B). Gravid females were allowed to continuously hatch clutches of eggs in culture until the emergence of copepod stage offspring at which time gravid females were removed. G1 and G2 cultures received seawater changes twice per week and continued to be fed *ad libitum* until cultures matured, mated, and produced gravid females. G1 and G2 cultures maintained mean salinities of 33.54 ± 0.53 ppt and 33.62 ± 0.52 ppt. Once 100 or more females within a culture became gravid, G2 was initiated using the same methods described for G1.

2.2. *Culturing of G3 sibships across high and low temperature* – n = 12 gravid G2 females per line were added to individual 20 mL cultures in 12-well plates where they were allowed to continuously hatch broods. Hatches were checked every 4 days and resulting G3, full sibling nauplii were split between two 50 mL cultures incubated at a target of 16.5 and 21.5 °C that were recorded to be 16.42 ± 0.37 °C and 21.55 ± 0.40 °C over the duration of G3 culture. Each gravid female continuously hatched until mortality or a maximum number of 100 larvae had hatched. If a gravid female died, it was replaced with another gravid female from the same line which was used to initiate new G3 cultures. Seawater in 50 mL G3 cultures was changed every 4 weeks, resulting in an average salinity of 35.15 ± 2.20 and 34.87 ± 2.26 ppt under 16.5 and 21.5 °C, respectively. Females were allowed to hatch over 4-weeks. The mean number of larvae per G3 culture equaled 40.52 ± 17.20 (Fig. S1), 24.68x lower than the upper density of *T. californicus in situ* (Powlik, 1996).

The developmental progress of G3 cultures was scored by tracking the maximum life history stage of cultures during hatch checks: stages were scored as achieving the naupliar larval stage, the juvenile copepod stage, or maturity upon the presence of sexually dimorphic mortality at stage-C6 molt as well as whether gravidity had been achieved by mature females. The generation time of each population x temperature group was then estimated using logit-transformed generalized linear models to determine the days post-hatch at which 50% of a population progenates the next generation at a given temperature. These generation times were then used to determine the relative ages of population x temperature groups (i.e., days post-gravidity).

2.3. *Assays of upper thermal tolerance, body size, and fecundity* – When cultures achieved an average age of 16 – 26 days post-gravidity (Fig. S2) and presented no evidence of mortality among stage-C6 adults, they were assayed for upper thermal tolerance and stored in 1% formalin-buffered seawater for morphometric measurements and counts of fecundity per brood. Upper thermal tolerance was measured using an LT₅₀ assay, a measure of the temperature at which 50% of a population or group has died. LT₅₀ was measured by adding mature males, mature females, and copepoids to 200 mL PCR tubes at an average density of 1-6 animals per tube. This density has been demonstrated to not result in an effect on LT₅₀ in *T. californicus* via oxygen depletion (M. W. Kelly et al., 2011). 8 tubes per sibship x temperature group were randomly distributed across thermal cycler plate where they were ramped up to a 3 °C temperature gradient over 2 hrs at a ramping rate of 0.2 °C per 1.5 min. BMR was exposed to a 34 – 37 °C gradient, SC and RMR to 35 – 38 °C, and PTD to 36 – 39 °C. Animals were then allowed to recover at room temperature for 1 hr. Following the recovery period, survival was scored for mature males, mature females, and copepoids from each sibship x temperature group. An average of 21.18 ± 9.8 animals per sibship x temperature group were assayed for LT₅₀. The LT₅₀ parameter of each sibship x temperature group was measured as an inflection point randomly varying across sibship x temperature groups predicted by a non-linear logistic regression modeling survival as a function of fixed effects for gradient temperature, age, season of collection and nested, random effects for pool of origin and population of origin. By accounting for sex, we were able to estimate LT₅₀ parameters controlling against the effects of variation in the proportion of sexes represented in each culture.

Total body length was measured in mature females via imaging on a compound light microscope under 40x magnification as the total linear distance from the anterior end of the cephalon to the caudal radius. Fecundity per brood was measured as a fitness-correlated trait by dissecting and counting eggs in brood sacs from formalin-preserved gravid females. Only egg sacs with matured eggs exhibiting nauplii morphology were dissected to avoid underestimation of fecundity per brood. Brood sac dissections were conducted on females collected up to 7 days after LT_{50} assays to maximize the number of fitness measurements per culture. The mean fecundity, female body length, and male body length were calculated for each sibship x temperature group. Thermal tolerance and thermal plasticity data were measured for 96 sibships reared across 2 temperatures for a total of 192 experimental cultures. Fecundity measures were successfully obtained for 109 cultures representing 68 sibships (Table 1).

2.4. Selection gradient modeling – Selection gradients acting on LT_{50} and its plasticity were estimated using a multivariate adaptation of the Lande and Arnold regression for measuring selection on correlating characters (Lande & Arnold, 1983). The multivariate model was fit using brms, an R interpreter of the Bayesian modeling language Stan (Bürkner, 2017a; Gelman et al., 2015). The two outcome variables of the multivariate model were mean clutch size and the plasticity of thermal tolerance. A multivariate approach was necessary to test both the physiological limit and fitness tradeoff hypotheses in one singular model and perform quantitative hypothesis testing, which is described in the next section of Methods. Mean egg clutch size of each sibship per temperature was modeled as a function of fixed effects for temperature, LT_{50} within a given temperature, the plasticity of LT_{50} across temperature, an interaction between the plasticity and baseline

LT₅₀, and nested random effects for sibship, pool of origin, population of origin, and season of collection (Fig. 2). Statistical non-independence between LT₅₀ intercepts (baseline thermal tolerance) and LT₅₀ slopes (plasticity of thermal tolerance) was controlled for using an orthogonal polynomial transformation of the intercept value following guidance for regressing intercepts and slopes from a single function (Kline, 2015).

An additional transformation on slopes was necessary to control against the effect of regression to the mean in driving negative correlations between baseline tolerance and plasticity. For example, if the baseline tolerance of a genotype is high it is likely that its thermal tolerance under warmer conditions will increase less. The probability that a high baseline tolerance is also accompanied by a large plastic change is inherently low because the secondary phenotypic measurement is likely to regress to the mean. A transformation controlling for regression to the mean for plastic traits was originally proposed by Kelly & Price and applied to thermal tolerance data by Gunderson & Revelle (Gunderson, 2023; Gunderson & Revell, 2022; C. Kelly & Price, 2005). Our application of this transformation was conducted in exact concordance with Gunderson, 2023.

Slope coefficients estimated by this model represent the direction and strength of directional selection acting on LT₅₀, its plasticity, and their interaction. An interaction may be antagonistic such that the fitness costs of trait's plasticity are greater among sibships with a higher trait value or synergistic such that positive directional selection on plasticity is strongest among sibships with high trait values (Fig. 1).

Before fitting the component of the multivariate model testing for a direct effect of baseline thermal tolerance on plasticity, two regressions predicting plasticity as a function of (i) tolerance and fitness (fecundity) or (ii) tolerance without fitness were fit to estimate

what percentage of tolerance's effect on plasticity is driven by fitness costs. Only 2.22% of baseline tolerance's effect on plasticity was explained by fitness. Thus, the multivariate model did not incorporate a predictor for fitness in the model of plasticity as a function of tolerance, visualized in blue in Fig. 2. Equations 1 and 2 describe the two outcomes of the multivariate model where ω = mean fecundity of sibship i under temperature e , S = season of collection, L = mean body length per gravid female, $X = LT_{50}$, int = the intercept of LT_{50} , pl = the plasticity of LT_{50} , and Z = random intercepts associated with nested effects (Fig. 2) or population alone in the case of Z_2 . Excluding fitness as a predictor also avoided a recursive model structure whereby fitness affected plasticity and plasticity affected fitness (Cortina, 2014; Kiiveri et al., 1984). Mean female body length was included as a fixed effect in order to control for its direct impact on egg clutch size (Voordouw et al., 2005).

$$\omega_{i,e} = \mu_1 + \beta_1 e_i + \beta_2 S_{i,e} + \beta_3 L_{i,e} + \beta_4 X_{i,e} + \beta_5 X_{i,e} + \beta_6 plX_i + \beta_7 intX_i plX_i + Z_1 b_i + \varepsilon \quad (Eq. 1)$$

$$plX_i = \mu_2 + \beta_8 intX_i + Z_2 b_i + \varepsilon \quad (Eq. 2)$$

The significance of fixed effects was evaluated across all models using a probability of direction test, a Bayesian corollary of the p-value for determining effect existence. Fixed effects were deemed significant if 95% confidence intervals of posterior distributions did not overlap 0 (Makowski, Ben-Shachar, Chen, et al., 2019a).

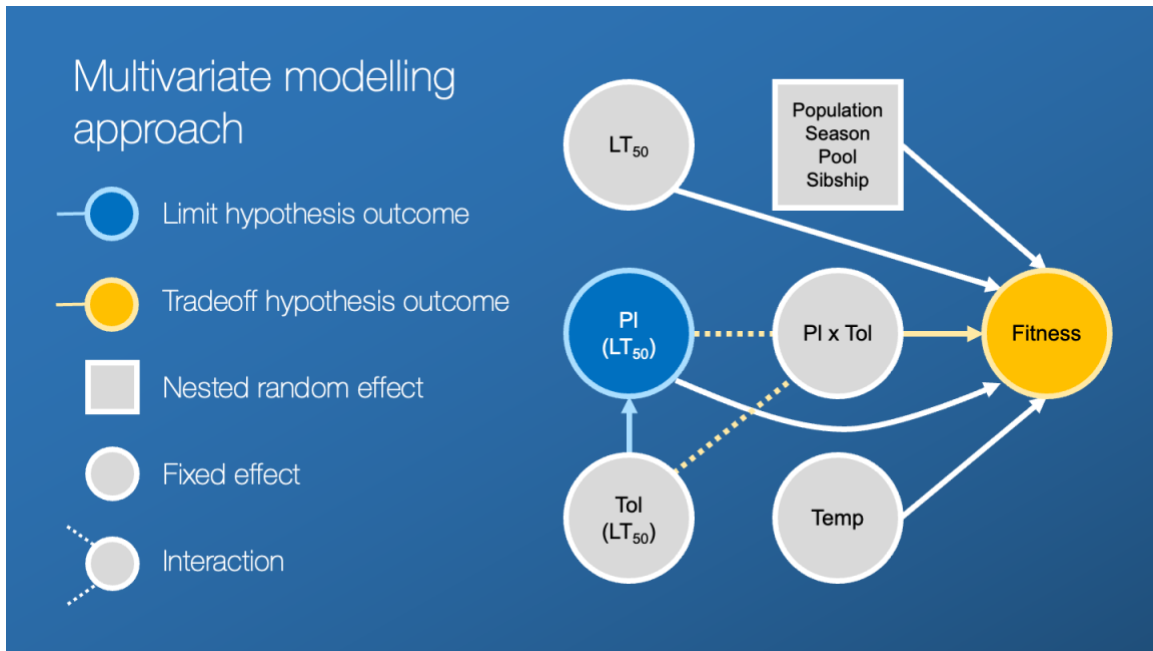


Figure 2 | *Visual representation of multivariate selection gradient model.* A directed acyclic diagram generalizing the multivariate model testing the physiological limit (blue) and fitness tradeoff (orange) hypotheses is shown. The limit hypothesis is evaluated by predicting the plasticity of thermal tolerance (PI) as a direct function of baseline thermal tolerance (e.g., the intercept of LT_{50} 's reaction norm; Tol). The tradeoff hypothesis is evaluated by fitting a Lande and Arnold-style selection gradient model that includes the fitness consequence of an interaction between LT_{50} pl and LT_{50} int. Not pictured are singular effects of season-of-collection, mean female body length, and interactive effects between 'Temp' and ' LT_{50} ', 'PI', and 'PI x Tol' predicting 'Fitness'. These parameters were excluded to simplify this visual generalization of the model.

2.5. *Quantitative hypothesis testing* – Quantitative support was measured and compared for the physiological limit and fitness tradeoff hypotheses in driving negative associations between thermal tolerance and its plasticity in *T. californicus*. Two distinct parameters were fit within the selection gradient model that were associated with the two hypotheses: $\beta_7 \text{int}X_i \text{pl}X_i$ and $\beta_8 \text{int}X_i$ (Eq. 1 & 2). The limit hypothesis was tested by predicting the plasticity of thermal tolerance as a function of baseline LT_{50} . The tradeoff hypothesis was tested by predicting mean clutch size as a function of interactive fitness costs between the baseline thermal tolerance and its plasticity. The marginal likelihood of the full model was compared to three alternative models that lacked one or both parameters using a Bayes factor test (Berger & Pericchi, 1996) in the R package bayestestR (Makowski, Ben-Shachar, & Lüdtke, 2019).

2.6. *Collection of in situ time series temperatures* – 3 TidBit MX5000 temperature loggers per site were installed in supralittoral pools containing *T. californicus* where they continuously recorded at a 10-minute interval between February, 2020 – April, 2022. Loggers were observed to be submerged 97.5% of the time during routine collections at each site. *T. californicus* were present in pools at the start and end of all recording periods included in the time series temperature dataset. If pools containing loggers had evaporated, data were filtered out of temperature time series until the last observed submersion. In the event of evaporation, active loggers were relocated and submerged in a new supralittoral pool containing *T. californicus*.

2.7. *Estimation of baseline thermal tolerance and thermal plasticity via logistic regression* – The LT_{50} of each sibship x temperature culture was estimated by fitting a quasibinomial logit-linked generalized linear mixed model to binary survival data collected

from thermal gradient assays. The model included fixed effects for interactions between gradient temperature and (i) sex, (ii) latitude, (iii) developmental temperature, (iv) an interaction between sex and developmental temperature, and (v) an interaction between latitude and developmental temperature. The model also included a nested random effect whereby culture sibships were nested within pools, pools were nested within collection seasons, and seasons were nested within populations. The logistic regression was fit using the `glmer()` function of the R package ‘nlme’ (Pinheiro et al., 2023).

The fixed ‘Sex’ variable included categories for females, males, and juvenile copepoids whose sexes were unknown. LT_{50} per culture was estimated from this model while controlling against variation in proportions of each sex across cultures. This was achieved by exporting fitted survival estimates per sibship x temperature culture assuming uniform sex within and across all cultures. The LT_{50} parameters of each culture were then individually modeled using the base `glm()` function of R and the `dose.p()` function of the R package ‘MASS’, which exports inflection point parameters from logistic regressions (Ripley et al., 2023). The LT_{50} estimates of culture’s reared under low developmental temperatures were used as a metric for a sibship’s’ baseline thermal tolerance in further analysis. Plasticity was measured as the change in LT_{50} across low to high temperature conditions for a given sibship.

2.8. Transformation of baseline thermal tolerance and thermal plasticity – Two statistical artifacts have the potential to inflate the negative effect of baseline thermal tolerance on thermal plasticity. Firstly, these two metrics are statistically non-independent because they are derived from the intercept and slope of a common linear function. If the intercept (baseline tolerance) of this function increases and LT_{50} of the high temperature

treatment is held constant, the slope (plasticity) must decrease. Non-independence was controlled for by transforming baseline tolerance as an orthogonal polynomial, reducing multicollinearity between baseline thermal tolerance and its plasticity (Smith & Sasaki, 1979). This transformation was achieved by centering baseline tolerance via subtraction of its mean and squaring the centered baseline tolerance variable. The product of the squared term's mean multiplied by the centered predictor variable was then subtracted from the centered variable itself, resulting in an orthogonal polynomial. This orthogonal polynomial was used a predictor variable representing baseline thermal tolerance in all subsequent regressions modeling baseline tolerance's effect on plasticity.

Regression to mean also has the potential to inflate negative associations between baseline thermal tolerance and its plasticity. If a replicate's reaction norm possesses high thermal tolerance under low temperature, it is unlikely that the subsequent value under high temperature increases by the average plasticity slope. It will more likely regress to the overall mean of thermal tolerance. We applied a method described by Kelly & Price (2003) and implemented by Gunderson (2023) on thermal tolerance data that estimates the proportion of observed plasticity explained by regression to the mean (D) and subtracts it from the plasticity measure such that

$$\widehat{D}^* = \hat{p}(X_1 - \bar{X}_1) - (X_2 - \bar{X}_2) \quad (\text{Eq. 3})$$

where p is the correlation between observation X_1 (phenotype in treatment one) and X_2 (phenotype in treatment two). The implementation of this approach for thermal tolerance data is detailed in an R script by Gunderson, 2023, (Gunderson, 2023; Gunderson & Revell, 2022; C. Kelly & Price, 2005).

The effect of baseline LT_{50} on the plasticity of LT_{50} was modelled using a linear mixed model predicting adjusted plasticity as a function of an orthogonal polynomial transformation of the LT_{50} intercept and a random nested effect assigning each observation to a sibship nested within a pool, nested within collection season, nested within population. This linear mixed model was fit using the methods described below under ‘Bayesian model fitting and specification’.

2.9. Bayesian model fitting and selection – Three groups of Bayesian linear mixed models (LMMs) were fit to predict the plasticity of LT_{50} and/or fecundity. These were (i) a univariate linear mixed model predicting baseline LT_{50} ’s effect on thermal plasticity described above, (ii) the multivariate linear mixed model predicting fitness costs of LT_{50} , its plasticity, and the effect of baseline LT_{50} on plasticity, and (iii) iterative versions of the multivariate model used for quantitative hypothesis testing. All LMMs were fit using a studentized gaussian distribution to reduce spurious or extreme model predictions. Student-t priors set to 3 degrees of freedom were assumed for all parameters (Bürkner, 2017b). Markov Monte Carlo chains were run using 40,000 iterations with a 10,000-iteration warmup. All continuous outcome and predictor effects were coded using a Z-score transformation prior to fitting to increase model convergence. The multivariate model of fecundity and thermal plasticity restricted against residual correlation.

Selection gradients acting on LT_{50} and its plasticity were fit using a multivariate model that also predicted plasticity as a function of baseline LT_{50} . Across all iterations subjected to selection, the selection gradient portion of the model predicted mean fecundity per sibship as a function of developmental temperature, season of collection, LT_{50} , the plasticity of LT_{50} , an interaction between baseline LT_{50} and its plasticity, and interactions

between developmental temperature and LT_{50} , its plasticity, and the interaction of the two terms. All iterations were also fit with a random effect nesting sibship, pool, season of collection, and population. Fixed and random effects held constant across all iterations are described in Fig. S5 as the ‘base model’. Because cultures varied in their mean body size of gravid females, mean age, and density, these three variables were also fit as alternative fixed effects in multiple model iterations (Fig. S5). Each iteration is listed in Figure S5, accompanied by a Bayes factor measuring its relative marginal likelihood (Berger & Pericchi, 1996). The model that generated the highest Bayes factor was selected and reported under Results.

3. Results

3.1. Environmental temperatures at collection sites – In situ temperature loggers deployed between 2019 – 2023 revealed an expected latitudinal thermal gradient such that temperatures increased toward lower latitudes. However, this cline was driven by the northern- and southern-most sites. The two mid-latitude locations (SC and RM) exhibited comparable mean annual temperature (Fig. 3). Diurnal and seasonal variation did not vary by latitude and were comparable across all sites except for RM, which exhibited less variation on both diurnal and seasonal timescales (Fig. 3B). Lastly, latitudinal clines in temperature were strongest during winter months and were weak-to-absent during summers.

3.2. *Intraspecific variation in performance traits and their thermal plasticity* –

Populations and sexes exhibited significant differences in upper thermal tolerance, organismal performance, and the plasticity of these traits across temperature. Upper thermal tolerance exhibited a significant latitudinal cline such that baseline tolerance decreased toward higher latitudes at a rate of -0.44 °C LT₅₀ per °N latitude. Females were also more thermally tolerant than males by an average of $+0.19$ °C LT₅₀. As the thermal tolerance of populations increased toward lower latitudes, the plasticity of thermal tolerance decreased. This negative correlation was primarily driven by females, which showed strong reductions in thermal plasticity as tolerance increased. In males, thermal plasticity was affected less by baseline tolerance (Fig. 4A). Scaling between sibships' thermal tolerance and its plasticity was significant after controlling against (i) non-independence between the intercept and slope of LT₅₀ reaction norms and (ii) regression to the mean. Non-independence only explained 0.48% of baseline tolerance's negative effect on plasticity while regression to the mean explained 47.97% of the effect.

Body size and generation time varied by population. Body size increased toward higher latitudes (Fig. S3A), while generation time did not exhibit a latitudinal pattern (Fig. S3B). In response to high temperature, body size and generation time both decreased. Body size was less plastic among northern populations, a latitudinal effect opposing the direction seen for the plasticity of thermal tolerance. The plasticity of generation time varied randomly across populations, with SC exhibiting no apparent thermal plasticity in this trait (Fig. S3B).

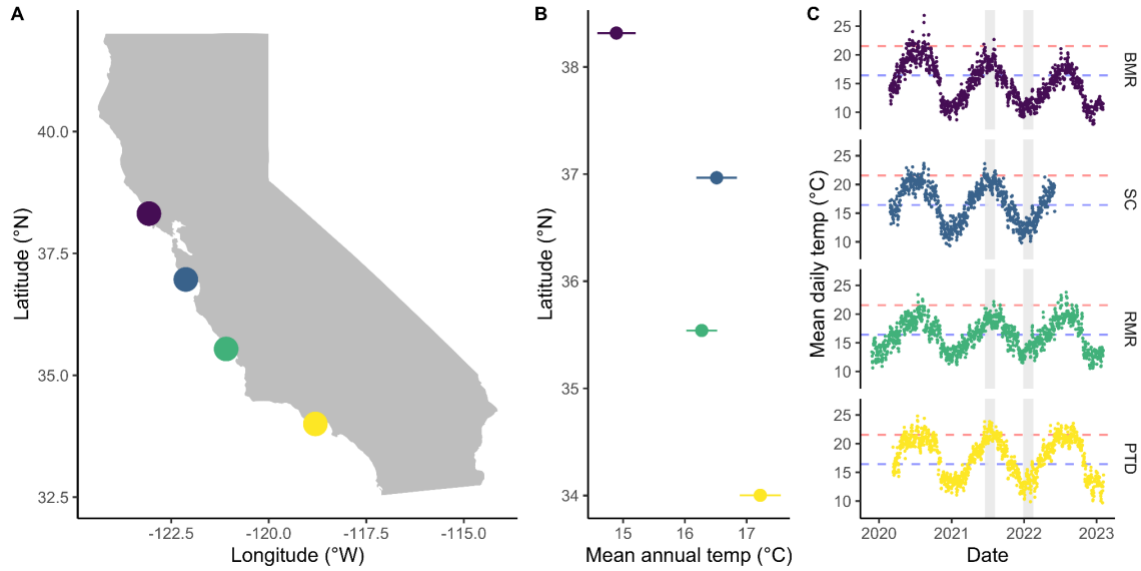


Figure 3 | *Tigriopus californicus* collection sites and thermal environments. (A) Coordinates of four intertidal collection sites in coastal California, USA. (B) Mean *in situ* temperatures recorded in supralittoral pools inhabited by *T. californicus* at each collection site over a two-year period between summer 2020 and summer 2022. Error bars depict \pm 95% CI. (C) Mean daily temperature is plotted across time in each collection site. Color depicts latitude in all panels. Horizontal dashed lines depict the high and low experimental temperatures used in this study. Vertical gray bars depict the 50-day period (the approximate minimum lifespan of *T. californicus*) prior to two field collections in August of 2021 and February of 2022.

3.3. *Fitness costs of thermal plasticity and their dependence on tolerance* – Upper thermal tolerance exhibited neutral selection under high temperature and significant positive directional selection at low temperature such that fecundity increased among more tolerant genotypes. Differences in selection on thermal tolerance between treatments was evidenced by a significant interaction between temperature and LT_{50} in the selection gradient model (Fig. 5A). An insignificant interaction between temperature and thermal plasticity also shaped fitness: a trend of negative directional selection acted on the plasticity under low temperature and yielded a neutral effect under high temperature (Fig. 5A). Positive selection on tolerance and negative selection on plasticity under low temperature were consistent with canalization.

Fitness costs of plasticity in thermal tolerance were significantly greater among thermally tolerant genotypes while plasticity bore neutral fitness effects among the least-tolerant genotypes. This tradeoff between basal tolerance and plasticity was strongest under low temperature as evidenced by a significant three-way interaction between temperature, basal thermal tolerance, and its plasticity. The two-way interaction between basal tolerance and plasticity was also significant. Selection gradients acting on thermal plasticity in the lowest and highest terciles of baseline thermal tolerance equaled -0.089 ± 0.099 . and -0.790 ± 0.181 , respectively, under low temperature. The dependence of thermal plasticity's costs on baseline tolerance was consistent with the fitness tradeoff hypothesis (Fig. 5B).

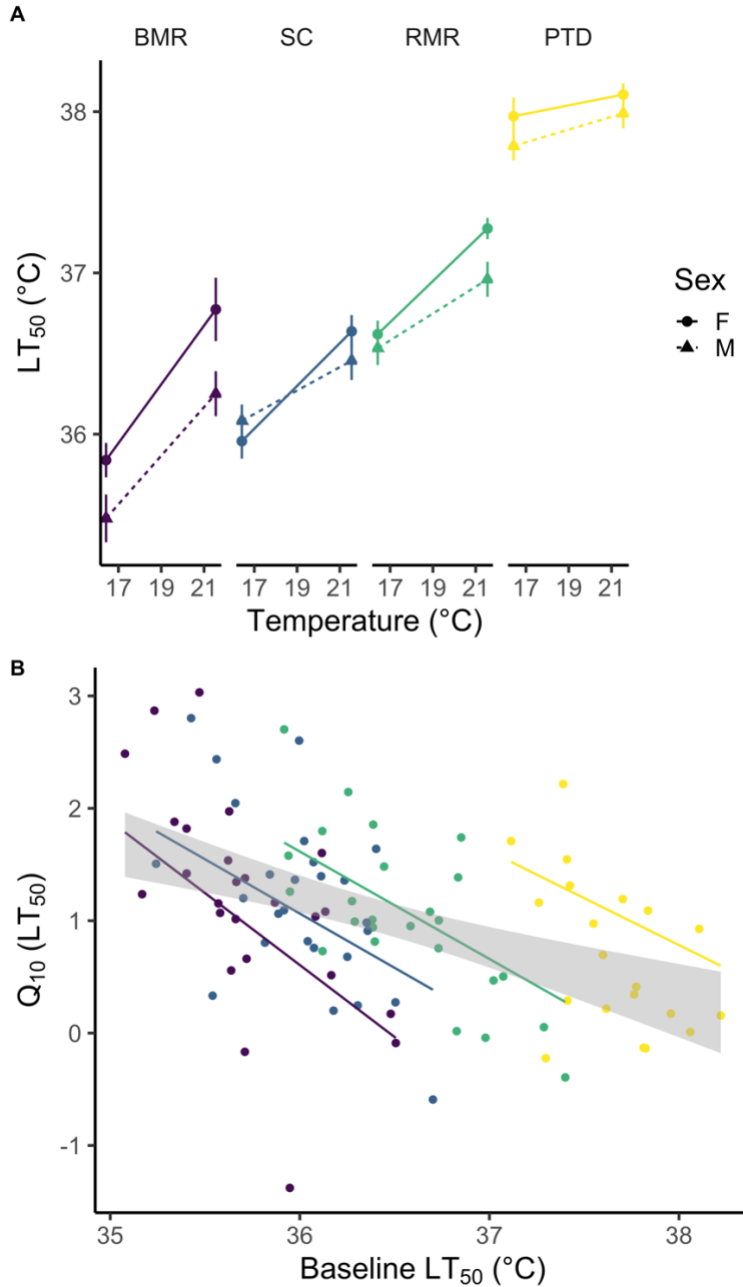


Figure 4 | *Negative correlation between baseline thermal tolerance and thermal plasticity at population and genotype levels.* (A) Upper thermal tolerance measured as LT_{50} is plotted across developmental temperatures grouped by population (color) and sex (shape and line type). Error bars represent 95% confidence intervals of LT_{50} parameter estimates. (B) Thermal plasticity, measured as the Q_{10} of LT_{50} , is plotted across baseline thermal tolerance measured as the intercept of LT_{50} 's reaction norm across developmental temperature. Each point depicts a single sibship whose population is represented by color. The gray confidence interval depicts best fit to raw Q_{10} of LT_{50} . Solid lines depict fitted regressions to adjusted Q_{10} of LT_{50} for each population, which are represented by color. This adjustment reduced the influence of regression to the mean on negative correlations between baseline thermal tolerance and its plasticity using the method described by Kelly & Price, 2005, and Gunderson, 2023.

Temperature and female body length significantly affected sibships' mean egg clutch size per brood. Including these parameters in the selection gradient model controlled for their effects on fecundity, permitting robust estimation of the fitness costs of LT_{50} and its plasticity. The high temperature treatment reduced egg clutch sizes by an average of 15.4% (3.25 eggs). Female body length had a positive but weak effect on fecundity (Fig. S4).

3.4. *Quantitative support for the limit versus tradeoff hypotheses* – The negative correlation between baseline thermal tolerance and its plasticity was attributable to a direct effect of tolerance on plasticity, controlling for fitness effects. After controlling for fitness, the direct effect of baseline tolerance on plasticity was responsible for only 2.22% of the negative association between the two traits. Using model selection and marginal likelihoods, quantitative support for the phenotypic limit and fitness tradeoff hypotheses were measured and compared. A 'limit' model incorporating parameters for direct effects of baseline thermal tolerance on thermal plasticity possessed a relative likelihood of 1.74x compared to a null model assuming neither hypothesis. A 'tradeoff' model, which included parameters predicting fitness costs of thermal plasticity conditional upon baseline tolerance, had a marginal likelihood of 6.74x compared to the null.

The 'limit + tradeoff' model outperformed iterations incorporating singular hypotheses and achieved a marginal likelihood of 11.37x compared to the null. These results demonstrated that our experimental observations of thermal plasticity and fecundity supported both the limit and tradeoff hypotheses, and that these two mechanisms are not mutually exclusive. Rather, the data were best explained by models jointly accounting for both hypotheses (Fig. 6).

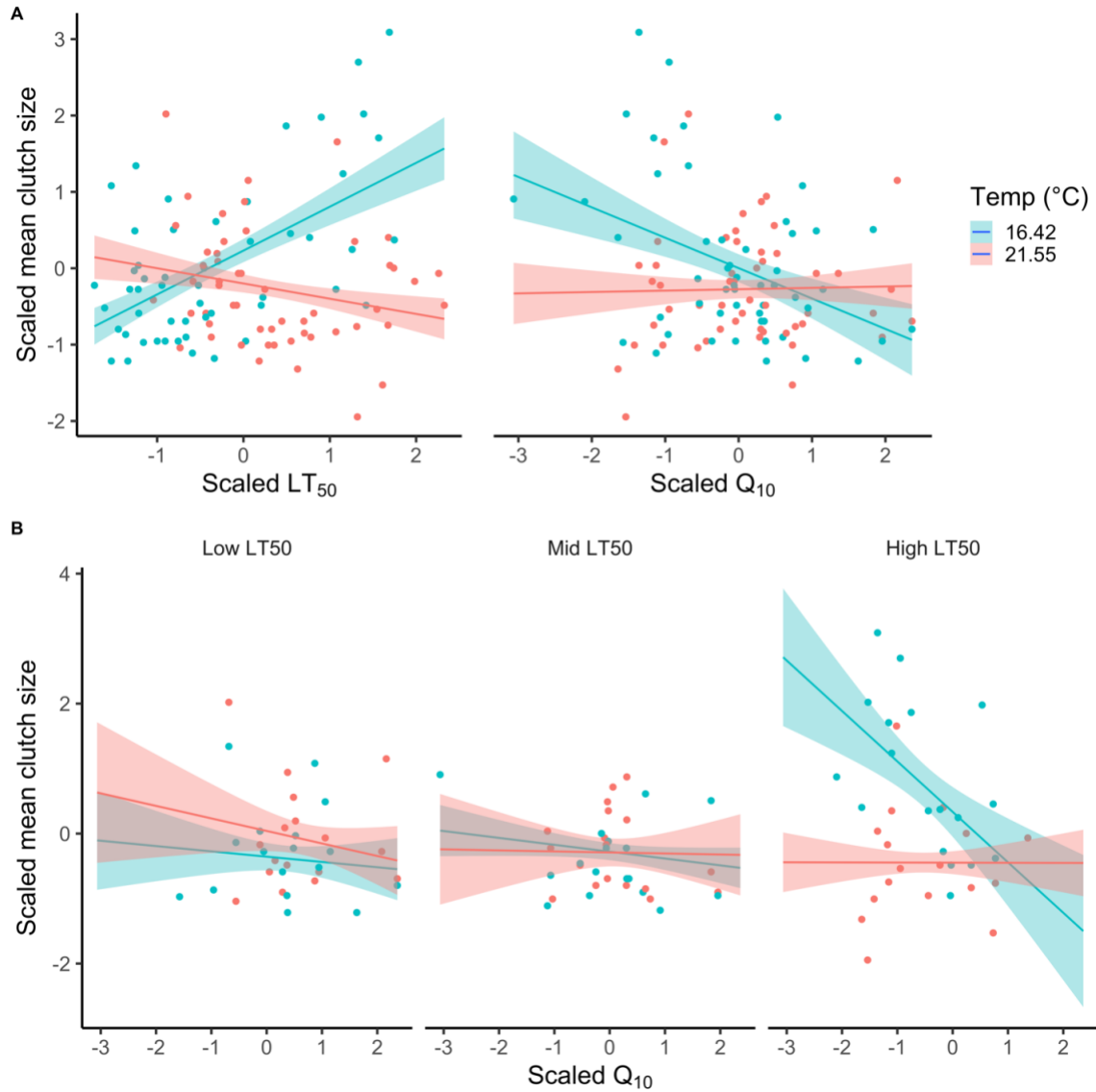


Figure 5 | *Selection gradients acting on upper thermal tolerance and its plasticity.* Each point represents a sibship within a given developmental temperature (low = blue; high = red). (A) Mean-standardized average clutch sizes per sibship x temperature culture are plotted across mean-standardized upper thermal tolerance (LT_{50}). Mean-standardized average clutch sizes per sibship x temperature culture are plotted across mean-standardized plasticity of upper thermal tolerance (Q_{10} of LT_{50}). (B) A fitness tradeoff between baseline thermal tolerance and its plasticity is visualized by plotting mean-standardized clutch sizes across the plasticity of thermal tolerance (Q_{10} of LT_{50}) grouped by tertiles of baseline thermal tolerance (low – high LT_{50}).

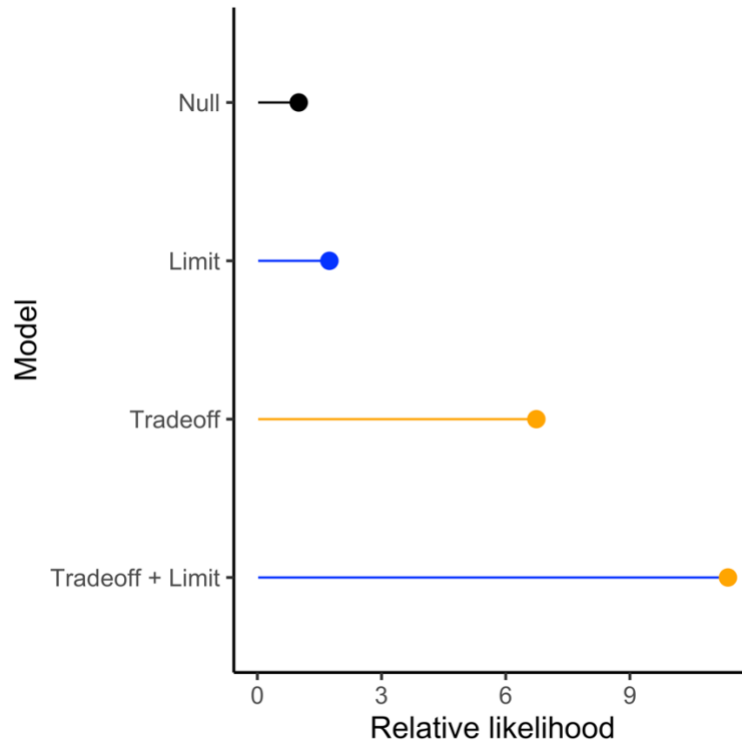


Figure 6 | *Quantitative hypothesis testing using model selection.* The marginal likelihoods of three models containing parameters associated with the fitness tradeoff (orange) and/or physiological limit hypothesis (blue) are plotted relative to a null model lacking these parameters. Marginal likelihoods relative to the null model were computed using the Bayes factor method.

4. Discussion

Whether and how thermal plasticity evolves via natural selection has remained an open question since first being discussed by Feder *et al.* and Huey & Kingsolver (Feder *et al.*, 1987; Huey & Kingsolver, 1989). Addressing this issue stands as a challenge in studies of adaptation to climate change and novel thermal environments (Arnold *et al.*, 2019; van Heerwaarden & Kellermann, 2020). We provide the first empirical support, to our knowledge, for the fitness tradeoff hypothesis in shaping negative associations between ectotherms' upper thermal tolerance and its plasticity by finding that fitness costs of thermal plasticity are greater among thermally tolerant genotypes. By measuring and comparing quantitative support for the fitness tradeoff and physiological limit hypotheses in driving this negative correlation in *T. californicus*, we contextualized the relative importance of fitness tradeoffs in shaping responses by thermal plasticity to directional selection in nature. Here we discuss our results as they relate to (i) the evolution of phenotypic plasticity at large, (ii) theory and prior empirical research regarding the co-evolution of thermal tolerance and its plasticity, and (iii) forecasts of adaptation by ectotherms to warmer, more variable climates.

4.1. Evolution and costs of phenotypic plasticity – Measuring and detecting fitness costs or benefits to phenotypic plasticity has proven challenging (Hendry, 2016; Van Buskirk & Steiner, 2009), and the plasticity of thermal performance is no exception (Arnold *et al.*, 2019). While plasticity should theoretically incur fitness effects, selection gradients acting on plasticity are frequently neutral (Van Buskirk & Steiner, 2009). It has been suggested that one source of plasticity's apparently neutral fitness effects is that they are conditional on traits and/or environmental variables untested in selection gradient

studies (Hendry, 2016; Van Buskirk & Steiner, 2009). With respect to the plasticity of thermal performance, these conditional variables may include (i) how predictable and variable temperature is during an experiment, such that moderate variation may dampen directional selection (Bitter et al., 2021), and (ii) intraspecific variation in fitness consequences attributed to sex, morphotypes, or other phenotypically distinct groups (Hangartner et al., 2022; Svensson et al., 2020).

Our ability to detect significant fitness consequences of thermal plasticity was likely improved by accounting for the contingencies of plasticity's fitness costs on developmental temperature and basal thermal tolerance. Our experiment employed static temperature conditions that likely contributed to the maladaptiveness of thermal plasticity evidenced by significant, negative selection gradients under low temperature (Fig. 5B). An absolute selection gradient of ≥ 0.2 is often regarded as a minimum threshold above which selection is considered evolutionary significant (Scheiner & Berrigan, 1998; Van Buskirk & Steiner, 2009). We measured thermal plasticity selection gradients of -0.397 ± 0.084 under low temperature and a neutral gradient of -0.006 ± 0.052 under high temperature. We also found that thermal plasticity's fitness costs exhibited intraspecific variation such that they significantly increased in more thermally tolerant families (Fig. 5B).

We did not predict that thermal tolerance and its plasticity would bear neutral fitness effects under high temperature. We posit two alternative explanations for this result. Firstly, the neutral effect of plasticity may have been the net effect of thermal plasticity's fitness benefit under high temperature and fitness costs incurred by the static, invariable nature of thermal conditions. Alternatively, high temperature had a direct negative effect on fecundity. This may have reduced fecundity to an extent that is washed out variation in

the selection gradient model attributable to the fitness costs of thermal tolerance and its plasticity. We also did not predict that LT_{50} would incur positive fitness effects under low temperature. One explanation of this result is that laboratory conditions often select for upper thermal tolerance even when developmental temperatures are low or ambient. Positive directional selection on LT_{50} resulting from laboratory selection has been observed during experimental evolution in the calanoid copepod *Acartia tonsa* (M. C. Sasaki & Dam, 2021).

4.2. Fitness tradeoffs shaping thermal plasticity – Fitness tradeoffs between thermal tolerance and its plasticity have become increasingly more discussed in global change biology and evolution, but experiments directly measuring interactive fitness effects of each trait have not been reported (van Heerwaarden & Kellermann, 2020) and are limited by traditional, univariate selection gradient models (Lande & Arnold, 1983). Experimental evolution studies exposing lines to positive selection for upper thermal tolerance have subsequently observed decreases in the plasticity of thermal tolerance (M. W. Kelly et al., 2011, 2017; Morgan et al., 2022; M. C. Sasaki & Dam, 2021). However, it cannot be determined from these studies whether fitness tradeoffs or physiological limits caused reductions to plasticity without measuring (i) the fitness costs of plasticity and tolerance and (ii) the direct effect of tolerance on plasticity, controlling for fitness effects. Furthermore, studies measuring thermal plasticity's fitness costs have largely detected neutral effect (Arnold et al., 2019). In instances where significant costs or benefits were detected, traditional selection gradient models prohibited the fitting of interactive costs between thermal tolerance and its plasticity because their non-independence must be statistically controlled (Kline, 2015). Our expansion of the Lande & Arnold regression

enabled us to determine for the first time whether significant costs of plasticity in thermal tolerance changed as baseline tolerance increased. With this easily executed modification of the Lande & Arnold regression, our method can be applied to existing data from selection gradient experiments to begin evaluating the prevalence of fitness tradeoffs between thermal tolerance and its plasticity.

Thermally tolerant genotypes of *T. californicus* in the top tercile of baseline LT_{50} exhibited a thermal plasticity selection gradient of -0.369, resulting in 3.27x greater negative directional selection on plasticity relative to the lowest tercile of baseline tolerance (Fig. 5C). This difference greatly exceeded a threshold of 0.2 representing significant variation in selection gradients (Scheiner & Berrigan, 1998; Van Buskirk & Steiner, 2009). Among studies on 9 species including plants, birds, and insects, directional or stabilizing selection on thermal plasticity was significant in 4 out of 14 reported traits and also greater than a gradient of 0.2 in 2 out of 14 traits (Arnold et al., 2019; Choi et al., 2019; De Lisle et al., 2022; Svensson et al., 2020; Valdés et al., 2019). Not only was the interactive fitness effect of thermal plasticity and baseline tolerance significant in our study, but it drove large increases in costs relative to the fitness effects of thermal plasticity measured in other systems.

Fitness tradeoffs between thermal tolerance and its plasticity may arise via two mechanisms termed ‘resource acquisition allocation’ (or ‘resource allocation’) and ‘genetic tradeoff’. Under the resource allocation model, energy is limiting and divided between biological processes such that investment in thermal tolerance and plasticity comes at the cost of investment in fitness-correlated traits like reproduction (Roff & Fairbairn, 2007; van Noordwijk & de Jong, 1986). Under a genetic tradeoff, thermal tolerance and its

plasticity share a negative genetic correlation attributed to (i) antagonistic pleiotropy where an allele increasing one phenotype has a decreasing effect on the other or (ii) linkage disequilibrium where distinct alleles have independent effects on tolerance and plasticity but are frequently co-inherited (Williams, 1957). Indeed, it has been long established that thermal tolerance and thermal plasticity/acclimation capacity can share negative genetic correlations (Debes et al., 2021; Ushakov, 1977). While our study's design was unable to determine whether significant fitness tradeoffs between thermal tolerance and its plasticity were attributed to resource allocation or a genetic tradeoff, pursuing this question is essential for determining how the fitness tradeoff may shape the evolution of thermal physiology. This is largely due to genetic tradeoffs being less prohibitive to the effects of drift and selection on phenotypic variation (Roff & Fairbairn, 2007).

4.3 Implications for adaptation to novel thermal environments – Under environments positively selecting for both upper thermal tolerance and thermal plasticity, adaptation may be constrained by physiological limits to tolerance (Kempes et al., 2019) and slowed by fitness tradeoffs between the two traits as visualized in Figure 1C (A. F. Agrawal & Stinchcombe, 2008; Stearns, 1989). We found evidence that both limits and tradeoffs may drive a negative association between thermal tolerance and its plasticity in *T. californicus*: the most likely model of thermal plasticity and fecundity incorporated parameters associated with both hypotheses rather than one or none (Fig. 6). One contributor to this result may be nonlinear reaction norms wherein more tolerant genotypes induce plasticity at temperatures greater than those used in our study. Expanding our study design to non-linear measurements of plasticity across thermal performance curves can eliminate this confounding effect (Schou et al., 2017). However, physiological limits

imposed a strong effect on variation in thermal plasticity and we expect that some of this relationship may remain after accounting for non-linear reaction norms. It therefore remains possible that both physiological limits and fitness tradeoffs jointly shape variance in thermal plasticity among *T. californicus*, but the relative effect strength of each process must be investigated further using experimental designs permitting non-linear thermal reaction norms.

In addition to empirical research, theoretical models represent an opportunity to study the joint influence of limits and tradeoffs on the evolution of thermal tolerance and plasticity (Buckley & Kingsolver, 2021; Gibert et al., 2019). Models of thermal tolerance and plasticity's effect on persistence under climate change have not accounted for these interactions and generally treat tolerance and plasticity as independent, additive effects (Chevin et al., 2010). By modeling the joint evolution of thermal tolerance and plasticity by varying parameters associated with their genetic correlation, physiological limits to plasticity, and the strength of their fitness tradeoffs, we may generate new theory regarding constraints on adaptation to novel thermal environments.

Numerous ectotherms exhibit negative associations between thermal tolerance and its plasticity and are threatened by climate change and/or are commercially significant. The domestication of Atlantic salmon, a fisheries species threatened by warming (Thorstad et al., 2021), has reduced CT_{max} and increased thermal plasticity (Debes et al., 2021). Similarly, Olympia oysters experiencing local extinction attributed to heatwaves and other factors (Raymond et al., 2022; zu Ermgassen et al., 2013) exhibit reduced thermal plasticity in tolerant populations (Bible et al., 2020). If fitness tradeoffs between thermal tolerance and plasticity are prevalent across other ectotherms, the breeding of 'heat hardened'

aquaculture stocks and assisted evolution efforts for species at risk under climate change must balance selection for basal thermal tolerance and plasticity (Gibbin et al., 2017). By accounting for fitness tradeoffs, optimal selection regimes may adaptively increase both traits to thresholds that do not reduce survival or reproduction.

We did not predict that thermal tolerance and its plasticity would bear neutral fitness effects under high temperature. The neutral cost of plasticity may have been the net effect of its fitness benefit under high temperature and fitness costs due to the static nature of thermal conditions. Alternatively, high temperature's direct negative effect on fecundity may have reduced fecundity to an extent that is washed out variation in the selection gradient model attributable to the fitness costs of thermal tolerance and its plasticity. We also did not predict that LT_{50} would incur positive fitness effects under low temperature. One explanation of this result is that laboratory conditions often select for upper thermal tolerance even when developmental temperatures are low or ambient. Positive directional selection on LT_{50} resulting from laboratory selection has been observed during experimental evolution in the calanoid copepod *Acartia tonsa* (M. C. Sasaki & Dam, 2021).

5. Conclusion

Negative correlations between ectotherms' upper thermal tolerance and thermal plasticity, though often observed, have received little mechanistic investigation. Due to a lack of statistically robust methods for accounting for the effect of regression-to-the-mean on this negative correlation across ectotherms (Gunderson, 2023; Gunderson & Revell, 2022), the significance of this phenomenon during thermal adaptation has remained an open question. The presence of a fitness tradeoff between thermal tolerance and its

plasticity in *T. californicus* signifies that these two traits are unlikely to evolve independently of one another, a relationship that may affect thermal adaptation in nature. Rather than simultaneous selection on thermal tolerance and its plasticity driving optimal changes in both traits, thermal tolerance and plasticity may evolve in a three-legged race by which adaptive change in one trait comes at the cost of the other. The generalizability of the effect of fitness tradeoffs on negative correlations between tolerance and plasticity should be investigated further in ectotherms to uncover the extent to which this mechanism shapes thermal physiology in metapopulations. Such studies should be performed with careful investigation of alternative hypotheses such as the physiological limit, which we found likely to operate alongside fitness tradeoffs in *T. californicus*.

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Chapter III: Contributions of phenotypic plasticity and evolution to seasonal variation in the thermal tolerance of *Tigriopus californicus*

Abstract

Adaptation to novel thermal environments depends on whether and how plastic versus genetic changes in thermal performance covary – e.g., whether their effects are additive, antagonistic, or synergistic. Studying 4 latitudinally distributed populations of *Tigriopus californicus*, a short-lived intertidal copepod exhibiting local adaptation to temperature, I measured the upper thermal tolerance (LT₅₀) of males, females, and juveniles from wild animals during 5 collections across 10 months of seasonal changes in temperature. I recorded time series temperatures across this 10-month duration and conducted point measures of dissolved oxygen (DO) and salinity during collections. By rearing wild-caught animals from high summer and low winter temperatures under common garden for 3 generations and remeasuring upper thermal tolerance, I calculated the proportion of seasonal and interpopulation variance in LT₅₀ attributed to genetic and environmental sources before modeling their covariance. Seasonal variation in temperature was comparable between sites, but autocorrelation between DO and salinity and temperature changed across latitude – southern sites exhibited higher salinity and lower DO following warming. Seasonal variation in LT₅₀ was non-linear in each population and decreased with latitude such that southern populations increased LT₅₀ toward a thermal optimum of ~16.0 °C before it declined toward maximum *in situ* temperatures. Plasticity accounted for 5.27 – 21.94% of total seasonal variation in LT₅₀ and largely opposed the direction of genetic change. Plastic and genetic changes in LT₅₀ across season shared

significant, negative covariance – evolved increases in LT_{50} during warming accompanied plastic decreases in LT_{50} that reduced its total variance. My results demonstrate that phenotypic plasticity and evolution jointly shape seasonal variation in thermal tolerance of *T. californicus* in nature in a manner consistent with countergradient variation, potentially due to compensation against maladaptive, plastic reductions in thermal tolerance.

1. Introduction

Populations and species will likely need to increase their thermal performance in response to warming by both evolving and plastically modifying these phenotypes to keep pace with rates of global climate change (Donelson et al., 2019; Sgrò et al., 2016). The ability of biodiversity to mount plastic and evolutionary responses to warming depends on (i) the extent to which plastic versus evolved changes in thermal performance occur in nature and (ii) how these two processes covary with one another (Diamond et al., 2017; Leonard & Lancaster, 2020; Schilthuizen & Kellermann, 2014; Sgrò et al., 2010). Plastic and evolutionary changes in phenotypes occur across dramatically different timescales, posing a challenge to investigating these two issues. Species with short life cycles experiencing strong seasonal variation in temperature are one set of study systems that can potentially overcome these obstacles (M. C. Sasaki & Dam, 2020; Warner & Shine, 2005). To this end, I studied plastic versus genetic contributions to seasonal variation in upper thermal tolerance in wild populations of the short-lived, intertidal copepod *Tigriopus californicus*.

The strength and direction of thermal plasticity accompanying evolved differences in thermal performance can impact adaptation to temperature (Fig. 7). Negative correlations between plasticity and evolutionary change in thermal tolerance may result from plasticity buffering against natural selection on trait means (Crispo, 2008; Oostra et al., 2018b). By contrast, plastic populations exhibiting greater evolutionary rates in thermal tolerance can be the product of plasticity first evolution, whereby plasticity promotes the expression of cryptic genetic variants that become subject to selection (Crispo, 2007; M. Kelly, 2019; Levis & Pfennig, 2016). Assuming plasticity and/or evolved changes in thermal tolerance in response to warming can be negative, negative covariance between the two processes would represent countergradient variation (CnGV), visualized in Figures 1B - 1C where maladaptive plasticity is genetically compensated for (Conover et al., 2009).

CnGV in thermal performance has been documented between populations of ectotherms across spatial variation in temperature (Dwane et al., 2023; Fanguie et al., 2009; Gardiner et al., 2010; Villeneuve et al., 2021). Covariance between thermal plasticity and the genetic effects on thermal performance (COV_{GE}) should also theoretically occur in response to temporal sources of thermal change such as heatwaves, but temporal studies of COV_{GE} measuring evolution in action remain sparse (Schaum et al., 2022). It is argued that understanding temporal COV_{GE} will provide meaningful improvements to predictions of how biodiversity will adapt to climate change by (i) determining whether environments induce phenotypic change toward or away from directions favored by natural selection and (ii) reducing genotype-environment interactions shaping genetic variation for plasticity (Albecker et al., 2022; Conover et al., 2009; Tüzün & Stoks, 2018).

Beyond its importance in nature, quantifying plastic versus genetic contributions to seasonal variation in thermal performance represents an important step in my thesis research on the evolution of thermal plasticity in *T. californicus*. The influence of thermal plasticity's evolution on organisms' persistence under climate change depends on the extent to which plasticity shapes intraspecific variation in thermal performance in wild populations. If seasonal fluctuations in thermal physiology are largely evolved, thermal plasticity may not be an important functional trait or contribute to adaptive responses to future climate change. However, phenotypic change associated with thermal stress in wild populations is rarely partitioned into plastic versus evolved responses (Acker et al., 2023; Schilthuizen & Kellermann, 2014).

Studying covariation between plastic and evolved sources of variation in thermal physiology across season is critical to my research on *T. californicus* and a necessary contribution to the fields of evolutionary and global change biology, particularly with respect to temporal co-gradient variation. In *T. californicus* for example, our only knowledge of co-gradient variation in plastic and evolved differences in thermal tolerance are derived from laboratory estimates and applied to spatial variation across populations (M. W. Kelly et al., 2011; Pereira et al., 2017). While space-for-time substitutions have been of immense value to studies of environmental adaptation (Blois et al., 2013; Wogan & Wang, 2018), evolutionary responses to temporal variation in temperature provide a more realistic example of how ectotherms will or won't adapt to novel thermal environments (Damgaard, 2019).

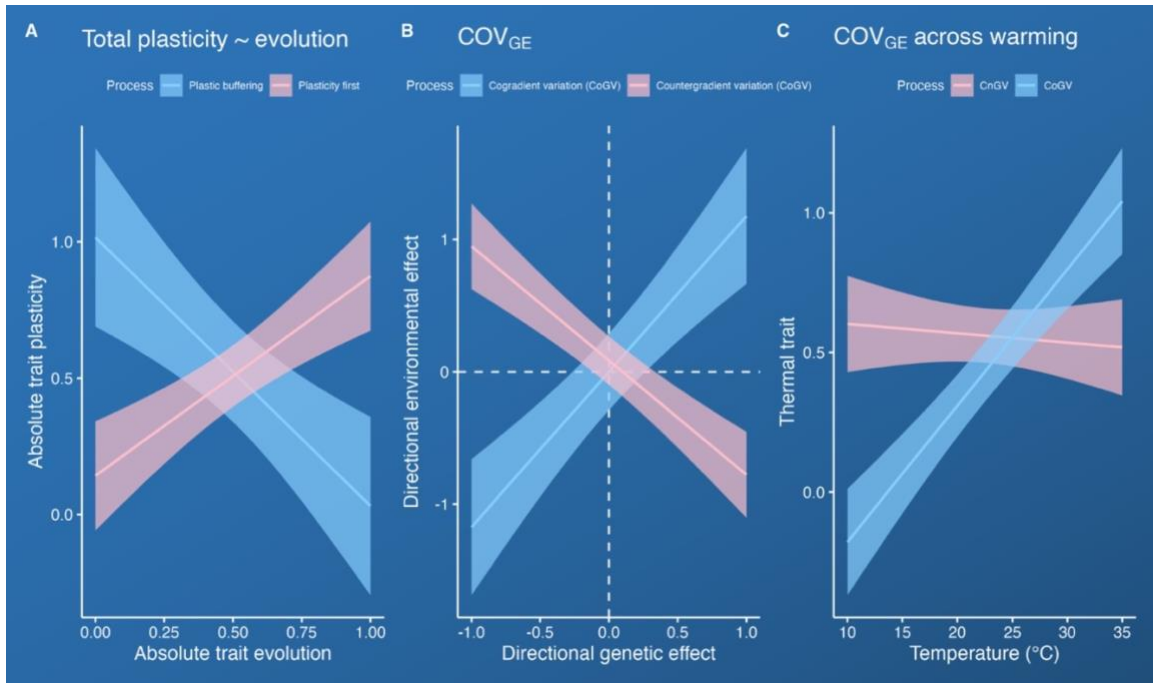


Figure 7 | Relationships between evolution, plasticity, and covariation in their effects. **(A)** Mock measures of absolute plasticity and absolute evolution of traits representing plasticity first evolution and plastic buffering against evolution are visualized. **(B)** Mock measures of covariance between directional genetic and environmental effects (COV_{GE}) on phenotype visualizing cogradient (CnGV) and countergradient (CnGV) evolution through space or time. **(C)** Effects of CoGV and CnGV on variation in a hypothetical thermal trait across spatial or temporal variation in temperature.

I measured variation in upper thermal tolerance across seasonal changes in temperature in 4 latitudinally distributed populations of *T. californicus*. By remeasuring the thermal tolerance of common garden lines derived from wild *T. californicus*, I estimated the proportions of seasonal change in thermal tolerance that resulted from phenotypic plasticity and genetic effects. Lastly, I modeled covariance between plastic and evolved changes in thermal tolerance in wild populations across season. These efforts determined the extent to which phenotypic plasticity shapes intraspecific variation in thermal tolerance of *T. californicus* across time and its potential role in shaping the evolution of thermal physiology.

2. Methods

2.1. Site selection and recording of time series temperatures – 4 rocky intertidal sites in coastal California distributed across 4.31 °N of latitude were selected as collection sites for this study due to (i) the status of two sites as protected, ecological research stations, (ii) their even distribution across latitude, (iii) similarities in the angle of their coastlines, which can affect diurnal warming rates (Seabra et al., 2011), and (iv) an abundance of *T. californicus* at each site. In order of decreasing latitude, these sites are the Bodega Marine Reserve in Bodega Bay, CA (BMR), Four Mile Beach in Santa Cruz, CA (SC), the Kenneth S. Norris Rancho Marino Reserve in Cambria, CA (RMR), and Pt. Dume State Beach in Malibu, CA (PTD). Coordinates of each site are listed in Chapter 2. TidBit MX 3000 temperature loggers (Onset Computer Corp.) were non-invasively installed in 3 pools per site at the beginning of the study and set to a 10-minute recording interval. Loggers were twice transferred to different pools at BMR due to evaporation.

2.2. *Collection of T. californicus and environmental point measures* – Collections were performed under California Fish and Wildlife scientific collecting specific use permit S-192200007-19260-001 between June, 2021, and April, 2022. 5 field collections of *T. californicus* were conducted at each site at a target interval of every other month. Following collection, animals were transported to laboratory facilities at UCSB for phenotyping and rearing of common garden lines (Table 1). Sites were visited once per day over a 4-day period during each collection excluding October, 2021, during which a final collection to BMR was prohibited due to flooding and extreme wave action, resulting in a delayed December, 2021 collection. To control for bias in the timing of collections, site visits during each collection period were arranged so the mean positions sites in the order of visits were equivalent at the end of the study. All collections were performed within a ± 2 hr window of low tide. Based on laboratory studies, this timespan incorporates approximately 2.5 – 6 generations of population turnover (Powers et al., 2020). This period likely included weak, include random variation in genetic and environmental effects on phenotype due to wave action, flooding of pools, and pool evaporation. However, pool evaporation only occurred in 3.33% of pools in which temperature loggers were deployed.

T. californicus were collected from 5 distinct supralittoral pools per site during each collection using noninvasive hand tools and transferred into 500 mL polystyrene cups. DO was measured in 10 pools per site per collection using an Oxyguard Polaris probe (Oxyguard International). Salinity was measured in the same 10 pools per collection using a refractometer whose measurements were calibrated against $n = 3$ seawater samples per site visit measured with a YSI salinity probe at UCSB laboratory facilities. 500 mL cups received seawater changes immediately following collection and were given oxygenated,

50 μm -filtered seawater controlled to a temperature ± 2 °C of the daily mean temperature of the site measured via TidBit MX 3000 loggers. Animals were fed ground 20% spirulina fish food flakes *ad libitum* upon seawater changes.

Animals were held in coolers during the 4 days of each collection trip and exposed to a 12 hr light:dark cycle regulated by LED lights installed within coolers. Cooler temperatures were monitored and manipulated with ice packs, resulting in transport temperatures that achieved a mean of ± 0.51 °C of *in situ* temperatures recorded in 21 d prior to collections, controlling against artificial effects of transportation temperature on seasonal variation in thermal performance. Each collection cup received daily seawater changes and feedings as described above.

2.3. Laboratory maintenance of collections, LT_{50} assays, and common garden rearing – On arrival to UCSB, animals were housed in incubators set to the mean temperature recorded at all 4 sites in the 21 d prior to collection and received daily seawater changes until phenotyping. LT_{50} assays were conducted on one population per day 4 d post-collection as described in Chapter 2 with the following adjustments. $n = 4$ females or males were combined per 200 μL assay tube on a 96 well plate. The distributions of females, males, and pools were randomly arranged across 96 well plates to control against positional effects during thermal gradient exposure. The pools animals were collected from were recorded during thermal tolerance scoring. Common garden lines were established from August, 2021, and February, 2022, collections and maintained for 3 generations before being phenotyped for LT_{50} . Methods for common garden rearing and phenotyping are described in Chapter 2. LT_{50} data used in this study were derived from low temperature

common garden cultures (16.42 °C) which were comparable to the annual average temperature of 16.51 °C recorded across all sites.

Survival assays of F1 offspring from each population under the common garden were conducted. N = 12 nauplii larvae from each of the 3 lines per population per month were reared in individual 50 mL culture housed in 12-well plates stored inside common garden incubators for 21 days. This period spanned metamorphosis into the copepod (juvenile) and sexual maturity. Survival was scored in each culture every 2 days. Seawater changes were conducted every 4 days. Animals were fed an ad libitum diet of 1 small 20% spirulina fish food flake per culture. Survivorship analysis was conducted in the R package ‘Survival’ v.3.5-5.

2.4. Modeling plastic versus genetic changes in LT_{50} – Variation in the LT_{50} of field collected *T. californicus* was modeled using a logit-linked, binomial generalized linear model that predicted survival as a function of thermal gradient exposure, latitude, sex (i.e., female, male, or unknown juvenile), a second-order polynomial of the mean temperature at populations’ collection sites 50 days prior to collection, and an interaction between a categorical population variable and the 50 d mean temperature polynomial. *In situ* temperatures were modeled using a polynomial because a likelihood ratio test comparing linear versus polynomial effects revealed that LT_{50} ’s association with temperature was significantly more likely to be non-linear. The interaction between temperature and population fitted population as categorical rather than a continuous effect of latitude to prevent bias toward predicting that seasonal variance in LT_{50} depended on latitude. The LT_{50} of common garden lines was modeled using the methods described in Chapter 2,

resulting in coefficients of seasonal, genetically fixed divergence in LT₅₀ for each population, sex, and population x sex group.

The proportion of seasonal variance in LT₅₀ explained by environmental effects was modeled using a Bayesian approach predicting August LT₅₀ – February LT₅₀ of field collected animals (V_i) as a function of a random intercept for each population (Z_i) and a fixed effect of genetic divergence in LT₅₀ between seasonal common garden lines of each population (β_1). Because total phenotypic variance is a function of genetic and plastic effects, the random intercepts for each population represent coefficients for plastic changes in LT₅₀ across seasons (Eq. 4). Bayesian model fitting is described in Section 2.5.

$$V_i = Z_i + \beta_1 X_i + \varepsilon \quad (\text{Eq. 4})$$

The proportion of seasonal LT₅₀ variance explained by plasticity was then calculated as the plasticity coefficient divided by change in LT₅₀ between August and February in field collected animals. Total seasonal variance in LT₅₀ explained by plasticity was calculated using the equation below, where E equals plasticity of population i , β_1 equals the linear parameter of *in situ* temperature's effect on LT₅₀ in that population, β_2 equals the quadratic parameter of temperature's effect on LT₅₀, and P equals the proportion of seasonal divergence in LT₅₀ explained by plasticity in that population (Eq. 5).

$$E_i = (\beta_{1,i} + \beta_{2,i}) * P_i \quad (\text{Eq. 5})$$

2.5. *Modeling covariance between plastic and genetic effects on LT_{50}* – Covariance in genetic and environmental effects on LT_{50} across seasonal fluctuations in temperature was modeled for all sex x population groups including juvenile unknowns to maximize the number of observations included in the model ($n = 12$). Evolved ΔLT_{50} was calculated as the difference in basal LT_{50} between seasonal common garden lines as described above. Plastic ΔLT_{50} was calculated per population x sex using a different method than described above for plasticity per population. There was not sufficient power to apply this Bayesian method to 12 sex-by-population groups as opposed to 4 populations. Seasonal plasticity was calculated as the total ΔLT_{50} of field collected animals across season - evolved ΔLT_{50} . Because estimations of a coefficient derived from another measure that it will be regressed with creates issues of non-independence and regression to the mean, this calculated difference was transformed using (i) a Kelly & Price transformation described in Chapter 2 to remove the effect of regression to the mean and (ii) an orthogonal polynomial transformation, also described in Chapter 2, controlling against statistical non-independence between plastic and evolved ΔLT_{50} (C. Kelly & Price, 2005; Kline, 2015).

Covariance between effects of adjusted, plastic ΔLT_{50} (V_E) and evolved ΔLT_{50} (V_G) on total ΔLT_{50} observed in the field (V_P) was modeled using a linear mixed model predicting V_P as a function of V_E , V_G , and a random effect of population and calculating covariance between the V_E and V_G parameters derived from this model using the `vcov()` function in R stats. Bootstrapping was used to determine the likelihood of measuring the observed COV_{GE} upon resampling using the ‘boot’ function of the R package `boot` v1.3-2.81 set to 10,000 resamples of data used to model observed COV_{GE} (Davison & Hinkley, 1997).

3. Results

3.1. Environmental variation across season and latitude – Variation in mean annual temperature across latitude is reported in Chapter 2 and decreased toward northern sites. Seasonal changes in temperature varied across sites but not as a function of latitude. In this chapter, I found that seasonal differences in mean temperature 50 days prior to August, 2021, and February, 2022, collections were highest at the southernmost site (PTD) and equaled 8.04 °C. SC, the second most-northerly site exhibited a seasonal difference of 7.55 °C and BMR, the most northern site, 7.49 °C. RMR exhibited the least seasonal variation in temperature equaling 4.94 °C, 35.73% lower than other sites. This dramatic reduction in temperature seasonality was driven by RMR's location within a region that receives the highest levels of marine fog along the range of this study (Iacobellis & Cayan, 2013). Fog and cloud cover may have limited maximum temperatures and reduced diurnal variation in temperature as well.

Diurnal variation in temperature was also unaffected by latitude (Fig. 8) and was highest in PTD at a mean difference of 20.00 °C \pm 1.17 95% CI between daily minima and maxima. Diurnal variation in temperature was second highest in SC (16.29 \pm 0.36 °C) followed by BMR (13.11 \pm 0.37 °C) and RMR (12.35 \pm 0.25 °C). Point measures of DO revealed that, as expected, oxygen increased in supralittoral pools between sunrise and midday. However, rates of diurnal increases in DO varied according to latitude such that midday DO maxima were highest in northern sites and limited in southern sites (Fig. 8). Point measures of salinity also revealed a latitudinal effect – when low tides occurred during midday and thus exposed supralittoral pools to greater evaporation (Geng et al., 2016), salinity increased more in southern, low latitude sites (Fig. 8). Interestingly,

latitudinal thermal gradients were strongest in winter months versus summer (Fig. 9). These results collectively demonstrate that latitudinal thermal gradients were variable across season and associated with greater DO and lower salinity toward higher latitudes.

3.2. *Intraspecific variation in LT₅₀ between populations, sexes, and seasons* – Latitude, sex, and seasonality each shaped intraspecific variation of upper thermal tolerance in *T. californicus*. LT₅₀ decreased with latitude at a rate of -0.5583 ± 0.0105 °C per °N ($p < 2e-16$; Fig. 10A) and was higher in females than males by 0.5664 ± 0.1437 °C ($p = 8.08e-05$; Fig 10B). LT₅₀ also exhibited non-linear variation across seasonal changes in temperature – LT₅₀ increased between annual thermal lows and a thermal optimum of ~ 16.0 °C, the approximate annual mean temperature across all sites, before decreasing toward annual maximum temperatures (Fig. 10B).

Seasonal variance in LT₅₀ decreased with latitude ($\chi^2 = 13.372$; $p = 0.0375$) such that southern populations exhibited positive relationships between *in situ* temperature and LT₅₀ while northern populations exhibited stronger, negative associations between temperature and LT₅₀. In Figure 11A, this effect is plotted as total seasonal variation in LT₅₀ per °C, which was estimated as the summed second-order polynomial coefficients fitted to each population's non-linear change in LT₅₀ across temperature. Here, the linear coefficient representing the directionality of temperature's effect on LT₅₀ was added to the quadratic coefficient representing the 'steepness' of their non-linear relationship. As a result, strong linear relationships with low rates of change across temperature amounted to less seasonal variance in LT₅₀ than populations that exhibited strong linear and quadratic effects (Fig. 11A).

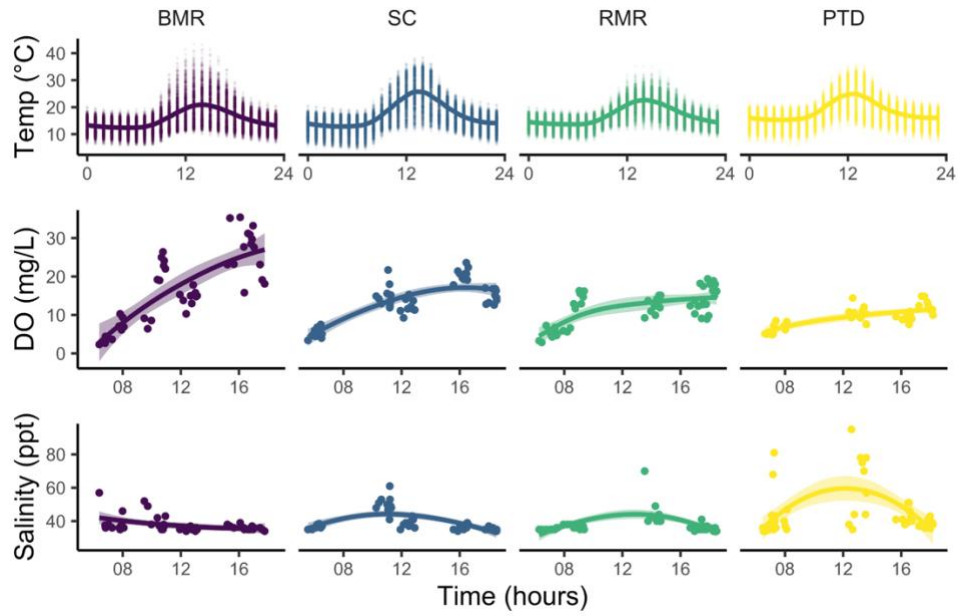


Figure 8 | *Diurnal abiotic variation across latitude.* Temperature (top), dissolved oxygen (DO; middle), and salinity (bottom) of *Tigriopus californicus* pools at all sites are plotted across time of day to visualize diurnal variation in abiotic factors. Temperature data are derived from time series measurements. DO and salinity values are derived from point measures recorded during *T. californicus* collections. Points represent individual measurements. Salinity changes in salinity across time represent an effect of the timing of low tide rather than true diurnal fluctuations.

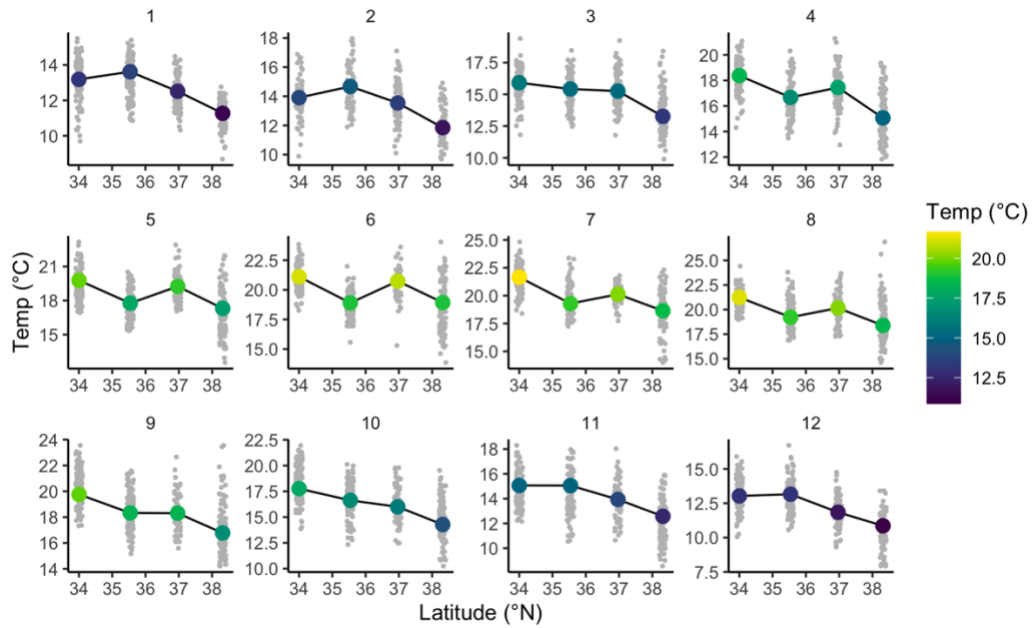


Figure 9 | Monthly latitudinal temperature gradients measured *in situ*. Grey points depict daily mean temperature. Large points depict mean monthly temperatures. All data are grouped by months listed in numerical order. Average monthly temperature is visualized by color.

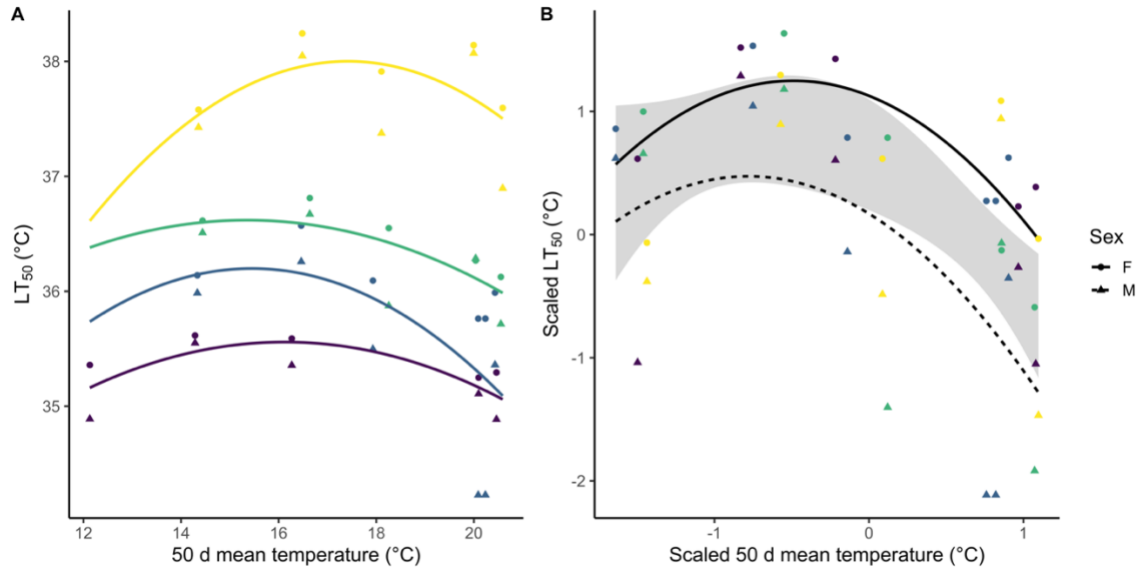


Figure 10 | *Seasonal variation in upper thermal tolerance.* Point shape represents sex. **(A)** Quadratic relationships between LT_{50} of females and males from each population (yellow = low latitude; blue = high latitude) are plotted across the mean temperature 50 days prior to collections. **(B)** Scaled estimates of LT_{50} and *in situ* temperatures plotted in panel A depict generalized, non-linear trends of seasonal warming's effect on LT_{50} across all populations. The grey region depicts 95% confidence intervals of the fitted curve across all points. Solid lines depict that fit to female LT_{50} . Dashed lines depict fit to male LT_{50} .

3.3. *Plastic and evolved contributions to seasonal variation in upper thermal tolerance* – Plastic changes in LT_{50} between seasons randomly varied across populations while the quotient of plastic variance in LT_{50} divided by its evolved divergence between seasons increased toward southern populations: BMR = -0.8354; SC = -0.4757; RMR = -0.1883; PTD = 2.8759. Multiplying total seasonal variance in LT_{50} per population by the proportion of variance explained by plastic effects revealed that directional plasticity in LT_{50} varied by latitude such that northern populations exhibited strong, negative plasticity of LT_{50} in response to warming while southern populations plastically increased LT_{50} (Fig. 11B).

Despite most populations exhibiting negative, linear relationships between *in situ* temperature and LT_{50} , almost all populations and sexes experienced positive associations between temperature and genetically fixed, seasonal changes in LT_{50} . The strength of evolved variation in LT_{50} differed between populations. BMR and PTD exhibited insignificant increases of $+0.0075 \pm 0.0162$ °C ($p = 0.6445$) and $+0.0204 \pm 0.0104$ °C ($p = 0.0533$) in LT_{50} per °C of thermal variation between August 2021 and February 2022. SC and RMR significantly increased genetically fixed LT_{50} by $+0.0574 \pm 0.0122$ °C ($p = 2.609e-06$) and $+0.0803 \pm 0.0157$ °C ($p = 3.104e-07$) per °C of seasonal variance (Fig. 11C). Populations with high LT_{50} exhibited significantly reduced survival under common garden conditions ($p = 0.0437$), an effect that was marginally worse among winter collections compared to summer ($p = 0.0752$).

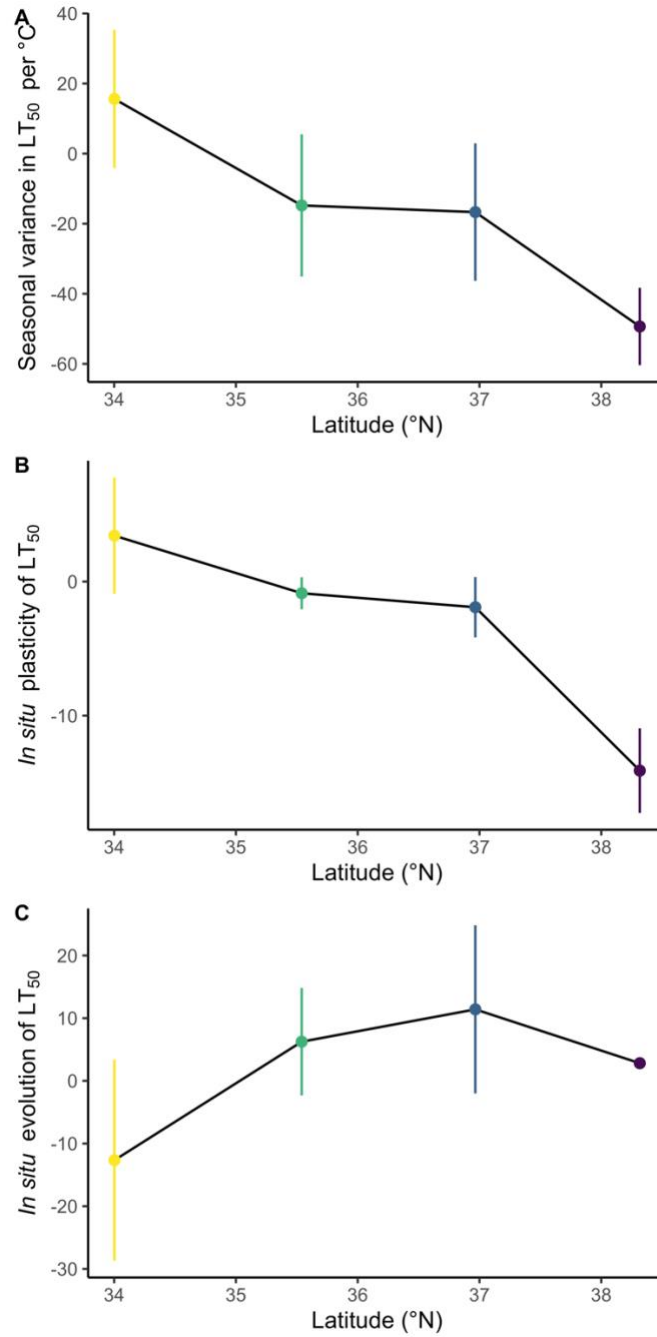


Figure 11 | *Genetically fixed and plastic variation in thermal tolerance across seasons.* Panels A, B, and C respectively visualize parameter estimates for total phenotypic variance, plasticity, and genetically fixed variance in LT_{50} across seasonal changes in temperature in each population. Error bars depict $\pm 95\%$ CI intervals of parameter estimates.

3.4. Covariance between plastic and genetic differences in upper thermal tolerance

– Plastic variation in LT_{50} between seasons estimated for all combinations of population x sex negatively covaried with genetically fixed changes (Fig. 12). On average, an evolved increase in LT_{50} of 1.0 °C was accompanied by a plastic decrease of -0.8588 ± 0.3179 °C (Q2.5 = -1.4459; Q97.5 = -0.1911). After correcting for regression to the mean and non-independence's effects on the relationship between genetic and plastic effects on LT_{50} using the Kelly & Price transformation (C. Kelly & Price, 2005) and orthogonal polynomial transformation (Kline, 2015), bootstrapped estimates of $LT_{50} COV_{GE}$ equaled a mean of -0.13193 ± 0.0975 SD. The distribution of bootstrapped COV_{GE} possessed 95% confidence intervals not overlapping with 0, demonstrating significant countergradient variation (Fig. 12B). Bootstrapped COV_{GE} was calculated by controlling for the effect of population because severe multimodality in COV_{GE} resulted from models that did perform this control. Sex was not included as a control and the resulting COV_{GE} distribution thus exhibits minor bimodality (Fig. 12B).

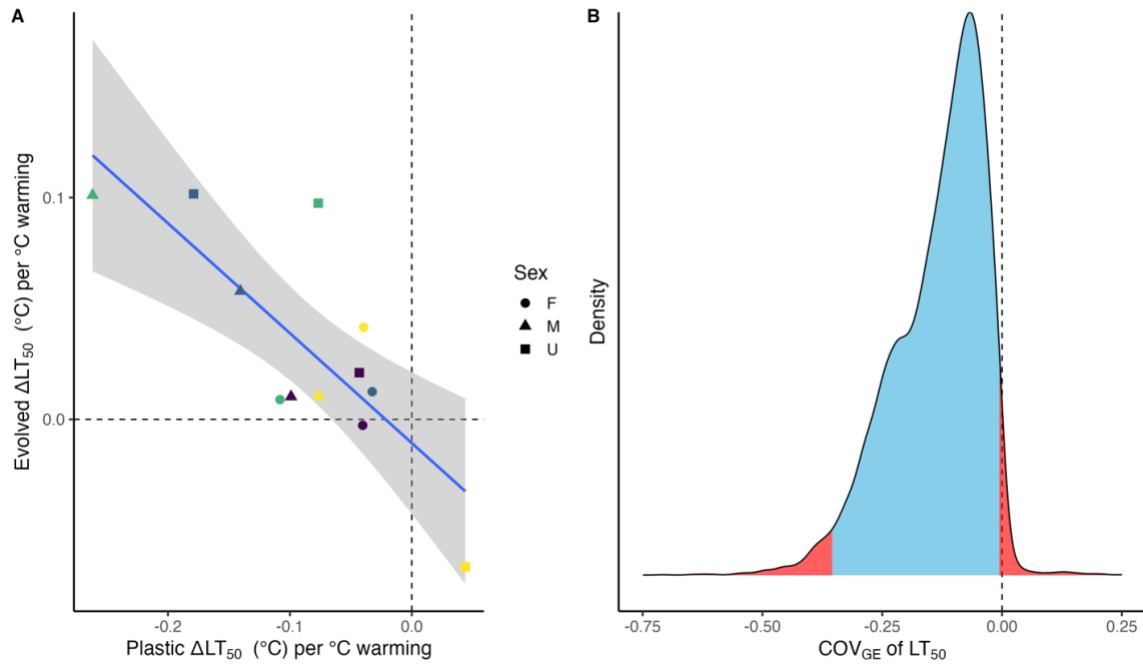


Figure 12 | Countergradient variation in temporal changes to thermal tolerance across seasons. **(A)** Untransformed estimates of evolved variation in LT_{50} per $^{\circ}C$ of seasonal warming are plotted across plastic variation in LT_{50} for all combinations of population and sex (F = female; M = male; U = unknown juvenile). **(B)** Density of bootstrapped COV_{GE} predictions for LT_{50} transformed to correct for the effect of regression to the mean and non-independence. Blue depicts the 95% density interval while red depicts the <2.5% and >97.25% intervals. A vertical dashed line is plotted across $COV_{GE} = 0$.

4. Discussion

Whether and how the plasticity of a trait covaries with its genetic variation affects its functional and evolutionary roles in populations (Urban et al., 2020) and the evolution of that trait and its plasticity under environmental change (Albecker et al., 2022; Stamp & Hadfield, 2020). Studying populations of *T. californicus* across seasonal changes in temperature during a single year, I observed patterns of temporal CnGV in upper thermal tolerance and quantitative support for the hypothesis that thermal tolerance's plasticity covaries with its genetic variation. Below I discuss potential causes and consequences of this CnGV in the context of adaptation to global change. I also highlight contrasts between the results of field and laboratory experiments in *T. californicus* that help explain this result before making recommendations for field and experimental research on thermal adaptation informed by these disparities.

4.1. Potential mechanisms of temporal, countergradient variation in thermal tolerance – CnGV is often discussed in the context of adaptive evolution as a process called genetic compensation, whereby maladaptive environmental effects on phenotype are compensated against by evolved divergence in trait means over space and time (Grether, 2005). While it has been argued that CnGV is necessarily driven by maladaptive plasticity (Albecker et al., 2022), it is possible that maladaptive genetic effects such as constraint on a phenotype due to genetic correlation with another phenotype under selection can be compensated for by adaptive, plastic changes. This process is called plastic compensation and is traditionally discussed as a secondary compensation against maladaptive plasticity (Morris & Rogers, 2013).

I observed that high LT_{50} came at a significant cost of larval survival that decreased under warmer temperatures to a marginally significant extent such that variation in LT_{50} was neutral during summer. Furthermore, survival is one component of total fitness. While high thermal tolerance may have a survival cost under cool temperatures, this does not require that high thermal tolerance is maladaptive under warm temperature. Thus, whether genetic variance in LT_{50} is adaptive, neutral, or maladaptive in wild populations of *T. californicus* requires further investigation. Because evolved increases in upper thermal tolerance following warming have generally been found to be adaptive in ectotherms (Diamond et al., 2018; Gilbert & Miles, 2017; Logan et al., 2014), I make that assumption throughout the remainder of this chapter and propose that observed CnGV may result from genetic compensation against maladaptive plasticity. This assumption permits the following hypothesis to explain CnGV in LT_{50} – thermal stress reduces upper thermal tolerance via condition dependence, where a reduction in energy or resources at high temperature prohibits investment toward LT_{50} (Buchanan et al., 2013; Rowe & Houle, 1997). As a result, positive directional selection on LT_{50} may compensate against maladaptive, condition-dependence (Grether, 2005). Determining how negative plasticity or condition-dependence in LT_{50} arises in natural populations, despite its thermal plasticity being exclusively positive in laboratory studies, is critical for understanding how CnGV of thermal tolerance arises in *T. californicus*.

4.2. *Negative thermal plasticity in the natural environment* – Thermal plasticity in wild populations of *T. californicus* was predominantly negative such that the linear coefficient of LT_{50} 's quadratic variation across *in situ* temperatures was negative in all populations except for PTD. This negative thermal plasticity contrasts positive plasticity

in LT_{50} with respect to temperature observed in the laboratory in Chapter 2 (Bogan et al., in revision) and by others (Healy et al., 2019; M. W. Kelly et al., 2011; Pereira et al., 2017). It is common for laboratory experiments to be uninformative of processes in the wild (Calisi & Bentley, 2009). As such, I expected the magnitude of thermal plasticity in LT_{50} to differ between field and laboratory measures of thermal plasticity, but contrasts in their directionality were unexpected. Interestingly, one published study in *T. californicus* does report negative thermal plasticity of upper thermal tolerance. Studying field-collected *T. californicus* with known, *in situ* thermal histories, Siegle et al. found that prior exposure to high accumulation of temperature (daily-degree hours) resulted in reduced survival under simulated heatwaves (Siegle et al., 2018). To my knowledge, Siegle et al. 2018 is the only published study of changes in thermal tolerance across temperature by *T. californicus* that simulates or accounts for high diurnal variance experienced by wild populations.

Three non-competing hypotheses may explain negative thermal plasticity of LT_{50} in wild *T. californicus*. Increased thermal variation can affect the strength and directionality of plasticity in thermal tolerance, which aligns with contrasting findings in *T. californicus* between studies leveraging static and variable temperature treatments (Healy et al., 2019; M. W. Kelly et al., 2011; Pereira et al., 2017; Siegle et al., 2018). Increasing the magnitude and frequency of variation in abiotic factors including temperature has been shown to reduce organismal performance (Kapsenberg et al., 2018; Morash et al., 2018) and influence the strength and direction of plasticity (K. E. Marshall et al., 2021; Morash et al., 2018).

Secondly, multiple stressors that autocorrelated with temperature in the natural environment may exacerbate negative effects of thermal stress on performance (Ban et al., 2014; Folt et al., 1999; Gunderson et al., 2016) or alter cues for phenotypic plasticity (Bonamour et al., 2019; Chevin & Lande, 2015). Indeed, I found that salinity and DO both shared autocorrelation with temperature in *T. californicus* pools in space or time. The combined effects of reduced oxygen, high salinity, and high temperature may collectively result in changes to the strength or direction of plasticity in thermal tolerance relative to single stressor experiments. Increases in salinity have been found to increase thermal tolerance in *T. californicus* (Denny & Dowd, 2022), but DO does not (Dinh et al., 2020). This effect of salinity may have contributed to why the southernmost site (PTD), which exhibited the largest increases in salinity (Fig. 8), was the only population to mount a positive, plastic change in LT_{50} in response to warming (Fig. 10).

Lastly, unmeasured biotic factors that can autocorrelated with temperature such as the quality and quantity of food resources can vary in the wild and affect thermal performance (Hardison et al., 2021) and its plasticity across temperature (Hardison et al., 2023). Latitudinal gradients in DO within supralittoral pools (Fig. 8) may have been due to site specific differences in wave exposure (Helmuth et al., 2006) and/or photosynthesis (Truchot & Duhamel-Jouve, 1980). Latitudinal gradients in wind driven wave forcing have been recorded across the range of sites sampled for this study in the California Current or Southern California Bite (Pares-Sierra et al., 1993; Pares-Sierra & O'Brien, 1989), but the quantity of difference in DO observed across season and latitude is greater than what should be caused by differences in wave action (Marks, 2008; Volaric et al., 2018). Determining the extent to which latitudinal gradients affect algal communities in the

supralittoral zone and how variation in these community-level interactions shape thermal tolerance may be of value for understanding variation in thermal physiology across latitudinally distributed populations.

4.3. *Evolutionary consequences of countergradient variation in thermal tolerance*
– CnGV in thermal tolerance by wild *T. californicus* populations suggests that adaptive plasticity may be limited in the species' natural environment, reducing its potential contributions to adaptive, evolved responses to novel thermal environments. If negative thermal plasticity in LT_{50} results from condition-dependence however, condition dependence is theoretically associated with the maintenance of genetic variation for trait means and their plasticity (De Lisle & Rowe, 2023; Rowe & Houle, 1997). *T. californicus* exhibited a high interpopulation variation in the strength and direction of thermal plasticity (Fig. 10). While the species exhibits low dispersal and gene flow between populations, metapopulations that do share connectivity or possess sufficient genetic diversity for LT_{50} reaction norms may have the potential for evolutionary changes in thermal plasticity under future temperatures (Hamilton & Miller, 2016). Additionally, Chapter 2 demonstrates that populations contain genetic variation for the plasticity of thermal tolerance.

COV_{GE} can affect the evolution of trait means and their plasticity by increasing or maintaining genetic variance in trait means (Conover et al., 2009) and reducing genetic variance for reaction norms or genotype x environment interactions. COV_{GE} and GxE are inversely related because COV_{GE} is driven by collinearity in reaction norms of populations or genotypes between environments (Albecker et al., 2022). The presence of CnGV for thermal tolerance in *T. californicus* may therefore be a constraint on the evolution of thermal plasticity while potentially aiding the evolution of baseline thermal tolerance.

However, any constraint of CnGV on genotype-environment interactions may be overpowered by the exceedingly greater variation for LT_{50} reaction norms across genotypes (Biological coefficient of variation, $BCV = 0.70$) than for basal LT_{50} ($BCV = 0.02$) as shown in Chapter 2. While some of the difference in genetic variance for reaction norms and trait means may be driven by varying estimation errors for the two traits, it is interesting that high GxE was evident in common garden lines while animals that developed in the wild exhibited COV_{GE} . This discrepancy may be an issue of scale. GxE was experimentally measured at a family level while COV_{GE} was measured at the population level. It also remains possible that COV_{GE} is stronger in nature than in laboratory studies and, thus, GxE is reduced in the wild. Studying the relative influence of GxE and COV_{GE} for thermal tolerance in natural populations of *T. californicus* will be an important step in understanding constraints on the evolution of thermal plasticity and acclimation under global change.

5. Conclusion

It is easy to rely on an adaptive narrative when discussing or studying thermal plasticity. When temperatures increase for a sustained period, we often expect that thermal acclimation should adaptively increase ectotherms' upper thermal tolerances. Laboratory studies have generally found this to be true, often relying on a putative assumption of positive thermal plasticity's fitness benefits. Here we show that in wild populations of *T. californicus*, the functional and evolutionary roles of thermal plasticity are far more complex than appreciated by experimental studies. *T. californicus* LT_{50} was plastically reduced rather than increased in response to high *in situ* temperatures. This reduction in

thermal tolerance was compensated against by evolved increases in LT_{50} in response to warming consistent with countergradient variation. If the negative plasticity of upper thermal tolerance is attributed to maladaptive stress and/or energetic limitation, my results demonstrate that adaptive responses to seasonal variation in temperature rely more on genetic rather than environmental effects. More broadly, this result supports a functional role for thermal plasticity that is not a silver bullet for adjusting physiology in response to thermal stress. Rather, thermal plasticity may be perturbed by thermal stress experienced in nature, requiring compensatory mechanisms preserving the distance of ectotherms upper thermal limit from environmental temperatures.

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Chapter IV: The heritability of adaptive molecular responses to upwelling across families of the purple sea urchin

Abstract

The evolution of adaptive phenotypic plasticity is theorized to promote persistence under environmental change. Plasticity's evolution hinges on sufficient heritability across phenomes and how this heritability covaries with its fitness effects. Some research has addressed this issue at the scale of singular or several traits, but a comprehensive understanding of genetic variation for adaptive and maladaptive plasticity is lacking. We conditioned parents and larvae of the purple sea urchin *Strongylocentrotus purpuratus* to experimental upwelling (an ecologically relevant stressor) and integrated RNA-seq, phenotyping of plastic organismal responses to stress, and measures of fitness-correlated traits in a quantitative genetic breeding design. We quantified (i) the heritability of transcriptional plasticity phenome wide, (ii) differential expression's effect on adaptive and maladaptive phenotypic plasticity, and (iii) covariance between the heritability of transcriptional plasticity and its associated fitness effects. In response to developmental exposure, larvae induced widespread differential expression, decreased biomineralization, and reduced body size. Models of selection on plasticity predicting developmental abnormality (a proxy for larval survival) detected strong fitness benefits for maintaining or increasing biomineralization under upwelling and weakly adaptive effects of reduced body size. Differential expression was predominantly associated with adaptive rather than maladaptive plasticity of both traits. Lastly, the heritability of differential expression significantly increased as the fitness benefit of its phenotypic effect grew. 50.42 – 54.31%

of differential expression associated with adaptive plasticity exhibited heritability. Our results demonstrate higher heritability of adaptive versus maladaptive phenotypic plasticity phenome wide and bear implications for evolution of acclimatory responses and phenotypic canalization under novel, future environments.

Introduction

The phenotypic plasticity of physiology and performance has received attention for its ability to facilitate adaptive organismal responses to environmental change on ecological timescales (Bozinovic & Pörtner, 2015; Hofmann & Todgham, 2010) and, increasingly, its potential to evolve and promote persistence under future climates (Corl et al., 2018; M. Kelly, 2019; Vinton et al., 2022). Plasticity can be adaptive, neutral, or maladaptive (Donelan et al., 2020). Its evolution hinges on the strength and direction of natural selection acting upon it (Hendry, 2016) and the degree of heritable genetic variance underpinning it, which can be epistatic to or uncorrelated with genetic variation in phenotypic means (Kovuri et al., 2023; Scheiner & Lyman, 1991). It has been stated for over a decade that we lack a comprehensive understanding of how plasticity's heritability varies for one trait across taxa or within a single organism across different traits (Bufford & Hulme, 2021; Napier et al., 2023; van Kleunen & Fischer, 2005). By extension, it is unknown whether there is generally greater or equal heritability for adaptive versus maladaptive plasticity (M. Kelly, 2019). Studying the purple sea urchin *Strongylocentrotus purpuratus*, we tested the hypothesis that phenotypic plasticity's heritability covaries with its fitness costs at a phenome wide scale.

A small number of empirical studies on singular traits or suites of multiple traits have uncovered patterns of genetic variation for (mal)adaptive plasticity that help explain its evolution. For example, heat tolerant populations of *Anolis* lizards exhibit canalization of transcriptional traits whose plasticity is maladaptive under acute thermal stress and possess greater interpopulation divergence at *cis*-regulatory sites of maladaptively regulated genes, suggesting that genetic variation in maladaptive plasticity fueled adaptive canalization of pathways related to thermal stress (Campbell-Staton et al., 2021). Similarly, species adapted to high altitudes exhibit the loss of ancestral plasticity in several pathways that, when induced under chronic low oxygen, result in maladaptive pathologies (Durmowicz et al., 1993; Velotta et al., 2018). In each example, it remains unclear whether canalization was driven by greater standing genetic variation underpinning maladaptive plasticity prior to selection or the shear strength of negative selection against maladaptive responses. This area of research would be improved by integrating fitness associated measures of plasticity across a multitude of traits with measures of genetic variation for plasticity across those traits.

A larger body of theoretical research has studied the joint influence of plasticity's genetic variance and fitness effects on adaptation. Adaptive plasticity possessing low genetic variation may enable populations to persist under novel environments at the cost of limiting adaptive evolution of trait means. Alternatively, maladaptive plasticity's may drive adaptation if it possesses a high degree of genetic variance and can unmask otherwise cryptic phenotypes that are subsequently canalized (Ghalambor et al., 2007). Adaptive plasticity may experience evolutionary increases and improve populations' abilities to cope with predictable environmental variation when plasticity is sufficiently heritable (Bitter et

al., 2021). Non-heritable, maladaptive plasticity may result in the evolution of genetic compensation to counteract its effects (Crispo, 2007; M. Kelly, 2019; Morris & Rogers, 2013). Essential to these predictions is whether and how the genetic variation and heritability of plasticity varies as a function of its fitness consequences and theoreticians have urged the need for empirical research in this area (Chevin & Hoffmann, 2017).

Two primary avenues exist for studying plasticity's heritability and adaptiveness across large biological scales. The response of singular phenotypes to a common environmental variable can be studied across diverse taxa through data synthesis and meta-analysis (van Kleunen & Fischer, 2005). Secondly, phenomic approaches can be leveraged to quantify these parameters for the plasticity of thousands of traits within a single species or populations. The former is obstructed by a low number of studies that collectively measure genetic variation and fitness costs of plasticity for common traits. -omic scale approaches such as transcriptomics, metabolomics, and high throughput morphological imaging (e.g., CT scanning and LiDAR) integrated with genotype-phenotype or quantitative genetic techniques have enabled estimation of phenome wide distributions of genetic variation (Blows et al., 2015; Blows & McGuigan, 2016; Pavlyshyn et al., 2022; Verma & Ritchie, 2017). To our knowledge, no studies have integrated phenome wide estimations of genetic variance for plasticity with measures of fitness-correlating traits to determine whether plasticity's heritability varies according to its fitness outcomes.

Studying the purple sea urchin *Strongylocentrotus purpuratus*, we integrated RNA-seq (producing a phenome wide array of plasticity in transcriptional phenotypes) with assays of performance and fitness-correlated traits in a quantitative genetic crossing design

of larval families exposed to ecologically relevant, abiotic stress. We quantified the heritability of transcriptional plasticity and its effect on adaptive phenotypic plasticity before modeling their covariance. Adults and larvae were conditioned to control and experimental treatments mimicking variation in temperature and $p\text{CO}_2$ under coastal upwelling frequently experienced by *S. purpuratus*, whereby wind-driven, upward movement of deep seawater lowers the temperature and increases the $p\text{CO}_2$ of surface oceans (Gruber et al., 2012).

2. Methods

2.1 Adult conditioning, crossing, and larval culture – Adult urchins were collected from 2 coastal sites in the Santa Barbara Channel in August and September of 2018: Naples Reef (34.4221216, -119.95154) on August 23, 2018, and from Arroyo Quemado Reef (34.46774988, -120.11905). Adults were distributed across 4 90 L tanks per parental treatment at a density of 10 urchins per tank. Adults acclimated to parental treatments for approximately 4 months: non-upwelling = 17°C and 596 $\mu\text{atm } p\text{CO}_2$; upwelling = 12.8°C and 1117 $\mu\text{atm } p\text{CO}_2$. Flow rates to adult tanks equaled 20 l h⁻¹. Adults were fed fresh *Macrocystis pyrifera ad libitum* with food changes and tank cleanings conducted once per week. Seawater temperature was controlled using heat pumps regulated by Nema 4X digital temperature controllers. $p\text{CO}_2$ was controlled using a flow-through CO₂ mixing system adapted from Fanguie *et al.* (Fanguie et al., 2010).

Fertilizations were conducted using a staggered cross-classified North Carolina II breeding design. During each phase of the staggered cross, 2 males and 2 females from a common adult condition were reciprocally crossed and their resulting offspring were

cultured under non-upwelling and upwelling conditions until the prism stage of larval development. Each cross x larval treatment group was reared using 2 technical replicate culture buckets, resulting in 16 larval cultures per staggered cross. This process was repeated 5x for non-upwelling and upwelling crosses, alternating in order between parental treatments, resulting in 40 crosses reared across 160 technical replicates and 80 biological replicates (Fig. 13).

Larvae were cultured in replicate flow-through 6 L nested buckets (i.e., an inner bucket with 30 μM mesh openings nested in an exterior bucket) at a flow rate of 3 L h^{-1} and a density of 10 larvae ml^{-1} until the early prism stage of larval development signified by the onset of tripartite gut differentiation. Temperature and pH were regulated in larval culture buckets as described above for adult conditioning. Point measures of temperature, salinity, total alkalinity, and pH for adult and larval tanks are described by Strader et al. 2022.

2.2. Phenotyping of performance and fitness-correlated traits – Three phenotypes were measured in larval cultures: (i) percent developmental abnormality (a corollary of survival), (ii) larval body size, and spicule length per unit body size (a corollary of biomineralization). Morphometric measurements of body size and spicule length were performed on $n \geq 30$ larvae per technical replicated stored in 2% formalin buffered with 100 mM NaBO_3 (pH 8.7) in filtered sea water. Body size was defined as the maximum linear distance of a prism body and spicule length defined as length from the tip of the body rod to the branching point of the post-oral rod. Abnormality was scored on $n \geq 30$ larvae during sampling and was measured as the percentage of larvae exhibiting unsuccessful gastrulation. Because RNA-seq was performed using pooled RNA samples per culture,

performance and fitness-correlating phenotypes were integrated with gene expression data using culture means rather than per-animal values.

2.3. RNA extraction, sequencing, and bioinformatic processing – Total RNA was extracted with Trizol from pooled samples of 6,000 prism-stage larvae per culture replicate. Extractions were performed on 1 technical replicate per cross x developmental treatment resulting in 80 RNA extractions. Total RNA quantity and quality was evaluated via Nanodrop, gel electrophoresis, and Qubit quantification before library preparation. RNA-seq libraries were prepared using polyA enrichment and were quality checked via LabChip GX. Strand specific PE 150 bp reads were sequenced on a single NovaSeq S4 lane.

Illumina Universal Adapters were removed from paired end reads using CutAdapt v4.4 (Martin, 2011) and reads were trimmed and quality filtered using Trimmomatic v0.39 set to paired end mode, trailing and leading clip = 3, sliding window = 4:15, minimum length = 36, and headcrop = 10 (Bolger et al., 2014). All trimmed reads passed quality check via FastQC v0.12.1 (Andrews, n.d.). Forward strands of paired reads were aligned to the ‘Spur_5.1’ reference genome assembly (Sodergren et al., 2006) using hisat2 v2.2.1 (Kim et al., 2019). Forward rather than paired reads were aligned to reduce computational load. Resulting SAM alignments were sorted and converted to BAM using SAMtools v1.6 (Li et al., 2009). Reads were counted per transcript from sorted BAM files using featureCounts v1.6.3 input with the ‘Spur_5.1’ gtf annotation set to a MAPQ alignment quality cutoff of 10 (Liao et al., 2014).

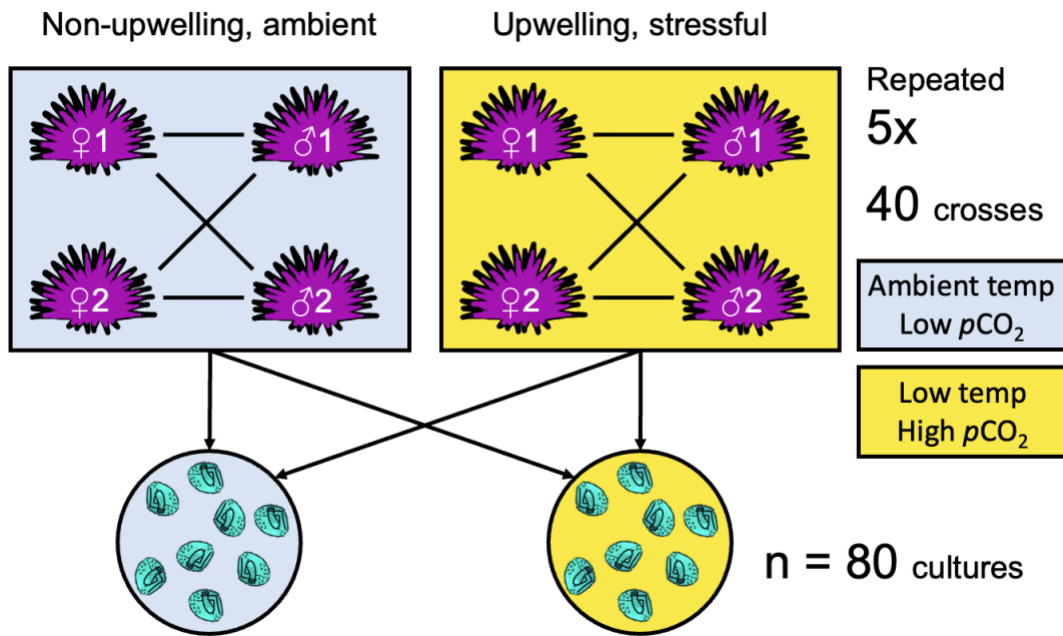


Figure 13 | *Crossing and experimental designs.* This graphic depicts adult and larval conditioning to ambient, non-upwelling (blue) and stressful, experimental upwelling conditions (yellow). Crosses between cohorts of conditioned adults are depicted with lines. Reciprocal rearing of offspring resulting from each cross is depicted with arrows directed toward larval non-upwelling and upwelling conditions.

2.4 Tests of differential expression – Transcript read counts were normalized in edgeR v3.40.2 as counts per million (CPM). Read counts were filtered to keep all transcripts exhibiting CPM > 0.5 in at least 75% of the 80 replicates. Differential expression (DE) was modeled using a negative binomial generalized linear model (glm) fitted with edgeR’s robust, tagwise dispersion parameter using the robust iteration of the model fitting function ‘GLMQLFit’ and the DE test function ‘GLMQLFTest’ (Robinson et al., 2010). Expression was predicted as a function of two non-interacting, categorical variables for parental and developmental environment. Models were fit with non-interacting environmental predictors because the study’s design only enabled the measurement of V_A for developmental rather than transgenerational plasticity. Fitting an interaction between both effects would confound interpretation of V_A for developmental plasticity. Significant DE was evaluated using FDR adjusted p-values ($\alpha < 0.05$). Functional enrichment was tested using a rank-based Mann Whitney U test of Gene Ontology terms input with logFC coefficients for DE. This test determines whether a given GO term’s logFC distribution is significantly skewed from the mean of the background, filtered transcriptome (Wright et al., 2015).

2.5 Measuring the adaptiveness of differential expression – The effect of DE on the plasticity of body size and biomineralization (body size-normalized spicule length) was measured using structural equation models (SEMs). SEMs were derived from two linear models: (i) phenotype predicted as a function of transcript abundance, developmental environment, and parental environment and (ii) scaled, signed transcript abundance predicted as a function of developmental and parental environments. Scaled transcript abundance was signed such that samples with low expression resulting in a negative scaled

value were multiplied by the direction of the transcript's DE under upwelling. The effect of developmental environment on phenotype mediated by DE was estimated for each transcript using mediation analysis performed with the 'mediate' function of the R package mediation v4.5.0 set to 1000 simulations (Tingley et al., 2014). Positive mediation effects indicated that changes in gene expression in the direction of DE were associated with higher levels of body size or biomineralization under upwelling. Negative mediation effects indicated that DE was associated with reduced phenotypic values under upwelling. To understand how the strength of DE impacted phenotypic outcomes, linear regressions were performed between transcriptome wide, absolute logFC and a second-order, quadratic polynomial for the phenotypic effects output by SEM.

The fitness costs and benefits of plasticity in body size and biomineralization were measured using a Lande & Arnold selection gradient model (Lande & Arnold, 1983) as described in Chapter Two, whereby a fitness correlated trait (proportion of normal development, a larval corollary of survival) was modeled as a function of developmental and parental environment, body size or biomineralization per cross in each environment, and the plasticity of body size or biomineralization of a cross between developmental environments. Selection gradient models included a random effect identifying each cross and controlling for genetic covariance using a relatedness matrix generated from the experimental pedigree using the R package nadiv v2.17.2 (Wolak, 2012). Selection gradient models were fitted in brms v2.19.6 (Bürkner, 2017a), an R interface to the Bayesian programming language Stan (Carpenter et al., 2017). Models assumed uniform priors, employed 40,000 MCMC iterations with a 5,000 iteration warm up, and a beta-distributed generalized linear regression model family. Beta distribution was selected

because the proportion of normal development is constrained between 0 and 1. A Bayesian approach was selected for fitting because of the flexibility of packages such as brms for accommodating relatedness matrices within a beta distributed model family. These selection gradient models predicted whether positive versus negative plasticity of larval body size and biomineralization promoted greater fitness under upwelling stress. The significance of fitness effects were tested using probability of direction, which determines whether the $\geq 95\%$ of posterior distribution falls above or below 0 (Makowski, Ben-Shachar, Chen, et al., 2019b). Selection gradient coefficients were then multiplied with the phenotypic effects of DE on plasticity for body size and biomineralization to calculate the associated fitness effect of transcriptional plasticity.

2.6 Estimating the heritability of gene expression and its plasticity – V_A for gene expression and DE were measured across all transcripts using animal models fit with the ‘relmatLmer’ function of the R package lme4qtl v0.2.2 (Ziyatdinov et al., 2018). Within animal models, DE was measured as the foldchange of gene expression across developmental environment for a given cross. Mean-standardized CPM (gene expression) was predicted as a function of fixed effects for developmental and parental environment, random effects for dam and sire, and a random effect for cross identity. Genetic covariance between crosses was estimated using the relatedness matrix described above. Mean-standardized DE fold changes were predicted using an identical animal model lacking a fixed effect for developmental environment. h^2 was derived from each model as the heritable proportion of total variance in gene expression or DE. Differences in h^2 of baseline gene expression and gene expression were modeled transcriptome wide using a beta distributed glm fitted using the R package betareg v3.1-4 (Grün et al., 2012).

Covariation between DE's heritability and adaptive, phenotypic effects were modelled using three different approaches addressing the questions (i) 'Does the probability of heritability ($I_A \geq 0.1$) vary as a function of DE's effect on adaptive plasticity in body size or biomineralization?', (ii) 'Does total heritability (I_A as a continuous variable) vary as a function of DE's phenotypic effects?', and (iii) 'Does total heritability vary according to the fitness costs of DE's combined effect on plasticity in body size and biomineralization?'. Tests of questions i and ii were performed by modelling the probability of $h^2 \geq 0.1$ using binomial glm's. Continuous h^2 was modeled as a beta-distributed glm. Each model type fitted two continuous predictor variables for the phenotypic effect of DE on body size and biomineralization signed toward the adaptive direction of that effect under parental upwelling, which induced yielded fitness effects of plasticity as opposed to neutral effects under parental non-upwelling. Models of continuous h^2 set parameters for DE's phenotypic effects as a second order polynomial to accommodate non-linear variation in h^2 across the parameter space. For question iii, DE's total effect on adaptive plasticity was calculated as the summation of the SEM-predicted coefficient for DE of transcript i 's effect (E) on body size (S) and biomineralization (B) multiplied by the selection gradient (β) acting on each plastic trait under upwelling, such that adaptive plasticity associated with transcript $i = (E_{S,i} \times \beta_{S,i}) + (E_{B,i} \times \beta_{B,i})$. Covariance between DE h^2 and its total adaptive effect on plasticity was modeled using a beta-distributed glm in the R package betareg v3.1-4 (Grün et al., 2012).

3. Results

Parental and developmental upwelling increased percent abnormality in larvae, particularly in cultures conditioned to the treatment transgenerationally and developmentally, indicating that upwelling induced greater stress (Strader et al., 2022). Developmental conditioning to experimental upwelling induced widespread differential expression enriched for GO terms involved in but not limited to cellular responses to stress, lipid and carbohydrate metabolism, oxidoreductase activity, and ribosomal organization and biogenesis. DE induced by developmental upwelling was primarily associated with decreases in larval body size and increases in biomineralization (Fig. 15). The plasticity of body size and biomineralization both yielded significant fitness effects measured as variance in the proportion of normal development among larval families – reductions in larval body size following parental upwelling were adaptive while plastic increases in biomineralization were adaptive. Thus, most DE was putatively adaptive while a significant but smaller fraction of gene expression changes were associated with maladaptive plasticity. Using a heritability threshold of $h^2 \geq 0.1$, 50.42 – 54.31% of adaptive transcriptional plasticity exhibited significant heritability. The heritability of DE did not vary between transcripts associated with adaptive versus maladaptive changes in biomineralization. However, DE associated with adaptive reductions in body size was significantly more heritable than DE associated with maladaptive increases in size (Fig. 16). We further describe these results below.

3.1. RNA-seq alignment, quality checking, and filtering – Following trimming, RNA-seq libraries achieved a mean size of 37.07 ± 5.09 million reads and a mean mapping efficiency of $74.14 \pm 1.80\%$. After filtering read-count filtering for transcripts with > 0.5

CPM in at least 75% of all samples, 12,953 transcripts were retained for downstream analysis. Outlier detection was performed with filtered read count data using arrayQualityMetrics v3.54.0 (Kauffmann et al., 2009), which flagged two half sibling crosses from parental and developmental upwelling treatments as significant outliers resulting in their removal from the dataset and resulting in $n = 78$. Mean variation in gene expression across samples equaled a BCV of 0.12.

3.2. Differential expression induced by parental and developmental upwelling – Parental conditioning to upwelling induced upregulation of 1,582 transcripts and downregulation of 1,539. These differentially expressed genes (DEGs) included 50 and 29 upregulated and downregulated transcripts with an absolute $\log_2FC > 1.0$ (Fig. 14). DEGs induced by parental upwelling were enriched for 71 biological process (BP) GO terms, 49 molecular function (MF) terms, and 30 cellular component (CC) terms. Upregulated transcripts included enrichment for the BP/MF terms involved in cellular signaling, cell adhesion, transmembrane and development localized to CC terms including the endoplasmic reticulum, cell membrane protein complexes, and extracellular region. Downregulated transcripts were enriched for the BP/MF terms involved in ATP metabolism, ribosomal structure/biogenesis, mitochondrial organization, and oxidoreductase activity localized to the ribosome, mitochondrial matrix, and cytosol.

Developmental exposure to upwelling induced upregulation of 2,246 transcript and downregulation of 2,205. With a >1.0 \log_2FC cutoff, these included 38 upregulated and 184 downregulated transcripts. 39.73% of DEGs induced by transgenerational effects were also differentially expressed in response to developmental conditioning (Fig. 14). Developmental upwelling DEGs were enriched for 89 BP GO terms, 53 MF terms, and 37

CC terms. Upregulated transcripts were enriched for BP/MF terms related to chromatin remodeling, mitochondrial organization, ATP metabolism, cellular responses to stress and DNA damage, ubiquitination, and ribosomal structure/biogenesis. These upregulated terms were localized to catalytic complexes, nuclear and organelle lumen, ribosomes, and the nucleolus. Downregulated transcripts were enriched for BP/MF terms related to cytoskeletal organization, cell adhesion, oxidoreductase activity, and metabolism of lipids and carbohydrates localized to the cytoskeleton, cell junctions, cell membranes, and endosomes.

Upregulated transcripts related to ribosomal function included several ribosomal subunits and 16 DEAD-box proteins involved in the initiation of translation. Included in cellular responses to stress was the significant upregulation of 8 heat shock proteins including 3 Hsp70 and 5 Hsp40 chaperones. Interestingly, 8 heat shock proteins were significantly downregulated in response to upwelling, indicating that they were more highly expressed under warmer conditions. These included all 3 Hsp90 isoforms present in the *S. purpuratus* genome as well as 2 Hsp70 and 3 Hsp40 chaperones. These functional enrichment results collectively demonstrate a suite of complex molecular responses to multivariate, abiotic environmental change brought on by experimental upwelling, a third of which were commonly induced by parental and larval conditioning.

3.3. Effects of differential expression on adaptive versus maladaptive plasticity – SEMs predicting DE's effect on plastic changes in body size and biomineralization responding to developmental upwelling identified 231 transcripts associated with larger body size, 564 with reduced body size, 125 associated with increased biomineralization, and 113 with decreased biomineralization. Selection gradient models predicting the

proportion of normal development per replicate culture (a fitness correlated trait) as a function of body size or biomineralization and their plasticity estimated fitness benefits of (i) plastic reductions in size under upwelling and maintenance of or plastic increases in biomineralization under upwelling. When larvae were spawned from parents exposed to upwelling, plastic reductions in body size incurred a selection gradient of 0.04 ± 0.01 percent normal development $\text{scaled plasticity}^{-1}$ and plastic increases in biomineralization a gradient of 0.25 ± 0.10 percent normal development $\text{scaled plasticity}^{-1}$, suggesting that fitness benefits of reductions in body size were weak (Fig. 15). Thus, DE associated with reductions in body size or increases in biomineralization were associated with adaptive or weakly adaptive phenotypic outcomes. Interestingly, the plasticity of both traits did not exhibit detectable fitness costs or benefits when larvae were spawned from non-upwelling parents.

As the absolute fold change of a transcript's DE increased, the adaptive effect of DE on reduced body size and/or increased biomineralization significantly increased. Associations between DE and effects on phenotypic plasticity toward maladaptive directions were weaker, demonstrating that DE induced by developmental upwelling was predominantly associated with adaptive plasticity (Fig. 15). Transcripts associated with reduced body size under upwelling were enriched for h BP/MF terms involved in cellular signaling, transmembrane transport, and ribosomal biogenesis localized to cell junctions and the nucleolus. In stark contrast to transcripts associated with plasticity of larval body size, DE driving increases or decreases in biomineralization exhibited not functional enrichment across all GO term categories.

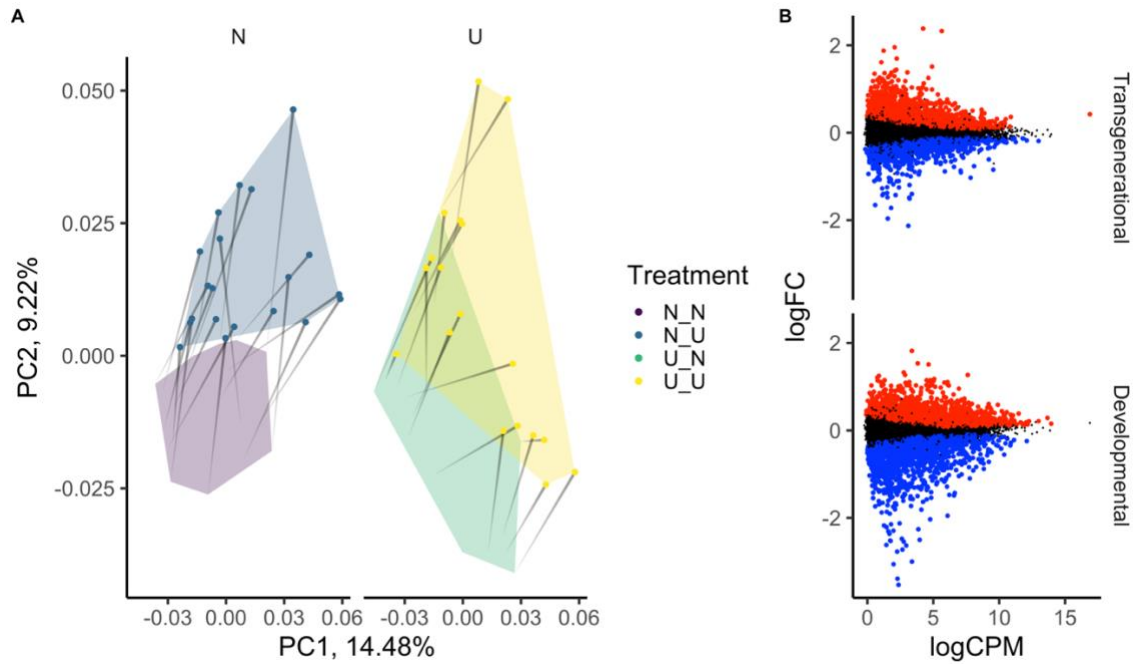


Figure 14 | *Differential expression induced by parental and developmental exposure to upwelling.* **(A)** Loading of samples to principal coordinate axes derived from filtered, normalized read counts. Parental and developmental treatments are represented by color. Paths connect single crosses and their change in loading between non-upwelling (“N”; no point) and upwelling (“U”; point) treatments. **(B)** Mean difference plots of transcript logFC across average CPM per transcript, grouped by parental and developmental effects of upwelling. Significant downregulation is depicted in blue and upregulation in red.

3.4. The phenome-wide heritability of adaptive versus maladaptive plasticity –

Baseline gene expression (CPM) was significantly less heritable than DE by a mean of $0.0803 \pm 0.0135 h^2$ ($p = 2.52e^{-09}$) and was uncorrelated with h^2 of DE (Fig. 14A). 52.82% of significant DE induced by developmental conditioning exhibited h^2 greater than or equal to 0.10 (i.e., at least 10% of in DE's variance was heritable). The average h^2 of DE equaled 0.2992 ± 0.1579 . 6.52% of DEGs were both heritable and associated with adaptive plasticity of body size, but 54.31% of adaptive DE related to body size was heritable. These transcripts exhibited a nearly identical mean h^2 relative to all DE equaling 0.2878 ± 0.1515 . Substantially less DE was heritable and associated with maladaptive plasticity of body size, equaling only 1.48% of all DEGs. Heritable, maladaptive DE related to body size exhibited a mean h^2 of 0.26 ± 0.14 .

1.35% of DEGs were heritable and associated with maladaptive increases in biomineralization under upwelling, which was observed to be more adaptive than plastic reductions in larval size (Fig. 15). This proportion was 4.83x lower than heritability among DEGs associated with adaptive plasticity in body size. 50.42% of DEGs associated with adaptive increases in biomineralization were heritable, achieving a mean h^2 of 0.2730 ± 0.1307 . 1.01% of DEGs were heritable and associated with maladaptive reductions in biomineralization.

DE associated with adaptive reductions in body size under upwelling was significantly more heritable than DE associated with maladaptive increases in body size. As the effect of DE on adaptive decreases in body size grew, h^2 remained constant ($\beta = 6.40$; $p = 1.05e^{-07}$) and the probability of $h^2 \geq 0.1$ significantly increased ($\beta = 136.95$; $p = 2.45e^{-05}$). h^2 and the probability of heritability decreased as maladaptive effects of DE on

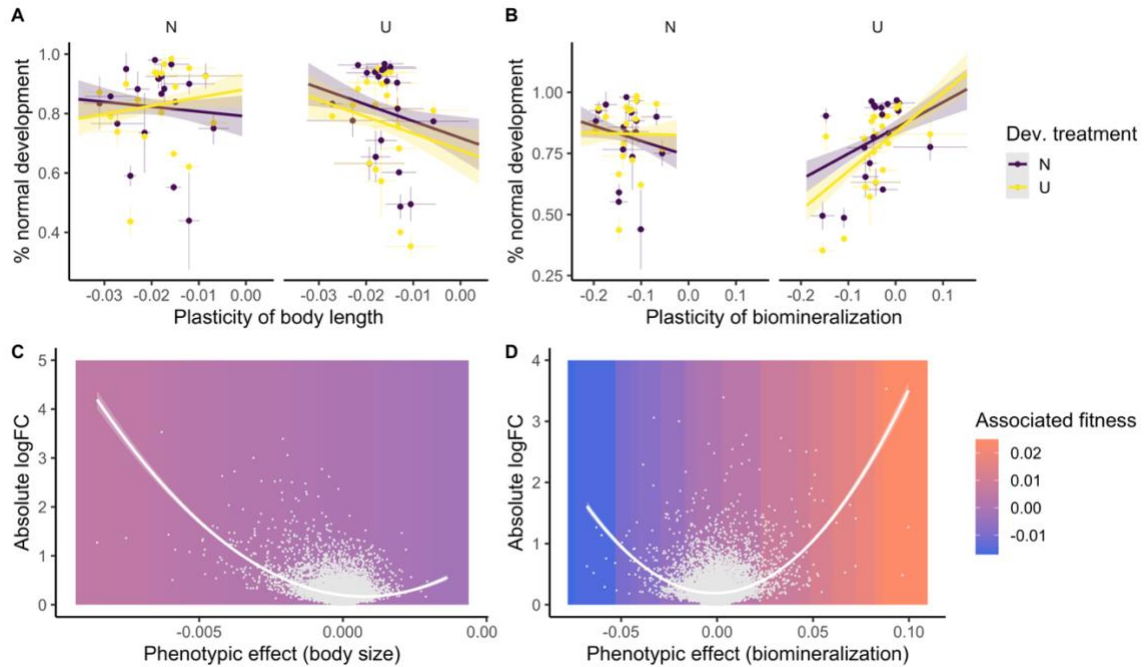


Figure 15 | *Effects of differential expression on adaptive plasticity.* (A – B) The effects of plasticity in body size and biomineralization on normal development grouped by parental environment are plotted in A and B, respectively. Points represent phenotypic means of crosses reared in each environment. Environments are depicted by color such that non-upwelling (N) is blue and upwelling (U) is yellow. Error bars depict standard deviation in each trait among replicates of each cross. (C – D) Absolute logFC of differential expression induced by developmental upwelling is plotted across differential expression’s association with upwelling effects on body size and biomineralization in A and B, respectively. Points represent transcripts. Points are colored according to whether differential expression drove phenotypic plasticity toward adaptive (red) or maladaptive (blue) directions, measured as the product of differential expression’s phenotypic effect and the selection gradient acting on that phenotype. Solid lines depict fitted quadratic curves \pm 95% CI. Plot background color corresponds to the product of differential expression’s phenotypic effect on the plasticity of body size or biomineralization and the selection gradient for plasticity of each trait, resulting in an inferred fitness level induced by differential expression (red = high; blue = low).

increased body size grew ($\beta = -5.47$; $p = 2.70e^{-06}$; Fig. 16B). DE's association with adaptive biomineralization plasticity had an insignificant, positive effect on its h^2 (Fig. 16B).

DE's summed effect on the adaptive plasticity of biomineralization and body size, measured as the summation of DE's phenotypic effects on both traits multiplied by selection gradients acting on their plasticity, was positively correlated with probability of heritability ($\beta = 0.04$; $p = 0.027$). Thus, DE was more heritable when associated with adaptive phenotypic plasticity. For illustrative purposes, variation in DE's logFC, h^2 , and total effect on adaptive plasticity are plotted as a fitness landscape in Figure 17 – positive fitness effects are enriched among transcripts with DE $h^2 > 0.1$ and absolute logFC > 1.0 .

Upregulated, adaptive DE was almost entirely heritable while downregulated, adaptive DE exhibited a larger proportion of $h^2 < 0.1$. Minimal changes in gene expression did not appear to result in strong effects on fitness, as evidenced by a valley of neutral fitness costs associated with DE in Figure 17 compared to fitness peaks and valleys that exist at high absolute logFC. These valleys, negative fitness effects associated with DE, were enriched within the most extreme downregulation (Fig. 17).

Transcripts with high DE heritability and strong negative, adaptive effects on body size under upwelling were enriched for the BP/MF GO terms related to ribosomal biogenesis, RNA processing, and transmembrane transport localized to the nucleolus. Heritable DE associated with maladaptive increases in body size was enriched for BP/MF terms related to amide formation, a component of peptide synthesis during translation, and ribosome structure localized to the cytosolic ribosome and large ribosomal subunit (Fig. 17). Heritable DE with strong adaptive effects on the plasticity of biomineralization was not enriched for any GO terms.

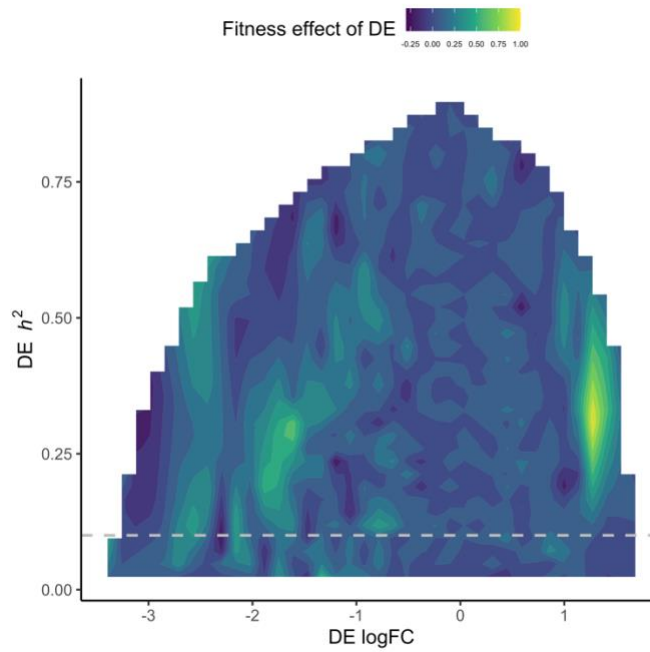


Figure 17 | *Transcriptome wide distribution of differential expression, its heritability, and associated fitness effects.* The distribution of heritability of differential expression (DE) in response to upwelling is plotted as h^2 across the distribution of DE's fold change (logFC). Color represents the fitness outcome associated with DE's effect on phenotype – the summed products of DE's phenotypic effects on body size and biomineralization multiplied by the selection gradients acting on the plasticity of each trait (yellow = high fitness; blue = low fitness).

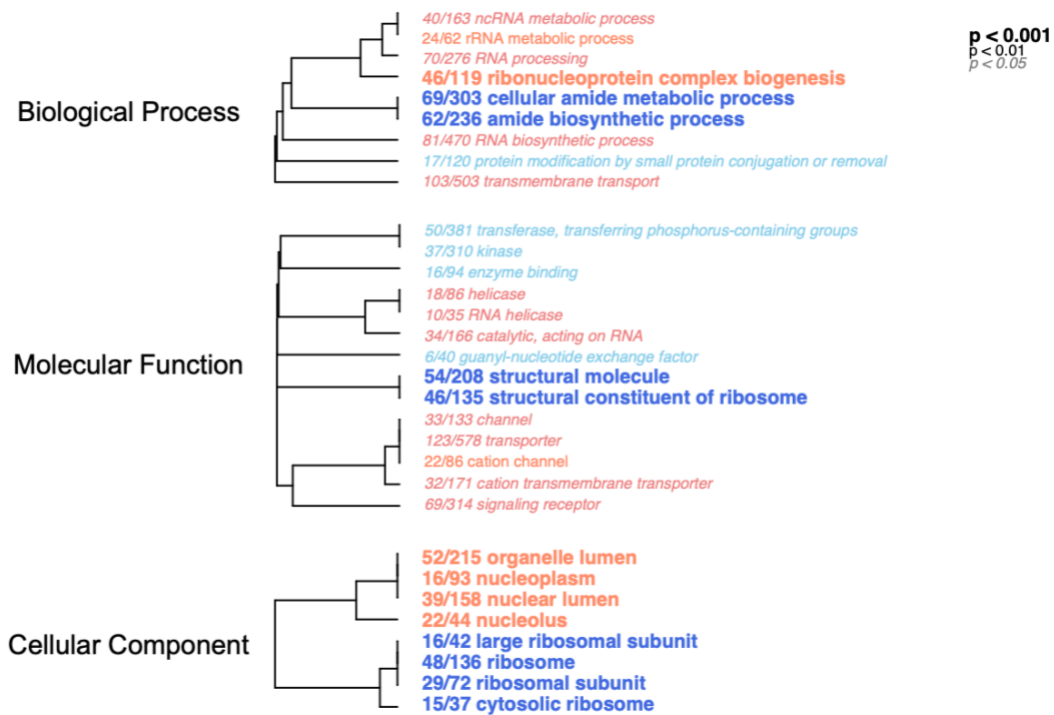


Figure 18 | *Functional enrichment of differentially expressed genes with high heritability and strong absolute effects on the plasticity of body size.* Enriched gene ontology (GO) terms are derived from a Mann Whitney U test of variation in the product of differential expression’s heritability and DE’s effect on adaptive plasticity of body size in response to upwelling. Trees depict clustering of GO terms based on shared transcripts. Red GO terms are enriched within heritable differential expression associated with adaptive reductions in body size in response to developmental upwelling exposure. Blue terms are enriched within heritable differential expression associated with maladaptive increases in body size under upwelling.

4. Discussion

4.1. *Greater heritability of adaptive versus maladaptive plasticity* – We observed that the phenome wide heritability of DE significantly increased when DE was associated with adaptive, phenotypic responses to developmental conditioning under upwelling (Figures 16 – 17). Additionally, 50.42 – 54.81% of DE and adaptive DE exhibited $h^2 \geq 0.1$. This degree of genetic variation for DE and its enrichment within transcripts exhibiting adaptive plasticity exceeds observations in other phenome-scale studies of plants and animals. Studying cross-environment genetic correlations (a measure of genetic variance in plasticity) for gene expression in a tropical butterfly, Oostra et al. measured low genetic variation for DE between two morphological phenotypes (Oostra et al., 2018a). Studying Anolis lizards, Campbell-Staton et al. found that higher frequencies of SNPs within *cis*-regulatory regions of genes associated with putatively maladaptive decreases in CTmax under thermal stress while *cis*-regulatory mutations proximal to genes with putatively adaptive DE did not vary relative to genomic background. This study's design differed from our own in that plasticity was measured at a population level – distinct genotypes were reared under each treatment rather than single genotypes or families being split across treatments. However, fitness effects of thermal plasticity were assumed and were thus putative (Campbell-Staton et al., 2021).

Multiple factors may contribute to high genetic variance and heritability for transcriptional plasticity under upwelling in *S. purpuratus*. *S. purpuratus* larvae are widely dispersed during their planktonic phase resulting in a high connectivity across spatial scales and high genetic diversity within populations (Edmands et al., 1996; Palumbi & Wilson, 1990; Pespeni & Palumbi, 2013). Its dispersal distances can be wide enough that larvae are

frequently transported across areas of major and minor upwelling in the California Current and Southern California Bight (Chan et al., 2017; Pespeni & Palumbi, 2013; Zaytsev et al., 2003). *S. purpuratus* populations exhibit evidence of local adaptation to regional differences in $p\text{CO}_2$ despite high rates of gene flow (Evans et al., 2013; Pespeni et al., 2013). These findings collectively suggest that an individual population should possess high genetic variation in trait means and/or plastic responses associated with coastal upwelling. A second reason we may have observed greater genetic variation for plasticity phenome wide is our study's fidelity to the multiple abiotic variables that autocorrelate with temperature in nature such as $p\text{CO}_2$. Plasticity frequently evolves as a response to multiple autocorrelating, environmental cues (Bonamour et al., 2019; Dore et al., 2018; Valladares et al., 2007) and experimental treatments mimicking natural variation may better illicit evolved, plastic responses possessing genetic underpinning. Lastly, genetic variation is often more highly expressed under stressful conditions (Hoffmann et al., 1999). We observed moderate-to-high levels of developmental abnormality in many larval crosses indicative of a baseline level of stress across replicates brought on by parental conditioning or stress incurred *in situ* prior to adult collection.

Heritability was not only high for DE induced by upwelling – it increased for DE yielding adaptive effects on phenotype (Figures 16 – 17). Ultimately, our study cannot pinpoint sources of genetic variants underpinning adaptive plasticity, but two hypotheses are worth highlighting. Firstly, plastic responses that were maladaptive in our study may incur strong fitness costs and stabilizing selection for reduce plasticity resulting in limited genetic variance (Price & Langen, 1992). Adaptive plasticity by larvae in response to upwelling could evolve if physiological processes promoting acclimation during plasticity

are not canalized by developmental constraint (Debat & David, 2001). If we assume responses to upwelling are under positive or stabilizing selection for high plasticity in some populations, high genetic variation for adaptive plasticity could be maintained by fluctuating selection caused by variation in the severity of coastal upwelling across the wide range and dispersal distance of *S. purpuratus*. For example, Hallson et al. observed that lines of seed beetles reared under fluctuating selection acting on thermal performance exhibited significantly greater genetic variance for thermal plasticity of developmental rate after 18 generations of selection (Hallsson & Björklund, 2012). However, it is important to contrast these empirical results with theory predicting decreased genetic variance for plasticity under more variable environments (Jong & Gavrillets, 2000). It is possible that fluctuating selection across the seascape acting on adaptive phenotypic plasticity has promoted increased genetic variation and subsequent heritability for transcripts associated with plasticity.

4.2. *Functional enrichment within heritable, adaptive plasticity* – Fitness benefits of plasticity during developmental conditioning to upwelling are likely contingent on whether affected pathways are developmentally canalized – e.g., whether DE can be induced by any process other than ontogeny (Siegal & Bergman, 2002). Under this framework, DE that compensates for variation in developmentally canalized processes or induces uncanalized changes to cellular functions are expected to be more associated with adaptive plasticity. Variation in ribosomal function during development bears harmful effects on organismal function (Freed et al., 2010; Ordas et al., 2008), and multiple GO terms enriched among transcripts differentially expressed in response to developmental upwelling exposure were indicative of cellular responses to ribotoxic stress.

Heritable and adaptive changes in gene expression were enriched for GO terms associated with ribosomal biogenesis while maladaptive, heritable DEGs retained enriched functions indicative of ribotoxic stress such as downregulated ribosomal subunits (Fig. 18). Induced ribotoxic stress can result in the downregulation of ribosomal subunits in tandem with Heat Shock Factor 1's induction of the cytosolic, 70 kda heat shock protein Hsc70 and Hsp40 chaperones (Albert et al., 2019). These are the two classes of heat shock proteins that were upregulated in response to developmental upwelling. However, hsp's were collectively split between being upregulated or downregulated in response to upwelling, potentially due to positive correlations between the expression of some chaperones and temperature, and thus require further scrutiny (Feder & Hofmann, 1999). Abiotic stress can perturbate ribosomal function via denaturation of ribosomal proteins/RNAs or misfolding of nascent proteins that disrupt proximal ribosomes (De & Mühlemann, 2022; Iordanov et al., 1998). The extent to which ribosomal biogenesis and structure was overrepresented among transcripts with heritable, (mal)adaptive plasticity suggests that maintenance of translation and ribotoxic stress responses may be critical for acclimation and adaptation to upwelling.

4.3. Evolutionary implications of heritable, adaptive plasticity – The heritability of phenotypic plasticity, and how this heritability covaries with its fitness effects, can impact the evolution of plasticity itself and the traits it acts on (Fox et al., 2019; West-Eberhard, 2003). Under climate change, these evolutionary effects are important for predicting whether future climates will drive canalization of tolerance or increase acclimatory potential via directional selection on heritable plasticity (Svensson et al., 2020; Van ASCH et al., 2007). Coastal upwelling is predicted to become more frequent and severe in the

California Current under climate change, primarily through increases in upwelling-favorable winds (Xiu et al., 2018). Our results suggest that there is high genetic variance and heritability for plastic responses by larval *S. purpuratus* to upwelling. This heritability should facilitate evolutionary increases in plasticity if these responses are adaptive under future upwelling such as they were in our study. However, alignment of plasticity's adaptiveness between our experimental conditions and future climates cannot be assumed. Firstly, upwelling yields highly multivariate changes in oxygen, organic nutrients, and physical processes that were not manipulated in our study (Huyer, 1983). Secondly, environmental cues inducing adaptive plasticity may be modified under climate change – specific abiotic cues that induce adaptive responses to upwelling may be muted or become unreliable indicators under future climates (Bonamour et al., 2019; Renaud et al., 2022).

The relationship between plasticity's adaptiveness and the probability of heritability was largely linear, and it is important to underscore the evolutionary implications of low genetic variation observed for maladaptive plasticity across the phenome. 29.07% and 52.32% of DEGs associated with plastic changes in body size and biomineralization drove phenotypic change toward maladaptive directions (Fig. 15). Non-heritable, maladaptive plasticity is generally predicted to result in the evolution of compensatory mechanisms such as those characterizing countergradient variation – when phenotypic change is maladaptive but cannot be removed by selection, secondary plasticity must evolve to neutralize net phenotypic variance, a process termed genetic compensation (Grether, 2005). Genetic compensation can be energetically costly (Morris & Rogers, 2013). Thus, genetic compensation's evolution is potentially suboptimal to equal genetic variation in adaptive and maladaptive plasticity resulting in canalization of traits whose

variance is costly. Our observed levels of heritability for adaptive plasticity suggest that the evolution of plasticity promoting genetic compensation may not be hindered by low, standing genetic variation.

Conclusion

Understanding plasticity's evolution as a function of its fitness costs and genetic variation will improve predictions of adaptive responses by biodiversity under climate change, but this pursuit faces an obstacle of scale – integrating measures of plasticity's heritability and fitness effects to a sample size of traits necessary for general inferences is challenging. By leveraging RNA-seq integrated with measures of organismal performance and fitness correlating traits, we resolved a phenome scale picture of plasticity's heritability in response to an ecologically relevant stressor. Our results demonstrate that, in our specific case study of a pervasive coastal herbivore, adaptive plasticity is associated with increased heritability across the phenome. Ecological and physiological research on *S. purpuratus* has demonstrated a high level of resilience to environmental perturbations such as marine heatwaves relative to other coastal fauna (Chamorro et al., 2023; Rogers-Bennett & Catton, 2019; Smale, 2020). Our findings further support the potential for *S. purpuratus* to maintain this resilience via evolution of phenotypic plasticity in multiple physiological pathways. More broadly, we hope that our results motivate the study genetic variance for plasticity across phenome-scale or biodiverse datasets so that we may better understand the evolution of plasticity across diverse taxa.

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Chapter V: Conclusion

Phenotypic plasticity's ability to both affect and evolve via natural selection has captivated evolutionary biologists and ecologists for a half century (Pigliucci, 2005; West-Eberhard, 2003). Progress in the study of these processes has been sporadic despite continual investigation since their theoretical origin (Sommer, 2020). The greatest biological obstacle to deriving meaningful, predictive results from studies of plasticity's evolution and evolutionary impact is arguably challenges to accounting for constraints such as its genetic variation (Kovuri et al., 2023; Munar-Delgado et al., 2023), limits, and fitness costs (Schneider, 2022). While the complex roles of plasticity in evolution has motivated its study (West-Eberhard, 1989), overcoming the obstacle of complexity is less an academic issue and more motivated by necessity – improving predictions or management of biodiversity's adaptation to climate change requires more accurate evolutionary models of variation in the plasticity of performance traits (Donelson et al., 2023; Reside et al., 2018). Such models require parameters drawn from empirical measures of plasticity's genetic variation and costs.

In chapters 2 – 4 of my dissertation, I demonstrated that the plasticity of physiological and molecular responses to global change drivers across populations and families of two coastal marine invertebrates exhibits sufficient additive genetic variation and/or fitness effects to evolve via natural selection under novel, future environments. However, genetic variation and fitness effects of plasticity were both limited by constraints whose detection represent novel contributions to evolutionary ecology and global change biology. Firstly, fitness costs of phenotypic plasticity depended on a trade off with trait intercepts evidenced by thermally tolerant genotypes of *T. californicus* incurring greater

reproductive costs of plasticity in thermal tolerance. Secondly, additive genetic variance and heritability of transcriptional plasticity in *S. purpuratus* varied as a function of its associated effects on phenotype and larval survival (i.e., proportion of normal development) such that maladaptive plasticity was less heritable than adaptive plasticity across the transcriptome. While there is potential for adaptive plasticity to evolve via natural selection under upwelling, the ecologically relevant stressor simulated in this study of *S. purpuratus*, limited heritability for maladaptive plasticity indicates that there is evidence of constraint on the evolution of transcriptional plasticity via natural selection (Crespi, 2000). In keeping with the history of convolutions to the study of plasticity's evolution, my study of fitness tradeoffs to thermal tolerance and its plasticity *in situ* nature suggest that my experimental evidence is not predictive of covariance between these two traits in nature. In this chapter, I expand further on the components of my research that have yielded meaningful or novel results for evolutionary ecology, contemporary populations, and future biodiversity before describing complex or equivocal results that motivate future areas of research.

1. Context dependence in plasticity's fitness effects and genetic variation – Fitness costs of phenotypic plasticity have been historically difficult to detect (Arnold et al., 2019; Hendry, 2016; Van Buskirk & Steiner, 2009). The detection and accurate measure of these variables was achieved in my thesis research by accounting for context dependence: ecological and evolutionary covariates included in my experimental design and models that explained a significant proportion of plasticity's fitness costs and heritability. For example, the fitness costs of LT_{50} 's plasticity in *T. californicus* depended on upper thermal tolerance and was only apparent in the most tolerant genotypes. Fitness effects of plasticity in

biomineralization and body size in *S. purpuratus* only arose in larvae spawned from stressed, upwelling conditioned parents. The heritability of transcriptional plasticity varied according to its associated phenotypic and fitness effect. Through experimental means, I found that understanding plasticity's adaptiveness and genetic variation requires accounting for complex, eco-evolutionary processes such as fitness tradeoffs between traits, transgenerational effects, and multiple modes of plasticity (e.g., stress and maladaptation versus inducible stress responses). These contingencies align with predictions posed by Marshall, and Van Buskirk & Steiner suggesting that detection and measurement of plasticity's fitness costs and genetic variation can be obscured by context-dependence on unmeasured ecological and evolutionary processes (D. J. Marshall, 2008; Van Buskirk & Steiner, 2009). In specific eco-evolutionary contexts such as parental exposure to stress, relatively high levels of selection on genetic variation for the plasticity of environmental performance exist.

2. *Translating experimental findings into predictions for natural populations* – This context dependence has implications for the evolution of phenotypic plasticity associated with unique ecologies of the species they were detected in. *S. purpuratus* and *T. californicus* exhibit contrasting life histories with respect to larval development. *S. purpuratus* larvae have a planktonic stage that is widely dispersed, resulting in levels of connectivity and gene flow between populations. The naupliar, crawl-away larvae of *T. californicus* are benthic and are only transported out of pools and between distinct populations during periods of extreme coastal sheering (Powlik, 1999), resulting in lower connectivity and high genetic structure among populations (Barreto et al., 2018; Edmands, 2001; Lima et al., 2019). Because fitness costs of plasticity by *S. purpuratus* arose in larvae

from upwelling conditioned parents, adaptation to the stressor via natural selection on plastic responses may be facilitated *in situ* by parental exposure to upwelling in source populations. Interestingly, regions of the California Current and Southern California Bight with the greatest frequencies and severity of wind driven upwelling also exhibit long sea surface transport of larvae (Bashevkin et al., 2020). This suggests that genetic variation for plastic responses to upwelling may be facilitated by positive covariance between parental conditioning to upwelling and larval transport in addition to its periodic, temporal variation. In *T. californicus*, the ability of fitness tradeoffs between thermal tolerance and its plasticity to shape their negative correlation across populations may be facilitated by canalization's ability to evolve more readily in populations with low-to-moderate genetic diversity and connectivity (Barreto et al., 2018; Lima et al., 2019).

Confirming these hypothesized effects of life history on the evolution of phenotypic plasticity, which are derived from the results of controlled experiments, will require field research. It is likely that the context dependence observed in my experiments is only a small representation of the complexity shaping natural selection on plasticity in nature, and the results of Chapter III underscore this point. While my field study of LT₅₀'s plasticity in *T. californicus* confirmed that its importance in shaping intraspecific variation in thermal tolerance, it laid bare the poor ability of experiments to predict plasticity's function in the natural environment.

My research and past laboratory studies of *T. californicus* have shown that conspecifics increase LT₅₀ following developmental conditioning to high temperature, and I show in Chapter III that seasonal temperatures and LT₅₀ generally share negative correlation in their natural habitat where temperature is more stochastic and covary with

salinity, dissolved oxygen, and community-level processes (Huggett & Griffiths, 1986). Furthermore, countergradient variation in LT_{50} existed between plastic and evolved responses to seasonal variation in temperature while Chapter II observed visual evidence of co-gradient variation (i.e., genetic and plastic variation in LT_{50} increased in populations or treatments with warmer temperatures).

Studies of context dependence in plasticity's fitness effects and genetic variation in natural populations must account for a great deal of complexity not reflected in laboratory experiments. My results from Chapter III suggest that understanding plasticity's evolution in natural populations can be aided by two approaches – conceptualizing thermal plasticity as a functional trait (i.e., how it varies and affects biology in nature) and accounting for multiple stressors, adding to a choir of their advocacy in the literature, particularly in global change biology (Gunderson et al., 2016; Heilmeier, 2019).

3. The evolution of adaptive plasticity under climate change – Climate change will increase environmental variation and, when that variation is predictable, may positively select for increased acclimation potential (Bitter et al., 2021). Alternatively, high environmental means may select against plasticity and drive the canalization of tolerance (Salachan & Sørensen, 2022). Whether biodiversity can adapt to novel climates depends in part on evolutionary rates of adaptive plasticity in environmental performance (Buckley & Kingsolver, 2021; M. Kelly, 2019; Kingsolver & Buckley, 2017; Matesanz et al., 2010). Rates of evolution can be slowed by fitness tradeoffs between traits and countergradient variation should reduce genotype-environment interactions associated with genetic variation in plasticity (A. F. Agrawal & Stinchcombe, 2008; Albecker et al., 2022; Stearns, 1989). The results of my dissertation suggest that the evolution of phenotypic plasticity via

natural selection is no exception. In *T. californicus*, fitness tradeoffs between thermal tolerance and its plasticity have the potential to slow rates of evolution in both traits when they are both under strong, positive directional selection. Countergradient variation in LT_{50} among wild populations is likely a constraint on genetic variation for thermal plasticity in nature.

The finding that breakdown and maintenance of ribosomal function represent genetically correlated, (mal)adaptive, plastic responses to environmental stress underscores the value of integrating ecophysiological and evolutionary approaches in a global change biology. The persistence of organisms under global change drivers depends in part on physiological pathways that breakdown and/or yield adaptive potential under global change drivers (Somero, 2010). Identifying these two pathways is not possible without integration of evolution and physiology that goes beyond a comparative framework and measures fitness corollaries and/or genetic variation for plasticity and acclimation. Identifying physiological responses to environmental change with potential for adaptation can aid management of threatened species (Donelson et al., 2023) and support efforts such as assisted evolution and climate proofing of food systems or threatened species by better identifying phenotypes for selection (Gibbin et al., 2017; van Oppen et al., 2015). As an example of the potential application of my thesis research toward such efforts, Chapters II and III suggest that heat hardening efforts for ectotherms selecting for upper thermal tolerance and/or thermal plasticity may need to account for fitness tradeoffs between the two traits to best optimize the fitness of selected lines by fine tuning their relative selection. Chapter III demonstrates that only some of the total extent of physiological responses to

stress are both heritable and adaptive and that the ability of these plastic phenotypes to be selected depends on their genetic correlation with maladaptive responses.

Integrating ecophysiology and evolution also raises questions about what is ultimately adaptive. In Chapter III, I observed that unexpected decreases in upper thermal tolerance during seasonal warming were associated with greater reproductive output by *T. californicus*. This calls into question whether increased thermal tolerance under warming, often deemed putatively adaptive, benefits fitness. When faced with environmental extremes, organisms may result to different strategies for persisting under such stress. By divesting from the probability of their survival under acute thermal extremes, *T. californicus* may divert energy toward reproduction and the probability of successfully progenating offspring. Predictions of biodiversity's adaptation to climate change, and studies of plasticity in general, often assume that increases in tolerance are adaptive and promote persistence across generations (Nettle & Bateson, 2015) and may benefit from assessing whether this assumption holds across taxa.

Motivated by plasticity's complexity, my thesis research has resolved the nature of its evolution and effects on evolution in areas previously untested or receiving little investigation, contributing to advances in the study of plasticity in ecology and evolution that have been hindered by its circuitous history. These findings bring value beyond evolutionary biology and have the potential to influence predictions and conservation of biodiversity threatened by climate change. Models and management efforts related to biodiversity's adaptation to global change drivers such as high, variable temperatures or increased coastal upwelling should (i) incorporate processes for the evolution of plastic responses to global change and (ii) take careful consideration in parameterizing limits to

the evolutionary rate of plasticity such as fitness tradeoffs between traits, varying levels of genetic variation for plasticity, and covariation between environmental and genetic variance.

References

- Acker, P., Daunt, F., Wanless, S., Burthe, S. J., Newell, M. A., Harris, M. P., Swann, R. L., Gunn, C., Morley, T. I., & Reid, J. M. (2023). Additive genetic and environmental variation interact to shape the dynamics of seasonal migration in a wild bird population. *Evolution*, qpad111. <https://doi.org/10.1093/evolut/qpad111>
- Agrawal, A., Conner, J., & Rasmann, S. (2010). Evolution Since Darwin: The First 150 Years. In *Chapter 10 Tradeoffs and Negative Correlations in Evolutionary Ecology* (pp. 243–268). Sinauer Associates, Inc.
- Agrawal, A. F., & Stinchcombe, J. R. (2008). How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences*, 276(1659), 1183–1191. <https://doi.org/10.1098/rspb.2008.1671>
- Albecker, M. A., Trussell, G. C., & Lotterhos, K. E. (2022). A novel analytical framework to quantify co-gradient and countergradient variation. *Ecology Letters*, 25(6), 1521–1533. <https://doi.org/10.1111/ele.14020>
- Albecker, M. A., Wilkins, L. G. E., Krueger-Hadfield, S. A., Bashevkin, S. M., Hahn, M. W., Hare, M. P., Kindsvater, H. K., Sewell, M. A., Lotterhos, K. E., & Reitzel, A. M. (2021). Does a complex life cycle affect adaptation to environmental change? Genome-informed insights for characterizing selection across complex life cycle. *Proceedings of the Royal Society B: Biological Sciences*, 288(1964), 20212122. <https://doi.org/10.1098/rspb.2021.2122>
- Albert, B., Kos-Braun, I. C., Henras, A. K., Dez, C., Rueda, M. P., Zhang, X., Gadal, O., Kos, M., & Shore, D. (2019). A ribosome assembly stress response regulates

- transcription to maintain proteome homeostasis. *ELife*, 8, e45002.
<https://doi.org/10.7554/eLife.45002>
- Amundson, R. (2005). *The Changing Role of the Embryo in Evolutionary Thought: Roots of Evo-Devo*. Cambridge University Press.
- Andrews, S. R. (n.d.). *Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data*. Retrieved July 7, 2023, from <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
- AR5 Synthesis Report: Climate Change 2014 — IPCC*. (n.d.). Retrieved August 14, 2023, from <https://www.ipcc.ch/report/ar5/syr/>
- Arnold, P. A., Nicotra, A. B., & Kruuk, L. E. B. (2019). Sparse evidence for selection on phenotypic plasticity in response to temperature. *Philosophical Transactions of the Royal Society B*, 374(1768), 20180185. <https://doi.org/10.1098/rstb.2018.0185>
- Baldwin, J. M. (1896). A New Factor in Evolution. *The American Naturalist*, 30(354), 441–451.
- Ban, S. S., Graham, N. A. J., & Connolly, S. R. (2014). Evidence for multiple stressor interactions and effects on coral reefs. *Global Change Biology*, 20(3), 681–697. <https://doi.org/10.1111/gcb.12453>
- Barley, J. M., Cheng, B. S., Sasaki, M., Gignoux-Wolfsohn, S., Hays, C. G., Putnam, A. B., Sheth, S., Villeneuve, A. R., & Kelly, M. (2021). Limited plasticity in thermally tolerant ectotherm populations: Evidence for a trade-off. *Proceedings of the Royal Society B: Biological Sciences*, 288(1958), 20210765. <https://doi.org/10.1098/rspb.2021.0765>

- Barreto, F. S., Watson, E. T., Lima, T. G., Willett, C. S., Edmands, S., Li, W., & Burton, R. S. (2018). Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus*. *Nature Ecology & Evolution*, 2(8), Article 8.
<https://doi.org/10.1038/s41559-018-0588-1>
- Bashevkin, S. M., Dibble, C. D., Dunn, R. P., Hollarsmith, J. A., Ng, G., Satterthwaite, E. V., & Morgan, S. G. (2020). Larval dispersal in a changing ocean with an emphasis on upwelling regions. *Ecosphere*, 11(1), e03015.
<https://doi.org/10.1002/ecs2.3015>
- Beldade, P., Mateus, A. R. A., & Keller, R. A. (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, 20(7), 1347–1363. <https://doi.org/10.1111/j.1365-294X.2011.05016.x>
- Berger, J. O., & Pericchi, L. R. (1996). The intrinsic Bayes factor for model selection and prediction. *J Am Stat Assoc*, 91(433), 109–122. <https://doi.org/10.2307/2291387>
- Bible, J. M., Evans, T. G., & Sanford, E. (2020). Differences in induced thermotolerance among populations of Olympia oysters. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 239, 110563.
<https://doi.org/10.1016/j.cbpa.2019.110563>
- Bitter, M. C., Wong, J. M., Dam, H. G., Donelan, S. C., Kenkel, C. D., Komoroske, L. M., Nickols, K. J., Rivest, E. B., Salinas, S., Burgess, S. C., & Lotterhos, K. E. (2021). Fluctuating selection and global change: A synthesis and review on disentangling the roles of climate amplitude, predictability and novelty. *Proceedings of the Royal Society B*, 288(1957), 20210727.
<https://doi.org/10.1098/rspb.2021.0727>

- Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T., & Ferrier, S. (2013). Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences*, *110*(23), 9374–9379. <https://doi.org/10.1073/pnas.1220228110>
- Blows, M. W., Allen, S. L., Collet, J. M., Chenoweth, S. F., & McGuigan, K. (2015). The Phenome-Wide Distribution of Genetic Variance. *The American Naturalist*, *186*(1), 15–30. <https://doi.org/10.1086/681645>
- Blows, M. W., & McGuigan, K. (2016). The Distribution of Genetic Variance Across Phenotypic Space and the Response to Selection. In *Invasion Genetics* (pp. 187–205). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119072799.ch11>
- Bodensteiner, B. L., Agudelo-Cantero, G. A., Arietta, A. Z. A., Gunderson, A. R., Muñoz, M. M., Refsnider, J. M., & Gangloff, E. J. (2021). Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(1), 173–194. <https://doi.org/10.1002/jez.2414>
- Bogan, S. N., Strader, M. E., & Hofmann, G. E. (2023). Associations between DNA methylation and gene regulation depend on chromatin accessibility during transgenerational plasticity. *BMC Biol.* <https://doi.org/10.1186/s12915-023-01645-8>
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>

- Bonamour, S., Chevin, L.-M., Charmantier, A., & Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: The importance of cue variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1768), 20180178. <https://doi.org/10.1098/rstb.2018.0178>
- Bozinovic, F., & Pörtner, H.-O. (2015). Physiological ecology meets climate change. *Ecology and Evolution*, 5(5), 1025–1030. <https://doi.org/10.1002/ece3.1403>
- Bradshaw, A. D. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants. In E. W. Caspari & J. M. Thoday (Eds.), *Advances in Genetics* (Vol. 13, pp. 115–155). Academic Press. [https://doi.org/10.1016/S0065-2660\(08\)60048-6](https://doi.org/10.1016/S0065-2660(08)60048-6)
- Buchanan, K. L., Grindstaff, J. L., & Pravosudov, V. V. (2013). Condition dependence, developmental plasticity, and cognition: Implications for ecology and evolution. *Trends in Ecology & Evolution*, 28(5), 290–296. <https://doi.org/10.1016/j.tree.2013.02.004>
- Buckley, L. B., & Kingsolver, J. G. (2021). Evolution of thermal sensitivity in changing and variable climates. *Annual Review of Ecology, Evolution, and Systematics*.
- Bufford, J. L., & Hulme, P. E. (2021). Increased adaptive phenotypic plasticity in the introduced range in alien weeds under drought and flooding. *Biological Invasions*, 23(8), 2675–2688. <https://doi.org/10.1007/s10530-021-02532-5>
- Bürkner, P.-C. (2017a). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, 80, 1–28. <https://doi.org/10.18637/jss.v080.i01>
- Bürkner, P.-C. (2017b). brms: An R Package for Bayesian Multilevel Models Using Stan. *Journal of Statistical Software*, 80, 1–28. <https://doi.org/10.18637/jss.v080.i01>

- Burton, R. S. (1985). Mating system of the intertidal copepod *Tigriopus californicus*. *Marine Biology*, 86(3), 247–252. <https://doi.org/10.1007/BF00397511>
- Calisi, R. M., & Bentley, G. E. (2009). Lab and field experiments: Are they the same animal? *Hormones and Behavior*, 56(1), 1–10. <https://doi.org/10.1016/j.yhbeh.2009.02.010>
- Cameron, R. A., Leahy, P. S., Britten, R. J., & Davidson, E. H. (1999). Microsatellite loci in wild-type and inbred *Strongylocentrotus purpuratus*. *Developmental Biology*, 208(2), 255–264. <https://doi.org/10.1006/dbio.1999.9224>
- Campbell-Staton, S. C., Velotta, J. P., & Winchell, K. M. (2021). Selection on adaptive and maladaptive gene expression plasticity during thermal adaptation to urban heat islands. *Nature Communications*, 12(1), Article 1. <https://doi.org/10.1038/s41467-021-26334-4>
- Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M., Guo, J., Li, P., & Riddell, A. (2017). Stan: A Probabilistic Programming Language. *Journal of Statistical Software*, 76, 1–32. <https://doi.org/10.18637/jss.v076.i01>
- Chamorro, J. D., Macdonald, A. M., & Hofmann, G. E. (2023). Transgenerational plasticity as a mechanism of response to marine heatwaves in the purple sea urchin, *Strongylocentrotus purpuratus*. *Frontiers in Marine Science*, 10. <https://doi.org/10.3389/fmars.2023.1212781>
- Chan, F., Barth, J. A., Blanchette, C. A., Byrne, R. H., Chavez, F., Cheriton, O., Feely, R. A., Friederich, G., Gaylord, B., & Gouhier, T. (2017). Persistent spatial

- structuring of coastal ocean acidification in the California Current System. *Scientific Reports*, 7(1), 2526.
- Chevin, L.-M., & Hoffmann, A. A. (2017). Evolution of phenotypic plasticity in extreme environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1723), 20160138. <https://doi.org/10.1098/rstb.2016.0138>
- Chevin, L.-M., & Lande, R. (2015). Evolution of environmental cues for phenotypic plasticity. *Evolution*, 69(10), 2767–2775. <https://doi.org/10.1111/evo.12755>
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biology*, 8(4), e1000357. <https://doi.org/10.1371/journal.pbio.1000357>
- Choi, B., Jeong, H., & Kim, E. (2019). Phenotypic plasticity of *Capsella bursa-pastoris* (Brassicaceae) and its effect on fitness in response to temperature and soil moisture. *Plant Species Biology*, 34(1), 5–10. <https://doi.org/10.1111/1442-1984.12227>
- Clusella-Trullas, S., Garcia, R. A., Terblanche, J. S., & Hoffmann, A. A. (2021). How useful are thermal vulnerability indices? *Trends in Ecology & Evolution*, 36(11), 1000–1010. <https://doi.org/10.1016/j.tree.2021.07.001>
- Conover, D. O., Duffy, T. A., & Hice, L. A. (2009). The Covariance between Genetic and Environmental Influences across Ecological Gradients. *Annals of the New York Academy of Sciences*, 1168(1), 100–129. <https://doi.org/10.1111/j.1749-6632.2009.04575.x>
- Corl, A., Bi, K., Luke, C., Challa, A. S., Stern, A. J., Sinervo, B., & Nielsen, R. (2018). The Genetic Basis of Adaptation following Plastic Changes in Coloration in a

- Novel Environment. *Current Biology*, 28(18), 2970-2977.e7.
<https://doi.org/10.1016/j.cub.2018.06.075>
- Cortina, L. M. (2014). Recursive Models. In *Wiley StatsRef: Statistics Reference Online*.
John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781118445112.stat06426>
- Crespi, B. J. (2000). The evolution of maladaptation. *Heredity*, 84(6), Article 6.
<https://doi.org/10.1046/j.1365-2540.2000.00746.x>
- Crispo, E. (2007). THE BALDWIN EFFECT AND GENETIC ASSIMILATION:
REVISITING TWO MECHANISMS OF EVOLUTIONARY CHANGE
MEDIATED BY PHENOTYPIC PLASTICITY. *Evolution*, 61(11), 2469–2479.
<https://doi.org/10.1111/j.1558-5646.2007.00203.x>
- Crispo, E. (2008). Modifying effects of phenotypic plasticity on interactions among
natural selection, adaptation and gene flow. *Journal of Evolutionary Biology*,
21(6), 1460–1469. <https://doi.org/10.1111/j.1420-9101.2008.01592.x>
- Damgaard, C. (2019). A Critique of the Space-for-Time Substitution Practice in
Community Ecology. *Trends in Ecology & Evolution*, 34(5), 416–421.
<https://doi.org/10.1016/j.tree.2019.01.013>
- Davison, A. C., & Hinkley, D. V. (1997). *Bootstrap Methods and their Application*.
Cambridge University Press. <https://doi.org/10.1017/CBO9780511802843>
- De Lisle, S. P., Mäenpää, M. I., & Svensson, E. I. (2022). Phenotypic plasticity is aligned
with phenological adaptation on both micro- and macroevolutionary timescales.
Ecology Letters, 25(4), 790–801. <https://doi.org/10.1111/ele.13953>

- De Lisle, S. P., & Rowe, L. (2023). Condition dependence and the paradox of missing plasticity costs. *Evolution Letters*, 7(2), 67–78.
<https://doi.org/10.1093/evlett/grad009>
- De, S., & Mühlemann, O. (2022). A comprehensive coverage insurance for cells: Revealing links between ribosome collisions, stress responses and mRNA surveillance. *RNA Biology*, 19(1), 609–621.
<https://doi.org/10.1080/15476286.2022.2065116>
- Debat, V., & David, P. (2001). Mapping phenotypes: Canalization, plasticity and developmental stability. *Trends in Ecology & Evolution*, 16(10), 555–561.
[https://doi.org/10.1016/S0169-5347\(01\)02266-2](https://doi.org/10.1016/S0169-5347(01)02266-2)
- Debes, P. V., Solberg, M. F., Matre, I. H., Dyrhovden, L., & Glover, K. A. (2021). Genetic variation for upper thermal tolerance diminishes within and between populations with increasing acclimation temperature in Atlantic salmon. *Heredity*, 127(5), 455–466. <https://doi.org/10.1038/s41437-021-00469-y>
- Denny, M. W., & Dowd, W. W. (2022). Elevated Salinity Rapidly Confers Cross-Tolerance to High Temperature in a Splash-Pool Copepod. *Integrative Organismal Biology*, 4(1), obac037. <https://doi.org/10.1093/iob/obac037>
- DeWitt, T. J., & Scheiner, S. M. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches*. Oxford University Press.
- DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2), 77–81. [https://doi.org/10.1016/S0169-5347\(97\)01274-3](https://doi.org/10.1016/S0169-5347(97)01274-3)

- Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: Parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180036.
<https://doi.org/10.1098/rspb.2018.0036>
- Diamond, S. E., Chick, L., Perez, A., Strickler, S. A., & Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean Society*, 121(2), 248–257.
<https://doi.org/10.1093/biolinnean/blw047>
- Dinh, K. V., Cuevas-Sanchez, A. Y., Buhl, K. S., Moeser, E. A., & Dowd, W. W. (2020). Heat tolerance and thermal preference of the copepod *Tigriopus californicus* are insensitive to ecologically relevant dissolved oxygen levels. *Scientific Reports*, 10(1), Article 1. <https://doi.org/10.1038/s41598-020-75635-z>
- Donelan, S. C., Hellmann, J. K., Bell, A. M., Luttbeg, B., Orrock, J. L., Sheriff, M. J., & Sih, A. (2020). Transgenerational Plasticity in Human-Altered Environments. *Trends in Ecology & Evolution*, 35(2), 115–124.
<https://doi.org/10.1016/j.tree.2019.09.003>
- Donelson, J. M., Gaitan-Espitia, J. D., Hobday, A. J., Mokany, K., Andrew, S. C., Boulter, S., Cook, C. N., Dickson, F., Macgregor, N. A., Mitchell, N. J., Pickup, M., & Fox, R. J. (2023). Putting plasticity into practice for effective conservation actions under climate change. *Nature Climate Change*, 13(7), Article 7.
<https://doi.org/10.1038/s41558-023-01706-4>

- Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., Leis, J. M., Ling, S. D., Marshall, D., Pandolfi, J. M., Pecl, G., Rodgers, G. G., Booth, D. J., & Munday, P. L. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180186. <https://doi.org/10.1098/rstb.2018.0186>
- Dore, A. A., McDowall, L., Rouse, J., Bretman, A., Gage, M. J. G., & Chapman, T. (2018). The role of complex cues in social and reproductive plasticity. *Behavioral Ecology and Sociobiology*, *72*(8), 124. <https://doi.org/10.1007/s00265-018-2539-x>
- Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *Journal of Experimental Biology*, *218*(12), 1956–1967. <https://doi.org/10.1242/jeb.114926>
- Durmowicz, A. G., Hofmeister, S., Kadyraliev, T. K., Aldashev, A. A., & Stenmark, K. R. (1993). Functional and structural adaptation of the yak pulmonary circulation to residence at high altitude. *Journal of Applied Physiology*, *74*(5), 2276–2285. <https://doi.org/10.1152/jappl.1993.74.5.2276>
- Dwane, C., Rezende, E. L., Tills, O., Galindo, J., Rolán-Alvarez, E., Rundle, S., & Truebano, M. (2023). Thermodynamic effects drive countergradient responses in the thermal performance of *Littorina saxatilis* across latitude. *Science of The Total Environment*, *863*, 160877. <https://doi.org/10.1016/j.scitotenv.2022.160877>
- Edmands, S. (2001). Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes.

- Molecular Ecology*, 10(7), 1743–1750. <https://doi.org/10.1046/j.0962-1083.2001.01306.x>
- Edmands, S., Moberg, P. E., & Burton, R. S. (1996). Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Marine Biology*, 126(3), 443–450. <https://doi.org/10.1007/BF00354626>
- Ehrenreich, I. M., & Pfennig, D. W. (2016). Genetic assimilation: A review of its potential proximate causes and evolutionary consequences. *Annals of Botany*, 117(5), 769–779. <https://doi.org/10.1093/aob/mcv130>
- Evans, T. G., Chan, F., Menge, B. A., & Hofmann, G. E. (2013). Transcriptomic responses to ocean acidification in larval sea urchins from a naturally variable pH environment. *Mol. Ecol.*, 22(6), 1609–1625.
- Evans, T. G., Pespeni, M. H., Hofmann, G. E., Palumbi, S. R., & Sanford, E. (2017). Transcriptomic responses to seawater acidification among sea urchin populations inhabiting a natural pH mosaic. *Mol. Ecol.*, 26(8), 2257–2275.
- Fangue, N. A., O'Donnell, M. J., Sewell, M. A., Matson, P. G., MacPherson, A. C., & Hofmann, G. E. (2010). A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. *Limnol. Oceanogr. Methods*, 8(8), 441–452.
- Fangue, N. A., Podrabsky, J. E., Crawshaw, L. I., & Schulte, P. M. (2009). Countergradient variation in temperature preference in populations of killifish *Fundulus heteroclitus*. *Physiological and Biochemical Zoology: PBZ*, 82(6), 776–786. <https://doi.org/10.1086/606030>

- Feder, M. E., Bennett, A. F., Burggren, W. W., & Huey, R. B. (1987). *New directions in ecological physiology*. Cambridge University Press.
- Feder, M. E., & Hofmann, G. E. (1999). HEAT-SHOCK PROTEINS, MOLECULAR CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological Physiology. *Annual Review of Physiology*, *61*(1), 243–282.
<https://doi.org/10.1146/annurev.physiol.61.1.243>
- Folt, C. L., Chen, C. Y., Moore, M. V., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, *44*(3part2), 864–877. https://doi.org/10.4319/lo.1999.44.3_part_2.0864
- Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180174. <https://doi.org/10.1098/rstb.2018.0174>
- Freed, E. F., Bleichert, F., Dutca, L. M., & Baserga, S. J. (2010). When ribosomes go bad: Diseases of ribosome biogenesis. *Molecular BioSystems*, *6*(3), 481–493.
<https://doi.org/10.1039/B919670F>
- Gardiner, N. M., Munday, P. L., & Nilsson, G. E. (2010). Counter-Gradient Variation in Respiratory Performance of Coral Reef Fishes at Elevated Temperatures. *PLOS ONE*, *5*(10), e13299. <https://doi.org/10.1371/journal.pone.0013299>
- Gelman, A., Lee, D., & Guo, J. (2015). Stan: A probabilistic programming language for Bayesian inference and optimization. *Journal of Educational and Behavioral Statistics*, *40*(5), 530–543. <https://doi.org/10.3102/1076998615606113>

- Geng, X., Boufadel, M. C., & Jackson, N. L. (2016). Evidence of salt accumulation in beach intertidal zone due to evaporation. *Scientific Reports*, *6*, 31486.
<https://doi.org/10.1038/srep31486>
- 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. *Functional Ecology*, *21*(3), 394–407.
<https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Gibbin, E. M., Massamba N'Siala, G., Chakravarti, L. J., Jarrold, M. D., & Calosi, P. (2017). The evolution of phenotypic plasticity under global change. *Scientific Reports*, *7*(1), Article 1. <https://doi.org/10.1038/s41598-017-17554-0>
- Gibert, P., Debat, V., & Ghalambor, C. K. (2019). Phenotypic plasticity, global change, and the speed of adaptive evolution. *Current Opinion in Insect Science*, *35*, 34–40. <https://doi.org/10.1016/j.cois.2019.06.007>
- Gilbert, A. L., & Miles, D. B. (2017). Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1860), 20170536.
<https://doi.org/10.1098/rspb.2017.0536>
- Grether, G. F. (2005). Environmental Change, Phenotypic Plasticity, and Genetic Compensation. *The American Naturalist*, *166*(4), E115–E123.
<https://doi.org/10.1086/432023>
- Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., & Plattner, G.-K. (2012). Rapid Progression of Ocean Acidification in the California Current System. *Science*, *337*(6091), 220–223. <https://doi.org/10.1126/science.1216773>

- Grün, B., Kosmidis, I., & Zeileis, A. (2012). Extended Beta Regression in R: Shaken, Stirred, Mixed, and Partitioned. *Journal of Statistical Software*, 48, 1–25.
<https://doi.org/10.18637/jss.v048.i11>
- Gunderson, A. R. (2023). Trade-offs between baseline thermal tolerance and thermal tolerance plasticity are much less common than it appears. *Global Change Biology*, 00, 1–6. <https://doi.org/10.1111/gcb.16710>
- 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. *Annual Review of Marine Science*, 8(1), 357–378. <https://doi.org/10.1146/annurev-marine-122414-033953>
- Gunderson, A. R., & Revell, L. J. (2022). Testing for genetic assimilation with phylogenetic comparative analysis: Conceptual, methodological, and statistical considerations. *Evolution*, 76(9), 1942–1952. <https://doi.org/10.1111/evo.14570>
- Hallsson, L. R., & Björklund, M. (2012). Selection in a fluctuating environment leads to decreased genetic variation and facilitates the evolution of phenotypic plasticity. *Journal of Evolutionary Biology*, 25(7), 1275–1290.
<https://doi.org/10.1111/j.1420-9101.2012.02512.x>
- Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology*, 30(1), 33–41. <https://doi.org/10.1111/cobi.12574>
- Handschumacher, L., Steinarsdóttir, M. B., Edmands, S., & Ingólfsson, A. (2010). Phylogeography of the rock-pool copepod *Tigriopus brevicornis* (Harpacticoida)

- in the northern North Atlantic, and its relationship to other species of the genus. *Marine Biology*, 157(6), 1357–1366. <https://doi.org/10.1007/s00227-010-1415-7>
- Hangartner, S., Sgrò, C. M., Connallon, T., & Booksmythe, I. (2022). Sexual dimorphism in phenotypic plasticity and persistence under environmental change: An extension of theory and meta-analysis of current data. *Ecology Letters*, 25(6), 1550–1565. <https://doi.org/10.1111/ele.14005>
- Hardison, E. A., Kraskura, K., Van Wert, J., Nguyen, T., & Eliason, E. J. (2021). Diet mediates thermal performance traits: Implications for marine ectotherms. *Journal of Experimental Biology*, 224(21), jeb242846. <https://doi.org/10.1242/jeb.242846>
- Hardison, E. A., Schwieterman, G. D., & Eliason, E. J. (2023). Diet changes thermal acclimation capacity, but not acclimation rate, in a marine ectotherm (*Girella nigricans*) during warming. *Proceedings of the Royal Society B: Biological Sciences*, 290(1995), 20222505. <https://doi.org/10.1098/rspb.2022.2505>
- Healy, T. M., Bock, A. K., & Burton, R. S. (2019). Variation in developmental temperature alters adulthood plasticity of thermal tolerance in *Tigriopus californicus*. *Journal of Experimental Biology*, 222(22), jeb213405. <https://doi.org/10.1242/jeb.213405>
- Heilmeier, H. (2019). Functional traits explaining plant responses to past and future climate changes. *Flora*, 254, 1–11. <https://doi.org/10.1016/j.flora.2019.04.004>
- 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. *Ecological Monographs*, 76(4), 461–479. [https://doi.org/10.1890/0012-9615\(2006\)076\[0461:MPOTSI\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2006)076[0461:MPOTSI]2.0.CO;2)
- Hendry, A. P. (2016). Key questions on the role of phenotypic plasticity in evolutionary dynamics. *Journal of Heredity*, 107(1), 25–41. <https://doi.org/10.1093/jhered/esv060>
- Hoffmann, A. A., Merilä, J., Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, 14(3), 96–101. [https://doi.org/10.1016/S0169-5347\(99\)01595-5](https://doi.org/10.1016/S0169-5347(99)01595-5)
- Hofmann, G. E., & Todgham, A. E. (2010). Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. *Annual Review of Physiology*, 72(1), 127–145. <https://doi.org/10.1146/annurev-physiol-021909-135900>
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), 1665–1679. <https://doi.org/10.1098/rstb.2012.0005>
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, 4(5), 131–135. [https://doi.org/10.1016/0169-5347\(89\)90211-5](https://doi.org/10.1016/0169-5347(89)90211-5)
- Huggett, J., & Griffiths, C. (1986). Some relationships between elevation, physico-chemical variables and biota of intertidal rock pools. *Marine Ecology Progress Series*, 29, 189–197. <https://doi.org/10.3354/meps029189>

- Huyer, A. (1983). Coastal upwelling in the California current system. *Progress in Oceanography*, 12(3), 259–284. [https://doi.org/10.1016/0079-6611\(83\)90010-1](https://doi.org/10.1016/0079-6611(83)90010-1)
- Iacobellis, S. F., & Cayan, D. R. (2013). The variability of California summertime marine stratus: Impacts on surface air temperatures. *Journal of Geophysical Research: Atmospheres*, 118(16), 9105–9122. <https://doi.org/10.1002/jgrd.50652>
- Iordanov, M. S., Pribnow, D., Magun, J. L., Dinh, T.-H., Pearson, J. A., & Magun, B. E. (1998). Ultraviolet Radiation Triggers the Ribotoxic Stress Response in Mammalian Cells *. *Journal of Biological Chemistry*, 273(25), 15794–15803. <https://doi.org/10.1074/jbc.273.25.15794>
- Jong, G. de, & Gavrillets, S. (2000). Maintenance of genetic variation in phenotypic plasticity: The role of environmental variation. *Genetics Research*, 76(3), 295–304. <https://doi.org/10.1017/S0016672300004729>
- Kapsenberg, L., Miglioli, A., Bitter, M. C., Tambutté, E., Dumollard, R., & Gattuso, J.-P. (2018). Ocean pH fluctuations affect mussel larvae at key developmental transitions. *Proceedings of the Royal Society B: Biological Sciences*, 285(1893), 20182381. <https://doi.org/10.1098/rspb.2018.2381>
- Kauffmann, A., Gentleman, R., & Huber, W. (2009). arrayQualityMetrics—A bioconductor package for quality assessment of microarray data. *Bioinformatics*, 25(3), 415–416. <https://doi.org/10.1093/bioinformatics/btn647>
- Kelly, C., & Price, T. D. (2005). Correcting for regression to the mean in behavior and ecology. *The American Naturalist*, 166(6), 700–707. <https://doi.org/10.1086/497402>

- Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1768), 20180176.
<https://doi.org/10.1098/rstb.2018.0176>
- Kelly, M. W., Padilla-Gamiño, J. L., & Hofmann, G. E. (2013). Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob. Chang. Biol.*, 19(8), 2536–2546.
- Kelly, M. W., Pankey, M. S., DeBiasse, M. B., & Plachetzki, D. C. (2017). Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. *Functional Ecology*, 31(2), 398–406. <https://doi.org/10.1111/1365-2435.12725>
- Kelly, M. W., Sanford, E., & Grosberg, R. K. (2011). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B: Biological Sciences*, 279(1727), 349–356.
<https://doi.org/10.1098/rspb.2011.0542>
- Kempes, C. P., Koehl, M. A. R., & West, G. B. (2019). The scales that limit: The physical boundaries of evolution. *Frontiers in Ecology and Evolution*, 7.
<https://www.frontiersin.org/articles/10.3389/fevo.2019.00242>
- Kiiveri, H., Speed, T. P., & Carlin, J. B. (1984). Recursive causal models. *Journal of the Australian Mathematical Society*, 36(1), 30–52.
<https://doi.org/10.1017/S1446788700027312>
- Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*, 37(8), Article 8. <https://doi.org/10.1038/s41587-019-0201-4>

- Kingsolver, J. G., & Buckley, L. B. (2017). Evolution of plasticity and adaptive responses to climate change along climate gradients. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1860), 20170386. <https://doi.org/10.1098/rspb.2017.0386>
- Kline, R. B. (2015). *Principles and practice of structural equation modeling, fourth edition*. Guilford Publications.
- Kovuri, P., Yadav, A., & Sinha, H. (2023). Role of genetic architecture in phenotypic plasticity. *Trends in Genetics*, *0*(0). <https://doi.org/10.1016/j.tig.2023.04.002>
- Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, *37*(6), 1210–1226. <https://doi.org/10.2307/2408842>
- Leahy, P. S. (1986). Chapter 1 Laboratory Culture of *Strongylocentrotus purpuratus* Adults, Embryos, and Larvae. In T. E. Schroeder (Ed.), *Methods in Cell Biology* (Vol. 27, pp. 1–13). Academic Press. [https://doi.org/10.1016/S0091-679X\(08\)60339-8](https://doi.org/10.1016/S0091-679X(08)60339-8)
- Leonard, A. M., & Lancaster, L. T. (2020). Maladaptive plasticity facilitates evolution of thermal tolerance during an experimental range shift. *BMC Evolutionary Biology*, *20*(1), 47. <https://doi.org/10.1186/s12862-020-1589-7>
- Levis, N. A., & Pfennig, D. W. (2016). Evaluating ‘Plasticity-First’ Evolution in Nature: Key Criteria and Empirical Approaches. *Trends in Ecology & Evolution*, *31*(7), 563–574. <https://doi.org/10.1016/j.tree.2016.03.012>
- Lewis, S. C., & King, A. D. (2017). Evolution of mean, variance and extremes in 21st century temperatures. *Weather and Climate Extremes*, *15*, 1–10. <https://doi.org/10.1016/j.wace.2016.11.002>

- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923–930. <https://doi.org/10.1093/bioinformatics/btt656>
- Lima, T. G., Burton, R. S., & Willett, C. S. (2019). Genomic scans reveal multiple mitochondrial incompatibilities in population crosses of the copepod *Tigriopus californicus*. *Evolution*, 73(3), 609–620. <https://doi.org/10.1111/evo.13690>
- Logan, M. L., Cox, R. M., & Calsbeek, R. (2014). Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences*, 111(39), 14165–14169. <https://doi.org/10.1073/pnas.1404885111>
- Makowski, D., Ben-Shachar, M., & Lüdtke, D. (2019). BayestestR: Describing effects and their uncertainty, existence and significance within the Bayesian framework. *The Journal of Open Source Software*, 4(40), 1541. <https://doi.org/10.21105/joss.01541>
- Makowski, D., Ben-Shachar, M. S., Chen, S. H. A., & Lüdtke, D. (2019a). Indices of effect existence and significance in the Bayesian framework. *Front Psychol*, 10, 2767. <https://doi.org/10.3389/fpsyg.2019.02767>

- Makowski, D., Ben-Shachar, M. S., Chen, S. H. A., & Lüdecke, D. (2019b). Indices of Effect Existence and Significance in the Bayesian Framework. *Frontiers in Psychology, 10*. <https://www.frontiersin.org/articles/10.3389/fpsyg.2019.02767>
- Marks, R. (2008). Dissolved oxygen supersaturation and its impact on bubble formation in the southern Baltic Sea coastal waters. *Hydrology Research, 39*(3), 229–236. <https://doi.org/10.2166/nh.2008.021>
- Marshall, D. J. (2008). Transgenerational Plasticity in the Sea: Context-Dependent Maternal Effects Across the Life History. *Ecology, 89*(2), 418–427. <https://doi.org/10.1890/07-0449.1>
- Marshall, D. J., Krug, P. J., Kupriyanova, E. K., Byrne, M., & Emler, R. B. (2012). The Biogeography of Marine Invertebrate Life Histories. *Annual Review of Ecology, Evolution, and Systematics, 43*(1), 97–114. <https://doi.org/10.1146/annurev-ecolsys-102710-145004>
- Marshall, K. E., Anderson, K. M., Brown, N. E. M., Dytner, J. K., Flynn, K. L., Bernhardt, J. R., Konecny, C. A., Gurney-Smith, H., & Harley, C. D. G. (2021). Whole-organism responses to constant temperatures do not predict responses to variable temperatures in the ecosystem engineer *Mytilus trossulus*. *Proceedings of the Royal Society B: Biological Sciences, 288*(1947), 20202968. <https://doi.org/10.1098/rspb.2020.2968>
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal, 17*(1), Article 1. <https://doi.org/10.14806/ej.17.1.200>

- Matesanz, S., Gianoli, E., & Valladares, F. (2010). Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*, *1206*(1), 35–55. <https://doi.org/10.1111/j.1749-6632.2010.05704.x>
- Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. *Journal of Experimental Biology*, *221*(14), jeb164673. <https://doi.org/10.1242/jeb.164673>
- Morgan, R., Andreassen, A. H., Åsheim, E. R., Finnøen, M. H., Dresler, G., Brembu, T., Loh, A., Miest, J. J., & Jutfelt, F. (2022). Reduced physiological plasticity in a fish adapted to stable temperatures. *Proceedings of the National Academy of Sciences*, *119*(22), e2201919119. <https://doi.org/10.1073/pnas.2201919119>
- Morris, M. R. J., & Rogers, S. M. (2013). Overcoming maladaptive plasticity through plastic compensation. *Current Zoology*, *59*(4), 526–536. <https://doi.org/10.1093/czoolo/59.4.526>
- Munar-Delgado, G., Araya-Ajoy, Y. G., & Edelaar, P. (2023). Estimation of additive genetic variance when there are gene–environment correlations: Pitfalls, solutions and unexplored questions. *Methods in Ecology and Evolution*, *14*(5), 1245–1258. <https://doi.org/10.1111/2041-210X.14098>
- Napier, J. D., Heckman, R. W., & Juenger, T. E. (2023). Gene-by-environment interactions in plants: Molecular mechanisms, environmental drivers, and adaptive plasticity. *The Plant Cell*, *35*(1), 109–124. <https://doi.org/10.1093/plcell/koac322>

- Nettle, D., & Bateson, M. (2015). Adaptive developmental plasticity: What is it, how can we recognize it and when can it evolve? *Proceedings of the Royal Society B: Biological Sciences*, 282(1812), 20151005.
<https://doi.org/10.1098/rspb.2015.1005>
- Oliver, E. C. J., Benthuyzen, J. A., Darmaraki, S., Donat, M. G., Hobday, A. J., Holbrook, N. J., Schlegel, R. W., & Sen Gupta, A. (2021). Marine Heatwaves. *Annual Review of Marine Science*, 13(1), 313–342.
<https://doi.org/10.1146/annurev-marine-032720-095144>
- Oostra, V., Saastamoinen, M., Zwaan, B. J., & Wheat, C. W. (2018a). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications*, 9(1), Article 1. <https://doi.org/10.1038/s41467-018-03384-9>
- Oostra, V., Saastamoinen, M., Zwaan, B. J., & Wheat, C. W. (2018b). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications*, 9(1), Article 1. <https://doi.org/10.1038/s41467-018-03384-9>
- Ordas, B., Malvar, R. A., & Hill, W. G. (2008). Genetic variation and quantitative trait loci associated with developmental stability and the environmental correlation between traits in maize. *Genetics Research*, 90(5), 385–395.
<https://doi.org/10.1017/S0016672308009762>
- Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19(8), 2373–2380.
<https://doi.org/10.1111/gcb.12240>

- Palumbi, S. R., & Wilson, A. C. (1990). Mitochondrial Dna Diversity in the Sea Urchins *Strongylocentrotus Purpuratus* and *S. Droebachiensis*. *Evolution*, *44*(2), 403–415.
<https://doi.org/10.1111/j.1558-5646.1990.tb05208.x>
- Pares-Sierra, A., & O'Brien, J. J. (1989). The seasonal and interannual variability of the California Current system: A numerical model. *Journal of Geophysical Research: Oceans*, *94*(C3), 3159–3180. <https://doi.org/10.1029/JC094iC03p03159>
- Pares-Sierra, A., White, W. B., & Tai, C.-K. (1993). Wind-driven Coastal Generation of Annual Mesoscale Eddy Activity in the California Current. *Journal of Physical Oceanography*, *23*(6), 1110–1121. [https://doi.org/10.1175/1520-0485\(1993\)023<1110:WDCGOA>2.0.CO;2](https://doi.org/10.1175/1520-0485(1993)023<1110:WDCGOA>2.0.CO;2)
- Parsons, K. J., McWhinnie, K., Pilakouta, N., & Walker, L. (2020). Does phenotypic plasticity initiate developmental bias? *Evolution & Development*, *22*(1–2), 56–70.
<https://doi.org/10.1111/ede.12304>
- Pavlyshyn, D., Johnstone, I. M., & Szepeanacz, J. L. (2022). *Comparison of REML methods for the study of phenome-wide genetic variation* (arXiv:2210.11709). arXiv. <https://doi.org/10.48550/arXiv.2210.11709>
- Pazzaglia, J., Reusch, T. B. H., Terlizzi, A., Marín-Guirao, L., & Procaccini, G. (2021). Phenotypic plasticity under rapid global changes: The intrinsic force for future seagrasses survival. *Evolutionary Applications*, *14*(5), 1181–1201.
<https://doi.org/10.1111/eva.13212>
- Pearse, J. S. (2006). Ecological role of purple sea urchins. *Science*, *314*(5801), 940–941.
<https://doi.org/10.1126/science.1131888>

- Pereira, R. J., Sasaki, M. C., & Burton, R. S. (2017). Adaptation to a latitudinal thermal gradient within a widespread copepod species: The contributions of genetic divergence and phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 284(1853), 20170236.
<https://doi.org/10.1098/rspb.2017.0236>
- Pespeni, M. H., Chan, F., Menge, B. A., & Palumbi, S. R. (2013). Signs of adaptation to local pH conditions across an environmental mosaic in the California Current Ecosystem. *Integr. Comp. Biol.*, 53(5), 857–870.
- Pespeni, M. H., & Palumbi, S. R. (2013). Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic. *Mol. Ecol.*, 22(13), 3580–3597.
- Pfennig, D. W. (Ed.). (2021). *Phenotypic Plasticity & Evolution: Causes, Consequences, Controversies*. Taylor & Francis. <https://doi.org/10.1201/9780429343001>
- Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. JHU Press.
- Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology & Evolution*, 20(9), 481–486.
<https://doi.org/10.1016/j.tree.2005.06.001>
- Pigliucci, M. (2006). Genetic Variance–covariance Matrices: A Critique of the Evolutionary Quantitative Genetics Research Program. *Biology and Philosophy*, 21(1), 1–23. <https://doi.org/10.1007/s10539-005-0399-z>
- Pinheiro, J., Bates, D., & R Core Team. (2023). *nlme: Linear and Nonlinear Mixed Effects Models*. <https://CRAN.R-project.org/package=nlme>

- Powers, M. J., Weaver, R. J., Heine, K. B., & Hill, G. E. (2020). Predicting adult lifespan and lifetime reproductive success from early-life reproductive events. *Marine Biology*, 167(10), 147. <https://doi.org/10.1007/s00227-020-03765-z>
- Powlik, J. J. (1996). *Ecology of Tigriopus californicus (Copepoda, Harpacticoida) in Barkley Sound, British Columbia* [University of British Columbia]. <https://doi.org/10.14288/1.0053324>
- Powlik, J. J. (1999). Habitat characters of *Tigriopus californicus* (Copepoda: Harpacticoida), with notes on the dispersal of supralittoral fauna. *Journal of the Marine Biological Association of the United Kingdom*, 79(1), 85–92. <https://doi.org/10.1017/S0025315498000095>
- Powlik, J. J., Lewis, A. G., & Spaeth, M. (1997). Development, body length, and feeding of *Tigriopus californicus* (Copepoda, Harpacticoida) in laboratory and field populations. *Crustaceana*, 70(3), 324–343. <http://www.jstor.org/stable/20105864>
- Price, T., & Langen, T. (1992). Evolution of correlated characters. *Trends in Ecology & Evolution*, 7(9), 307–310. [https://doi.org/10.1016/0169-5347\(92\)90229-5](https://doi.org/10.1016/0169-5347(92)90229-5)
- Raymond, W., Barber, J., Dethier, M., Hayford, H., Harley, C., King, T., Paul, B., Speck, C., Tobin, E., Raymond, A., & McDonald, P. (2022). Assessment of the impacts of an unprecedented heatwave on intertidal shellfish of the Salish Sea. *Ecology*, 103. <https://doi.org/10.1002/ecy.3798>
- Renaud, L.-A., Festa-Bianchet, M., & Pelletier, F. (2022). Testing the match–mismatch hypothesis in bighorn sheep in the context of climate change. *Global Change Biology*, 28(1), 21–32. <https://doi.org/10.1111/gcb.15923>

- Reside, A. E., Butt, N., & Adams, V. M. (2018). Adapting systematic conservation planning for climate change. *Biodiversity and Conservation*, 27(1), 1–29. <https://doi.org/10.1007/s10531-017-1442-5>
- Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters*, 9(8), 981–993. <https://doi.org/10.1111/j.1461-0248.2006.00950.x>
- Ripley, B., Venables, B., Bates, D. M., ca 1998), K. H. (partial port, ca 1998), A. G. (partial port, & Firth, D. (2023). *MASS: Support Functions and Datasets for Venables and Ripley's MASS (7.3-60)* [Computer software]. <https://cran.r-project.org/web/packages/MASS/index.html>
- Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26(1), 139–140.
- Roff, D. A., & Fairbairn, D. J. (2007). The evolution of trade-offs: Where are we? *Journal of Evolutionary Biology*, 20(2), 433–447. <https://doi.org/10.1111/j.1420-9101.2006.01255.x>
- Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, 9(1), Article 1. <https://doi.org/10.1038/s41598-019-51114-y>
- Rowe, L., & Houle, D. (1997). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London. Series B:*

- Biological Sciences*, 263(1375), 1415–1421.
<https://doi.org/10.1098/rspb.1996.0207>
- Salachan, P. V., & Sørensen, J. G. (2022). Molecular mechanisms underlying plasticity in a thermally varying environment. *Molecular Ecology*, 31(11), 3174–3191.
<https://doi.org/10.1111/mec.16463>
- Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science*, 3, 509–535. <https://doi.org/10.1146/annurev-marine-120709-142756>
- Sasaki, M., Barley, J. M., Gignoux-Wolfsohn, S., Hays, C. G., Kelly, M. W., Putnam, A. B., Sheth, S. N., Villeneuve, A. R., & Cheng, B. S. (2022). Greater evolutionary divergence of thermal limits within marine than terrestrial species. *Nature Climate Change*, 12(12), Article 12. <https://doi.org/10.1038/s41558-022-01534-y>
- Sasaki, M. C., & Dam, H. G. (2020). Genetic differentiation underlies seasonal variation in thermal tolerance, body size, and plasticity in a short-lived copepod. *Ecology and Evolution*, 10(21), 12200–12210. <https://doi.org/10.1002/ece3.6851>
- Sasaki, M. C., & Dam, H. G. (2021). Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. *Evolutionary Applications*, 14(8), 2114–2123.
<https://doi.org/10.1111/eva.13270>
- Schaum, C.-E., Buckling, A., Smirnoff, N., & Yvon-Durocher, G. (2022). Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proc. Biol. Sci.*, 289(1980), 20220834.

- Scheiner, S. M. (1993). Genetics and Evolution of Phenotypic Plasticity. *Annual Review of Ecology and Systematics*, 24(1), 35–68.
<https://doi.org/10.1146/annurev.es.24.110193.000343>
- Scheiner, S. M., & Berrigan, D. (1998). The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. *Evolution*, 52(2), 368–378.
- Scheiner, S. M., & Lyman, R. F. (1991). The genetics of phenotypic plasticity. II. Response to selection. *Journal of Evolutionary Biology*, 4(1), 23–50.
<https://doi.org/10.1046/j.1420-9101.1991.4010023.x>
- Schilthuizen, M., & Kellermann, V. (2014). Contemporary climate change and terrestrial invertebrates: Evolutionary versus plastic changes. *Evolutionary Applications*, 7(1), 56–67. <https://doi.org/10.1111/eva.12116>
- Schneider, H. M. (2022). Characterization, costs, cues and future perspectives of phenotypic plasticity. *Annals of Botany*, 130(2), 131–148.
<https://doi.org/10.1093/aob/mcac087>
- Schou, M. F., Mouridsen, M. B., Sørensen, J. G., & Loeschcke, V. (2017). Linear reaction norms of thermal limits in *Drosophila*: Predictable plasticity in cold but not in heat tolerance. *Functional Ecology*, 31(4), 934–945.
<https://doi.org/10.1111/1365-2435.12782>
- Seabra, R., Wetthey, D. S., Santos, A. M., & Lima, F. P. (2011). Side matters: Microhabitat influence on intertidal heat stress over a large geographical scale. *Journal of Experimental Marine Biology and Ecology*, 400(1), 200–208.
<https://doi.org/10.1016/j.jembe.2011.02.010>

- Sgrò, C. M., Overgaard, J., Kristensen, T. N., Mitchell, K. A., Cockerell, F. E., & Hoffmann, A. A. (2010). A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *Journal of Evolutionary Biology*, *23*(11), 2484–2493. <https://doi.org/10.1111/j.1420-9101.2010.02110.x>
- Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What Can Plasticity Contribute to Insect Responses to Climate Change? *Annual Review of Entomology*, *61*(1), 433–451. <https://doi.org/10.1146/annurev-ento-010715-023859>
- Siegal, M. L., & Bergman, A. (2002). Waddington's canalization revisited: Developmental stability and evolution. *Proceedings of the National Academy of Sciences*, *99*(16), 10528–10532. <https://doi.org/10.1073/pnas.102303999>
- Siegle, M. R., Taylor, E. B., & O'Connor, M. I. (2018). Prior heat accumulation reduces survival during subsequent experimental heat waves. *Journal of Experimental Marine Biology and Ecology*, *501*, 109–117. <https://doi.org/10.1016/j.jembe.2018.01.012>
- Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Overview. *The Quarterly Review of Biology*, *79*(3), 241–277. <https://doi.org/10.1086/422893>
- Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*, *225*(4), 1447–1454. <https://doi.org/10.1111/nph.16107>

- Smith, K. W., & Sasaki, M. S. (1979). Decreasing Multicollinearity: A Method for Models with Multiplicative Functions. *Sociological Methods and Research*, 8, 35–56.
- Sodergren, E., Weinstock, G. M., Davidson, E. H., Cameron, R. A., Gibbs, R. A., Angerer, R. C., Angerer, L. M., Arnone, M. I., Burgess, D. R., Burke, R. D., Coffman, J. A., Dean, M., Elphick, M. R., Ettensohn, C. A., Foltz, K. R., Hamdoun, A., Hynes, R. O., Klein, W. H., Marzluff, W., ... Wright, R. (2006). The Genome of the Sea Urchin *Strongylocentrotus purpuratus*. *Science*, 314(5801), 941–952. <https://doi.org/10.1126/science.1133609>
- Somero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers.’ *Journal of Experimental Biology*, 213(6), 912–920. <https://doi.org/10.1242/jeb.037473>
- Sommer, R. J. (2020). Phenotypic Plasticity: From Theory and Genetics to Current and Future Challenges. *Genetics*, 215(1), 1–13. <https://doi.org/10.1534/genetics.120.303163>
- Stamp, M. A., & Hadfield, J. D. (2020). The relative importance of plasticity versus genetic differentiation in explaining between population differences; a meta-analysis. *Ecology Letters*, 23(10), 1432–1441. <https://doi.org/10.1111/ele.13565>
- Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. *BioScience*, 39(7), 436–445. <https://doi.org/10.2307/1311135>
- Stevens, D. R., Wund, M. A., & Mathis, K. A. (2023). Integrating environmental complexity and the plasticity-first hypothesis to study responses to human-altered

- habitats. *Animal Behaviour*, 200, 273–284.
<https://doi.org/10.1016/j.anbehav.2022.12.005>
- Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, 301(5629), 65–65. <https://doi.org/10.1126/science.1083073>
- Strader, M. E., Kozal, L. C., Leach, T. S., Wong, J. M., Chamorro, J. D., Housh, M. J., & Hofmann, G. E. (2020). Examining the role of DNA methylation in transcriptomic plasticity of early stage sea urchins: Developmental and maternal effects in a kelp forest herbivore. In *Frontiers in Marine Science* (Vol. 7, p. 205). <https://doi.org/10.3389/fmars.2020.00205>
- Strader, M. E., Wolak, M. E., Simon, O. M., & Hofmann, G. E. (2022). Genetic variation underlies plastic responses to global change drivers in the purple sea urchin, *Strongylocentrotus purpuratus*. *Proceedings. Biological Sciences*, 289(1981), 20221249. <https://doi.org/10.1098/rspb.2022.1249>
- Strader, M. E., Wong, J. M., Kozal, L. C., Leach, T. S., & Hofmann, G. E. (2019). Parental environments alter DNA methylation in offspring of the purple sea urchin, *Strongylocentrotus purpuratus*. *J. Exp. Mar. Bio. Ecol.*, 517, 54–64.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*. <https://doi.org/10.1098/rspb.2010.1295>
- Svensson, E. I., Gomez-Llano, M., & Waller, J. T. (2020). Selection on phenotypic plasticity favors thermal canalization. *Proceedings of the National Academy of Sciences of the United States of America*, 117(47), 29767–29774.
<https://doi.org/10.1073/pnas.2012454117>

- Swaegers, J., De Cupere, S., Gaens, N., Lancaster, L. T., Carbonell, J. A., Sánchez Guillén, R. A., & Stoks, R. (2023). Plasticity and associated epigenetic mechanisms play a role in thermal evolution during range expansion. *Evolution Letters*, qrac007. <https://doi.org/10.1093/evlett/qrac007>
- Terblanche, J. S., & Hoffmann, A. A. (2020). Validating measurements of acclimation for climate change adaptation. *Current Opinion in Insect Science*, 41, 7–16. <https://doi.org/10.1016/j.cois.2020.04.005>
- Thorstad, E. B., Bliss, D., Breau, C., Damon-Randall, K., Sundt-Hansen, L. E., Hatfield, E. M. C., Horsburgh, G., Hansen, H., Maoiléidigh, N. Ó., Sheehan, T., & Sutton, S. G. (2021). Atlantic salmon in a rapidly changing environment—Facing the challenges of reduced marine survival and climate change. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(9), 2654–2665. <https://doi.org/10.1002/aqc.3624>
- Tingley, D., Yamamoto, T., Hirose, K., Keele, L., & Imai, K. (2014). mediation: R package for causal mediation analysis. *UCLA Statistics/American Statistical Association*. <https://dspace.mit.edu/handle/1721.1/91154>
- Truchot, J.-P., & Duhamel-Jouve, A. (1980). Oxygen and carbon dioxide in the marine intertidal environment: Diurnal and tidal changes in rockpools. *Respiration Physiology*, 39(3), 241–254. [https://doi.org/10.1016/0034-5687\(80\)90056-0](https://doi.org/10.1016/0034-5687(80)90056-0)
- Tüzün, N., & Stoks, R. (2018). Evolution of geographic variation in thermal performance curves in the face of climate change and implications for biotic interactions. *Current Opinion in Insect Science*, 29, 78–84. <https://doi.org/10.1016/j.cois.2018.07.004>

- Urban, M. C., Strauss, S. Y., Pelletier, F., Palkovacs, E. P., Leibold, M. A., Hendry, A. P., De Meester, L., Carlson, S. M., Angert, A. L., & Giery, S. T. (2020). Evolutionary origins for ecological patterns in space. *Proceedings of the National Academy of Sciences*, *117*(30), 17482–17490. <https://doi.org/10.1073/pnas.1918960117>
- Ushakov, B. P. (1977). The environmental temperature and physiological polymorphism of populations. 4. The effect of heat acclimation on the intensity and the genetic effectiveness of selection caused by heating. *Journal of Thermal Biology*, *2*(4), 177–182. [https://doi.org/10.1016/0306-4565\(77\)90027-4](https://doi.org/10.1016/0306-4565(77)90027-4)
- Usui, T., Lerner, D., Eckert, I., Angert, A. L., Garroway, C. J., Hargreaves, A., Lancaster, L. T., Lessard, J.-P., Riva, F., Schmidt, C., van der Burg, K., & Marshall, K. E. (2023). The evolution of plasticity at geographic range edges. *Trends in Ecology & Evolution*, *38*(9), 831–842. <https://doi.org/10.1016/j.tree.2023.04.004>
- Valdés, A., Marteinsdóttir, B., & Ehrlén, J. (2019). A natural heating experiment: Phenotypic and genotypic responses of plant phenology to geothermal soil warming. *Global Change Biology*, *25*(3), 954–962. <https://doi.org/10.1111/gcb.14525>
- Valladares, F., Gianoli, E., & Gómez, J. M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist*, *176*(4), 749–763. <https://doi.org/10.1111/j.1469-8137.2007.02275.x>
- Van ASCH, M., Van TIENDEREN, P. H., Holleman, L. J. M., & Visser, M. E. (2007). Predicting adaptation of phenology in response to climate change, an insect

- herbivore example. *Global Change Biology*, 13(8), 1596–1604.
<https://doi.org/10.1111/j.1365-2486.2007.01400.x>
- Van Buskirk, J., & Steiner, U. K. (2009). The fitness costs of developmental canalization and plasticity. *Journal of Evolutionary Biology*, 22(4), 852–860.
<https://doi.org/10.1111/j.1420-9101.2009.01685.x>
- van Heerwaarden, B., & Kellermann, V. (2020). Does plasticity trade off with basal heat tolerance? *Trends in Ecology & Evolution*, 35(10), 874–885.
<https://doi.org/10.1016/j.tree.2020.05.006>
- van Heerwaarden, B., Kellermann, V., & Sgrò, C. M. (2016). Limited scope for plasticity to increase upper thermal limits. *Functional Ecology*, 30(12), 1947–1956.
<https://doi.org/10.1111/1365-2435.12687>
- van Kleunen, M., & Fischer, M. (2005). Constraints on the evolution of adaptive phenotypic plasticity in plants. *The New Phytologist*, 166(1), 49–60.
<https://doi.org/10.1111/j.1469-8137.2004.01296.x>
- van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, 128(1), 137–142. <https://www.jstor.org/stable/2461293>
- van Oppen, M. J. H., Oliver, J. K., Putnam, H. M., & Gates, R. D. (2015). Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, 112(8), 2307–2313. <https://doi.org/10.1073/pnas.1422301112>
- Velotta, J. P., Ivy, C. M., Wolf, C. J., Scott, G. R., & Cheviron, Z. A. (2018). Maladaptive phenotypic plasticity in cardiac muscle growth is suppressed in high-

- altitude deer mice. *Evolution; International Journal of Organic Evolution*, 72(12), 2712–2727. <https://doi.org/10.1111/evo.13626>
- Verma, A., & Ritchie, M. D. (2017). Current Scope and Challenges in Phenome-Wide Association Studies. *Current Epidemiology Reports*, 4(4), 321–329. <https://doi.org/10.1007/s40471-017-0127-7>
- Via, S., Gomulkiewicz, R., Jong, G. D., Scheiner, S. M., Schlichting, C. D., & Tienderen, P. H. V. (1995). Adaptive phenotypic plasticity: Consensus and controversy. *Trends in Ecology & Evolution*, 10(5), 212–217. [https://doi.org/10.1016/S0169-5347\(00\)89061-8](https://doi.org/10.1016/S0169-5347(00)89061-8)
- Villeneuve, A. R., Komoroske, L. M., & Cheng, B. S. (2021). Environment and phenology shape local adaptation in thermal performance. *Proceedings of the Royal Society B: Biological Sciences*, 288(1955), 20210741. <https://doi.org/10.1098/rspb.2021.0741>
- Vinton, A. C., Gascoigne, S. J. L., Sepil, I., & Salguero-Gómez, R. (2022). Plasticity's role in adaptive evolution depends on environmental change components. *Trends in Ecology & Evolution*, 37(12), 1067–1078. <https://doi.org/10.1016/j.tree.2022.08.008>
- Volaric, M. P., Berg, P., & Reidenbach, M. A. (2018). Oxygen metabolism of intertidal oyster reefs measured by aquatic eddy covariance. *Marine Ecology Progress Series*, 599, 75–91. <https://doi.org/10.3354/meps12627>
- Voordouw, M. J., Robinson, H. E., Stebbins, G., Albert, A. Y. K., & Anholt, B. R. (2005). Larval density and the Charnov–Bull model of adaptive environmental

- sex determination in a copepod. *Canadian Journal of Zoology*, 83(7), 943–954.
<https://doi.org/10.1139/z05-088>
- Waddington, C. H. (1957). *The strategy of genes*. George Allen & Unwin, Ltd.
- Waddington, C. H. (1977). *The Evolution of an Evolutionist*.
<https://philpapers.org/rec/WADTEO-3>
- Walter, G. M., Clark, J., Terranova, D., Cozzolino, S., Cristaudo, A., Hiscock, S. J., & Bridle, J. (2023). Hidden genetic variation in plasticity provides the potential for rapid adaptation to novel environments. *New Phytologist*, 239(1), 374–387.
<https://doi.org/10.1111/nph.18744>
- Warner, D. A., & Shine, R. (2005). The Adaptive Significance of Temperature-Dependent Sex Determination: Experimental Tests with a Short-Lived Lizard. *Evolution*, 59(10), 2209–2221. <https://doi.org/10.1111/j.0014-3820.2005.tb00929.x>
- West-Eberhard, M. J. (1989). Phenotypic Plasticity and the Origins of Diversity. *Annual Review of Ecology and Systematics*, 20(1), 249–278.
<https://doi.org/10.1146/annurev.es.20.110189.001341>
- West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press.
- Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, 11(1), 398–411. <https://doi.org/10.2307/2406060>
- Wogan, G. O. U., & Wang, I. J. (2018). The value of space-for-time substitution for studying fine-scale microevolutionary processes. *Ecography*, 41(9), 1456–1468.
<https://doi.org/10.1111/ecog.03235>

- Wolak, M. E. (2012). *nadiv*: An R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods in Ecology and Evolution*, 3(5), 792–796. <https://doi.org/10.1111/j.2041-210X.2012.00213.x>
- Wong, J. M., Johnson, K. M., Kelly, M. W., & Hofmann, G. E. (2018). Transcriptomics reveal transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling conditions alters the response of their progeny to differential pCO₂ levels. *Mol. Ecol.*, 27(5), 1120–1137.
- Wong, J. M., Kozal, L. C., Leach, T. S., Hoshijima, U., & Hofmann, G. E. (2019). Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling conditions alters maternal provisioning and progeny phenotype. *J. Exp. Mar. Bio. Ecol.*, 517, 65–77.
- Wright, R. M., Aglyamova, G. V., Meyer, E., & Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. *BMC Genomics*, 16, 371.
- Xiu, P., Chai, F., Curchitser, E. N., & Castruccio, F. S. (2018). Future changes in coastal upwelling ecosystems with global warming: The case of the California Current System. *Scientific Reports*, 8(1), Article 1. <https://doi.org/10.1038/s41598-018-21247-7>
- Zaytsev, O., Cervantes-Duarte, R., Montante, O., & Gallegos-Garcia, A. (2003). Coastal Upwelling Activity on the Pacific Shelf of the Baja California Peninsula. *Journal of Oceanography*, 59(4), 489–502. <https://doi.org/10.1023/A:1025544700632>
- Ziyatdinov, A., Vázquez-Santiago, M., Brunel, H., Martínez-Pérez, A., Aschard, H., & Soria, J. M. (2018). *lme4qtl*: Linear mixed models with flexible covariance

structure for genetic studies of related individuals. *BMC Bioinformatics*, 19(1), 68. <https://doi.org/10.1186/s12859-018-2057-x>

zu Ermgassen, P. S. E., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. (2013).

Quantifying the loss of a marine ecosystem service: Filtration by the eastern oyster in US estuaries. *Estuaries and Coasts*, 36(1), 36–43.

<https://doi.org/10.1007/s12237-012-9559-y>

Appendix

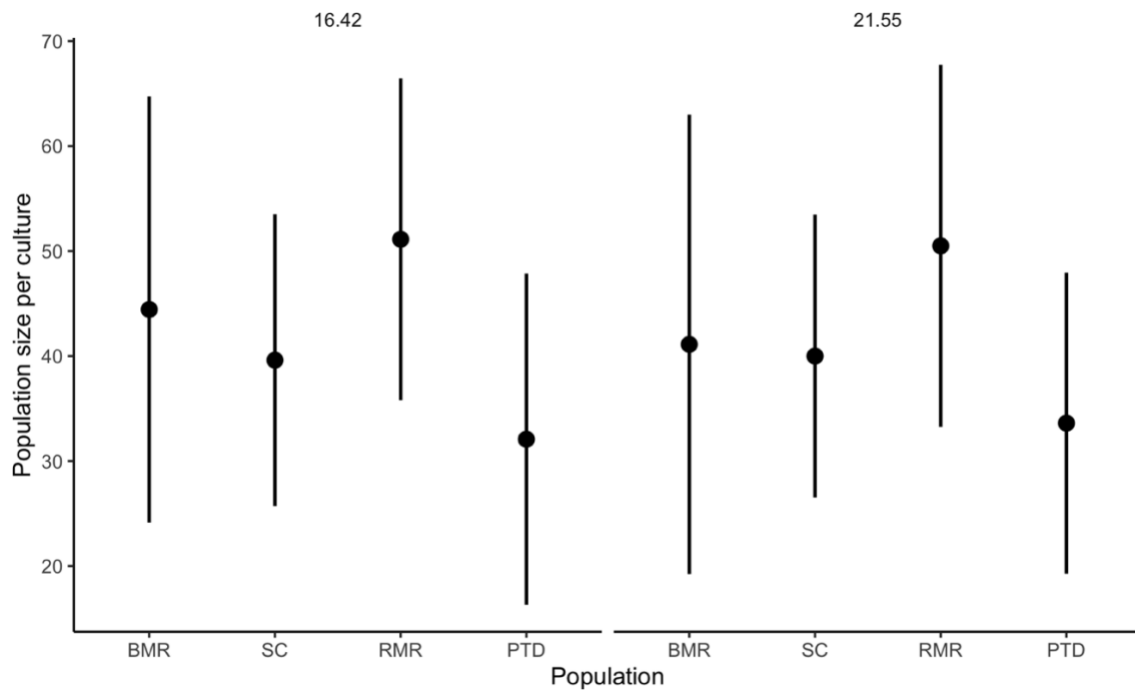


Figure S1 | *Mean density of Tigriopus californicus per culture.* Error bars depict standard deviation. The left and right columns denote low (16.55) and high (21.55) developmental temperatures.

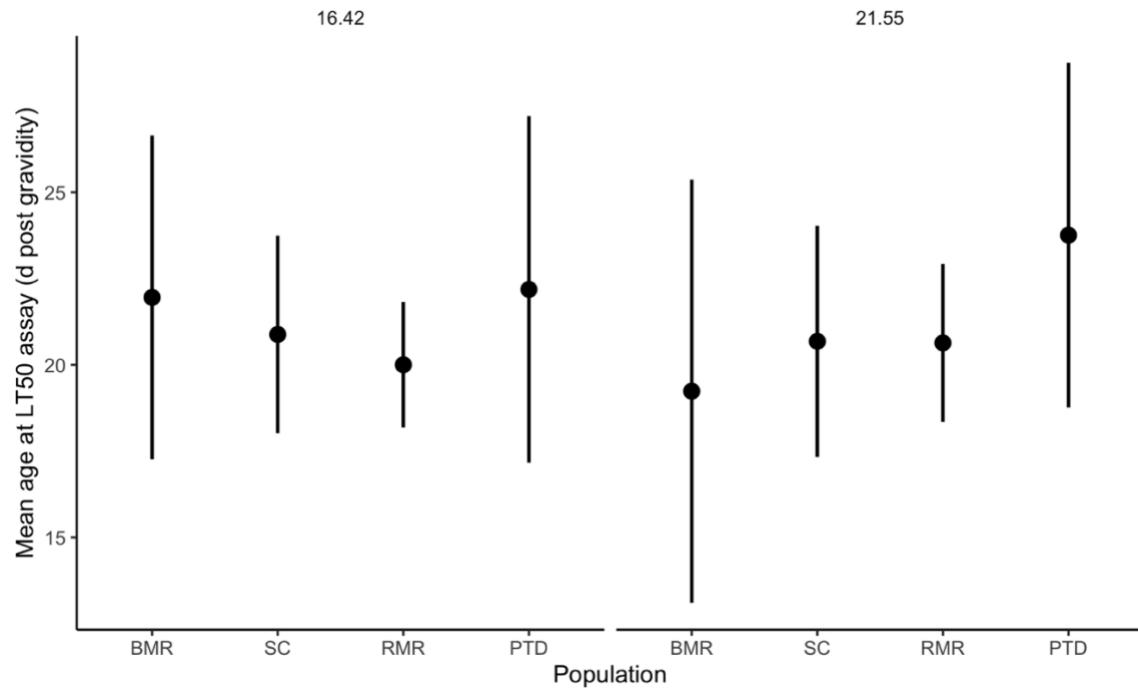


Figure S2 | *Mean culture ages at time of LT_{50} assay.* Error bars depict standard deviation. The left and right columns denote low (16.55) and high (21.55) developmental temperatures.

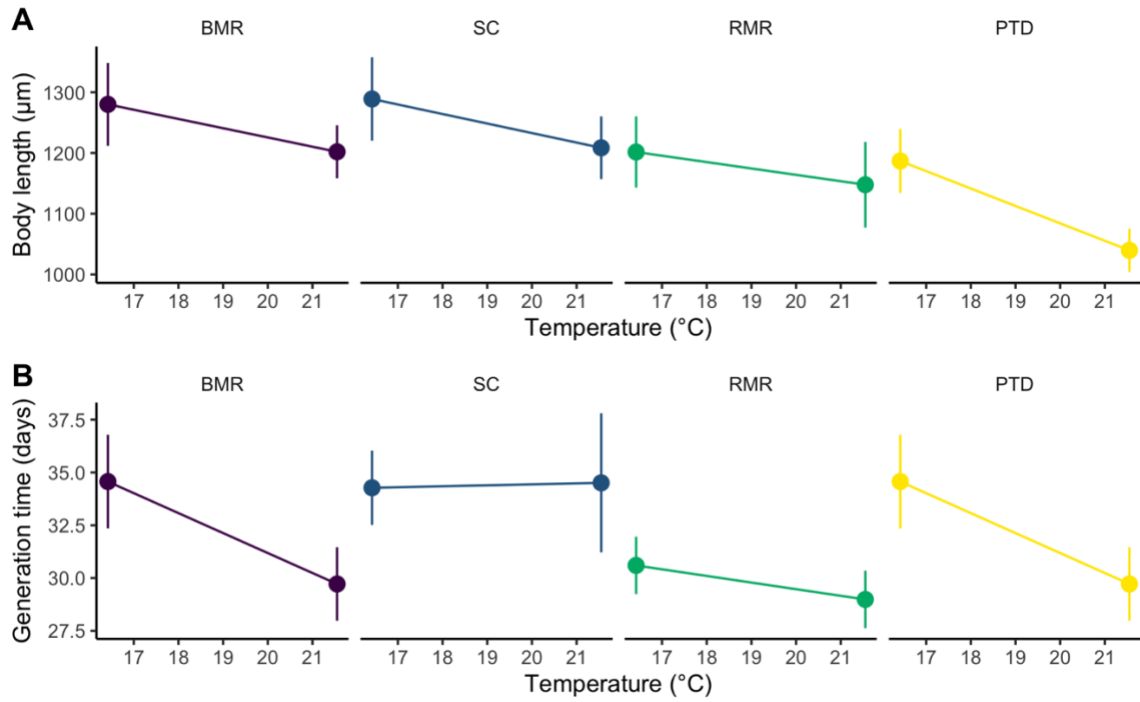


Figure S3 | *Thermal plasticity of generation time and body size.* Points represent means and error bars depict standard error of the mean. (A) Total body length is plotted for females used in selection gradient model predicting fecundity. (B) Generation time was measured per population as the number of days by which 50% of a population exhibited gravidity within cultures following hatching and initiation.

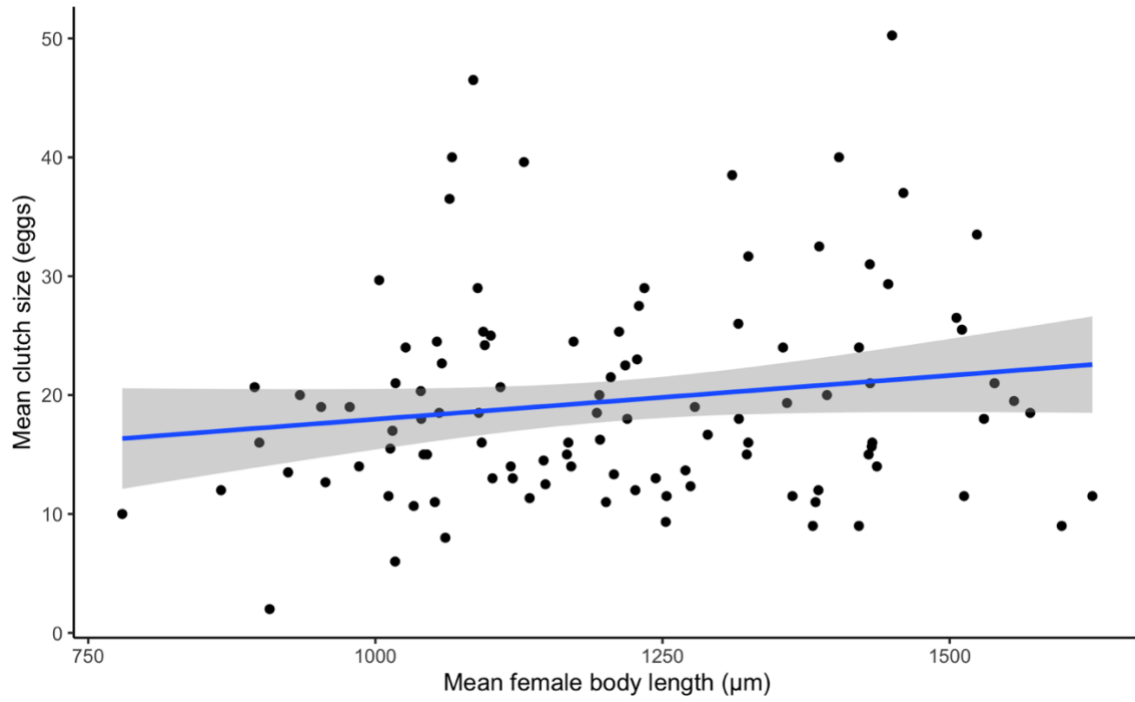


Figure S4 | *Weak but positive effect of mean female body size on fecundity per brood.* Each points represents a single sibship x temperature culture replicate. Mean female body length is measured from gravid females for which egg sack dissections were conducted in order to measure mean clutch size.

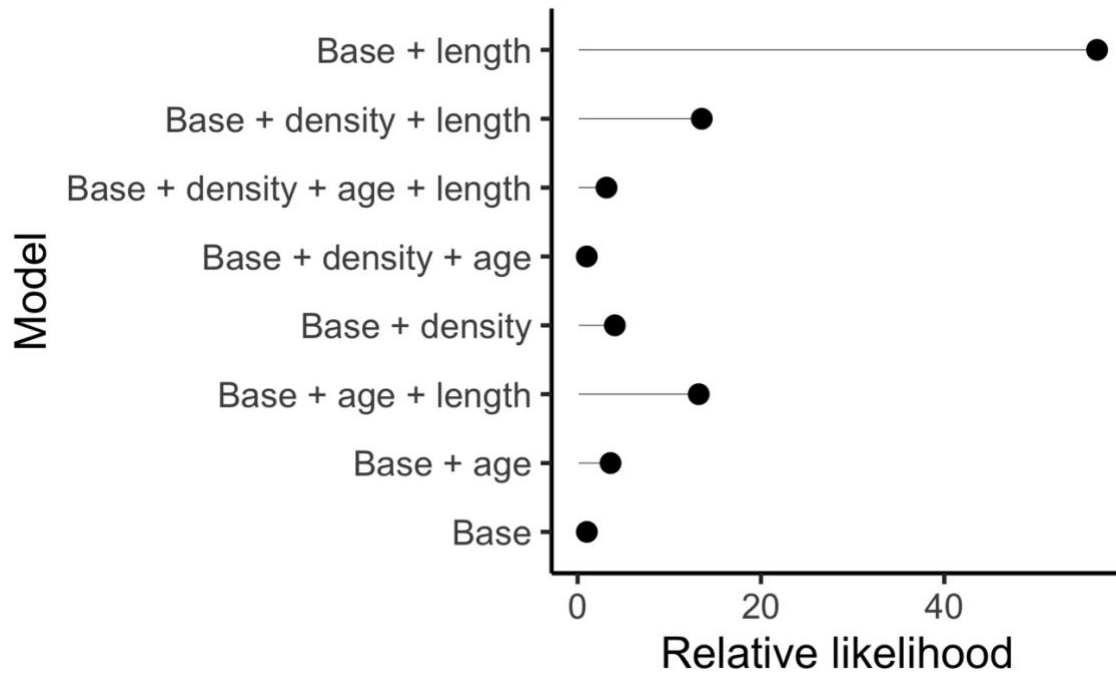


Figure S5 | Selection of multivariate selection gradient models with and without parameters associated with mean culture age and density. ‘Base’ refers to the selection gradient model reported in the main body of the manuscript whose specifications are described in detail under Methods. ‘Base +...’ refers to iterations of this model that contained additional parameters for either (i) mean culture age measured in days post-gravidity or (ii) mean culture density.