MONASH University

Evolutionary consequences of fertilisation and early development in warming seas

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BSc (Hons)

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Table of contents

Thesis Abstract

Natural populations are experiencing stressful changes to their environments due to global warming, but their capacity to respond remains unclear. Evolutionary adaptation may allow populations to recover and maintain fitness (i.e., survival and reproduction) under the environmental changes experienced within their native ranges over extended periods of time (i.e., several generations). To predict the capacity for adaptation to new environmental conditions, we need to understand whether populations have the genetic variation in components of fitness that is required for adaptation, and which biologically-important traits related to fitness will be involved in adaptation. Currently, our understanding of such factors has been largely skewed towards terrestrial populations, while similar insights for marine populations remain relatively scarce. Yet, marine populations are expected to be particularly susceptible to projected warming during life stages involved in fertilisation and early development, which may act as bottlenecks for population persistence. The overarching aim of my thesis was to improve our understanding of how these life stages may adapt to projected ocean warming. Focusing on natural populations of marine invertebrates, I undertook five empirical studies that I present in two distinct halves. In the first half of my thesis (Chapters 2 to 4), I use quantitative genetic approaches to examine the adaptive evolutionary potential of survival during early life stages under projected levels of warming. In particular, I focus on the underappreciated impacts of exposure to warming during preceding life stages, or generations, on adaptive potential. My findings show that the environment experienced by parents, or by gametes at fertilisation, can impact not only the susceptibility of early offspring stages to projected warming but also the genetic variation needed to adapt to it. In the second half of my thesis (Chapters 5 and 6), I measure how selection acts on traits involved in fertilisation and early development, and how it promotes the adaptive evolution of such traits under projected warming. In doing so, I provide a rare disentanglement of how selection acts on size and development time at key early life stages, and show the first empirical example of how selection on gamete traits may be modified by projected warming. Through these five studies, my thesis presents novel insights into how marine populations will adapt under ocean warming, and offers new ideas that may help guide conservation strategies for natural populations more broadly.

Publications during enrolment

Chirgwin, E., Marshall, D.J., Sgrò, C.M., and Monro, K. 2018. How does parental environment influence the potential for adaptation to global change? Proceedings of the Royal Society B: Biological Sciences **285**.

Chirgwin, E., Marshall, D.J., Sgrò, C.M., and Monro, K. 2017. The other 96%: Can neglected sources of fitness variation offer new insights into adaptation to global change? Evolutionary Applications **10**: 267-275.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. This thesis is contains two peer-reviewed and published papers (Chapters 2 and 3), and three manuscripts yet to be submitted for publication (Chapter 4, 5, and 6). The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Dr Keyne Monro, and Profs Dustin Marshall and Carla Sgrò. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers. In Chapters 2, 3, 4, 5, and 6 my contribution to the work involved the following:

Thesis Chapter	Publication title	Status	Nature and % of student contribution	Co-author names(s) and % of co-author's contribution	Co-Author(s), Monash student Y/N
2	*The other 96%: Can neglected sources of fitness variation offer new insights into adaptation to global change?*	Published	73%. Concept, design, data collection, data analysis, writing of manuscript.	20%. K. Monro, concept, analyses, edits to manuscript 5%. D.J. Marshall, concept, design, edits to manuscript 2%. C. M. Sgrò, concept, design, edits to manuscript.	No
3	*How does parental environment influence potential for adaptation to global change?*	Published	73%. Concept, design, data collection, data analysis, writing of manuscript.	20%. K. Monro, concept, analyses, design, edits to manuscript 5%. D.J. Marshall, concept, design, edits to manuscript 2%. C. M. Sgrò, concept, edits to manuscript.	No
4	*Fertilisation under projected ocean warming decreases adaptive potential in an external fertiliser*	Not submitted	80%. Concept, design, data collection, data analysis, writing of manuscript.	20%. K. Monro, concept, design, analyses, edits to manuscript	No

5	*Development time is targeted by selection, independent of initial size but not subsequent size*	Not submitted	75%. Concept, design, data collection, data analysis, writing of manuscript.	20%. K. Monro, concept, analyses, design, edits to manuscript 5%. D.J. Marshall, concept, edits to manuscript	No
6	*Dual physical and physiological impacts of ocean warming alter phenotypic selection on sperm morphology*	Not submitted	75%. Concept, design, data collection, data analysis, writing of manuscript.	20% K. Monro, concept, analyses, design, edits to manuscript 5% D.J. Marshall, concept, design, edits to manuscript	No

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Evatt Chirgwin

Student signature: [redacted] **Date: 12/06/2019**

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Keyne Monro

Main Supervisor signature: [redacted] **Date: 12/06/2019**

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Note on the thesis

This thesis is comprised of a general introduction (Chapter 1), five data-based chapters (Chapters 2 to 6), and a general discussion (Chapter 7). Note that the data set analysed in Chapter 2 comes from an experiment partly published in Chirgwin et al. (2015), but the two pieces of work had distinctly different research questions and Chapter 2 focuses on different experimental results that were not analysed or reported in Chirgwin et al. (2015). The inclusion of Chapter 2 as part of this thesis was supported by my PhD advisory panel on the basis that it was entirely conceptualised, analysed, and written up during my PhD candidature. Also note that I was responsible for the planning, experimental design, data collection, analysis, and manuscript preparation for each chapter, but first-person plural is used in Chapters 2 to 6 to reflect the collaborative nature of the research.

Chapter 1

General introduction

Responding to warming seas

Natural populations are experiencing rapid and stressful changes to their environments because of anthropogenic warming (Scheffers et al. 2016). Populations can move to escape the stress, use phenotypic plasticity to buffer against the stress, or evolve to adapt to the stress (Hoffmann and Sgrò 2011). Yet change is happening so fast, and is so widespread, that many populations cannot escape or cope *in situ* using plasticity alone (Gienapp et al. 2008). As such, their long-term persistence will often depend on their potential to undergo adaptive evolution. Understanding the processes that determine how populations will adaptively evolve in response to projected warming can enhance our ability to forecast biodiversity loss and direct conservation strategies (Chevin et al. 2013, Pujol et al. 2018).

Terrestrial biologists have a rich tradition of studying the adaptive evolution of populations, but similar traditions are far weaker amongst marine biologists (Munday et al. 2013). While research attention has increased recent years (e.g, Kelly et al. 2013, Chirgwin et al. 2015, Dixon et al. 2015, Foo and Byrne 2016, Marshall et al. 2016, Munday et al. 2017), our ability to predict the adaptive evolution of marine populations in response to future warming lags far behind that of terrestrial populations (Munday et al. 2013). Marine populations will regularly need to adapt in response to projected warming because several elements of their demography and life history make them particularly vulnerable (Foo and Byrne 2016, Donelson et al. 2019, Pinsky et al. 2019). Perhaps most importantly, unlike terrestrial species where fertilisation and early (i.e., embryonic) development generally occurs within the female reproductive tract, most marine species (e.g., most fish and marine invertebrates) spawn their gametes into the water column, where fertilisation and early development are directly exposed to stressors in the external environment (Strathmann 1990, Byrne 2011, Monro and Marshall 2015). Crucially, fertilisation and particularly early development are often the most susceptible stages in the life cycle to environmental stress, and are therefore anticipated to regularly be 'weak links' in the capacity of marine populations to persist under future warming (Byrne 2011, Pandori and Sorte 2019).

The overarching aim of my thesis was to improve our understanding of how the life stages involved in fertilisation and early development will adaptively evolve under projected warming. To do so, I carried out five empirical studies that used natural populations of the tubeworm *Galeolaria caespitosa* or the ascidian *Pyura dalbyi* (henceforth referred to by genus name), which were chosen because of their ecological importance, and because their lifecycles — a motile larval stage and a sessile adult stage that reproduces via external fertilisation — are characteristic of marine invertebrates (Strathmann 1990, Monro and Marshall 2015). I present my thesis as two distinct halves. For the first half (Chapters 2, 3, and 4), I examine the genetic basis of survival, during early developmental stages, under a range of experimental conditions that allow me to predict the potential for adaptive evolutionary responses to projected warming. In each of these studies, I use a quantitative genetic breeding design to estimate the genetic basis of survival based on the resemblance among relatives, as components of fitness (i.e., survival and reproduction) are quantitative traits determined by many genes whose individual effects are difficult to identify (Lynch and Walsh 1998, Charmantier et al. 2014, Shaw 2018). For the second half of my thesis (Chapters 5 and 6), I consider how selection acts on traits involved in fertilisation and early development, and how novel selection pressures will drive traits to adaptively evolve under projected warming.

Predicting the potential for adaptive evolution to projected conditions

For any population, the potential for adaptive evolution requires individuals that vary in fitness, and that this variation has a genetic basis (Arnold and Wade 1984). More specifically, the maximum rate of adaptive evolution is determined by the additive genetic variation in fitness (Fisher 1930), which accounts for the proportion of fitness variation that is consistently inherited from one generation to the next (Price 1972). However, variation in fitness is also the product of nonadditive genetic effects due to allele interactions within loci (dominance) and among loci (epistasis), as well as several sources of environmental variation (e.g., parental effects on offspring outside of gene transmission; Lynch and Walsh 1998).

Fitness, along with the genetic and environmental factors that underpin it, are often environmentally dependant (Via and Lande 1985, Rowinski and Rogell 2017). Consequently, the adaptive potential of populations under projected warming is dependent on the genetic variation expressed in fitness under these conditions. Fitness can also be genetically correlated across multiple environments, whereby the same alleles underpin how fitness is expressed under different environmental conditions (e.g., current temperatures and projected warming; Chirgwin et al 2015). Such genetic correlations will constrain adaptation if alleles have antagonistic effects on fitness in different environments, or speed up adaptation if alleles are beneficial across multiple environments (Lande 1979). In turn, an increasing number of studies are providing valuable estimates of additive genetic variation and across-environment correlations in key traits (i.e., those linked to fitness) under projected levels of warming (e.g., Foo et al. 2012, Kelly et al. 2013, Lymbery and Evans 2013, Blackburn et al. 2014, Chirgwin et al. 2015, Kristensen et al. 2015, Munoz et al. 2015, Martins et al. 2019). Conversely, nonadditive genetic and parental environmental effects on traits or fitness have rarely been estimated for natural populations, and rarer still are estimates of these effects under projected levels of warming (Hendry 2013, Rudin-Bitterli et al. 2018, Vega-Trejo et al. 2018). Furthermore, past studies have usually examined how experiencing projected warming during a single generation impacts the genetic variation in traits, whilst largely ignoring if the environment experienced during preceding generations or fertilisation (which links one generation to the next) carryover to impact genetic variation in traits (Donelson et al. 2018).

In Chapter 2, I examine how nonadditive genetic and maternal environmental effects influence survival during early development under current-day conditions and projected levels of warming. While the contribution of nonadditive genetic and maternal environmental effects to fitness in natural populations is poorly understood, theory and data argue that they can have important impacts on the evolutionary trajectories of populations and the amount of gene flow between populations (Kirkpatrick and Lande 1989, Wang et al. 1998, Wade 2002, Rasanen and Kruuk 2007, Hendry 2013, Dey et al. 2016). In this chapter, I use *Galeolaria* in a quantitative genetic breeding design to estimate nonadditive genetic and maternal environmental effects on survival during early development stages

across three temperature environments. I find that both effects, but nonadditive genetic effects in particular, had substantial and temperature-dependent influences on survival. Moreover, positive nonadditive genetic correlation between thermal environments, indicate that parental combinations that improve survival at one temperature also improve survival at elevated temperatures. Therefore, while the role of nonadditive genetic effects in adaptive evolution has largely been neglected, my findings suggest they merit greater research attention in a warming world.

In Chapter 3, I test how parental exposure to projected warming impacts the survival, and genetic variation for survival, of offspring during early developmental stages under current and projected temperatures. An individual's fitness often depends not only the environment they experience during their lifetime, but also the environment experienced by their parents (Agrawal et al. 1999, Uller et al. 2013, Donelson et al. 2018). However, whether or not genetic variation in fitness also depends on parental environment has received little empirical examination (Munday et al. 2017, Donelson et al. 2018). Indeed, if parental environments impact how genetic variation is expressed in the subsequent offspring generation, not accounting for these impacts could lead us to misjudge the adaptive potential of populations. To address this gap in knowledge, I exposed a parental generation of *Galeolaria* to either current conditions or projected ocean warming before crossing them in a quantitative genetic breeding design and measuring the survival of their offspring under current conditions and projected warming. My results suggest parental environment has more extensive impacts on adaptive potential than presently appreciated, not only reducing the susceptibility of offspring to projected warming but also reshaping the genetic variation needed for adaptive evolutionary responses to it.

In Chapter 4, I consider the missing link between parental and early offspring stages that I was unable to access in Chapter 3, by testing if genetic variation for early offspring survival also depends on the environmental conditions under which fertilisation takes place. Since fertilisation is necessary for the ongoing persistence of all sexual populations, understanding how the fertilisation environment impacts adaptive potential is a key step in predicting their vulnerability to projected warming (Walsh et al. 2019). Here, using *Galeolaria*, I combine a quantitative genetic breeding design with a factorial

manipulation of fertilisation and development temperatures under current conditions and projected warming. I show that the fertilisation under projected levels of warming impacts early offspring survival, and leads to major changes in genetic variation and across-environment genetic correlations in offspring survival under current and projected conditions. In doing so, my results highlight that accounting for fertilisation environment may be a key step in predicting the adaptive potential of sexual populations under future warming.

Measuring phenotypic selection under current day and projected conditions

Phenotypic selection acts when individuals with different phenotypes differ in components of fitness, and is the primary cause of adaptive evolution in natural populations (Lande and Arnold 1983, Kingsolver and Pfennig 2007). Projected warming is expected to alter existing selection pressures, impose new ones, acting to shape optimal phenotypes in natural populations (Chevin et al. 2010, Hoffmann and Sgrò 2011). For instance, in terrestrial systems, recent climatic changes have lead several populations to experience novel selection on the timing of seasonal activities such as mating, migration, or hibernation (Réale et al. 2003, Franks et al. 2007, Husby et al. 2011, Gienapp et al. 2014, Visser et al. 2015, Siepielski et al. 2017). Yet, in marine systems, we still have a weak grasp on how selection currently acts on most traits (including those involved in fertilisation and early development), let alone how selection on traits will be altered by projected warming.

In Chapter 5, I assess how selection targets the fundamental traits of body size and development time (the time between distinct life stages). Macroevolutionary patterns have led some to argue that variation in species' development times is the evolutionary by-product of allometric and physiological constraints arising from variation in size (Gillooly 2000, Gillooly et al. 2002). In contrast, life history theory predicts size and development time are both under strong selection, and should evolve adaptively within the constraints set by a trade-off in their effects on fitness (Stearns 1992, Dmitriew 2011). However, to test this prediction of life history theory requires empirical estimates that disentangle how selection directly and indirectly acts on size and development time, which has rarely been achieved by past studies. Using *Pyura*, I measure selection on size and development time at key

early developmental stages, owing to their effects on survival under field conditions. I find development time (from fertilisation to hatching) is not only a direct target of selection, but also shapes how selection acts upon on size before (i.e. embryo) after development (i.e. hatching). However, selection does not entirely explain the phenotypic relationship between size and development time, suggesting that it may act alongside allometric and physiological constraints to jointly direct the evolution of size and development time.

Finally, In Chapter 6, I provide the first test of how projected warming will alter selection on gametes. Male fertility relies on the ability of sperm to find and fuse with eggs, and consequently sperm traits that are involved in this vital step in reproduction are expected to be key targets of selection (Fitzpatrick et al. 2012, Monro and Marshall 2016, Levitan 2018). Projected warming in marine (and aquatic) environments will expose the sperm of external fertilising species to the physiological challenges of higher temperature and the physical challenges of lower viscosity (Kupriyanova and Havenhand 2005), but how either will influence selection on sperm traits is unknown. In the final study of my thesis, I created a suite of fertilisation environments to disentangle the physical effects of projected warming from the physiological, and use *Galeolaria* to measure how male fertility and selection, acting via fertility, on the sperm morphology differs between these fertilisation environments. My findings suggest that the physical and physiological impacts associated with projected warming each independently affect male fertility, and jointly alter how selection acts upon sperm morphology. In doing so, I highlight the neglected impacts that the physical effects of projected ocean warming will have alongside the physiological, and I provide new insights into how populations may adapt in response to these impacts.

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Chapter 2

The other 96%: can neglected sources of fitness variation offer new insights into adaptation to global change?

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Abstract

Mounting research considers whether populations may adapt to global change based on additive genetic variance in fitness. Yet selection acts on phenotypes, not additive genetic variance alone, meaning that persistence and evolutionary potential in the near-term, at least, may be influenced by other sources of fitness variation, including nonadditive genetic and maternal environmental effects. The fitness consequences of these effects, and their environmental sensitivity, are largely unknown. Here, applying a quantitative genetic breeding design to an ecologically-important marine tubeworm, we examined nonadditive genetic and maternal environmental effects on fitness (larval survival) across three thermal environments. We found that these effects are non-trivial and environment-dependent, explaining at least 44% of all parentally-derived effects on survival at any temperature, and 96% of parental effects at the most stressful temperature. Unlike maternal environmental effects, which manifested at the latter temperature only, nonadditive genetic effects were consistently significant and covaried positively across temperatures (i.e., parental combinations that enhanced survival at one temperature also enhanced survival at elevated temperatures). Thus, while nonadditive genetic and maternal environmental effects have long been neglected because their evolutionary consequences are complex, unpredictable, or seen as transient, we argue that they warrant further attention in a rapidly warming world.

Introduction

Anthropogenic global change is causing populations to encounter changes in selection above natural rates and scales (Davis et al. 2005, Merilä and Hendry 2014). Populations can evade extinction by one or a combination of three mechanisms: migration to more favorable habitats, phenotypic plasticity, or adaptive evolution (Holt 1990, Williams et al. 2008). The relative importance of each mechanism will vary among species according to their life histories and the time scale considered (Gienapp et al. 2008). For instance, migration is only feasible for species with an alternative habitat and sufficient dispersal capacity to reach it (Hughes 2000). Furthermore, phenotypic plasticity is predicted to be vital for populations enduring short-term fluctuations in selection, whereas long-term directional selection pressures are predicted to require phenotypic responses beyond the limits of plasticity alone (Munday et al. 2013, Reusch 2014). Given the limitations of migration and phenotypic plasticity, the ability of many populations to withstand the impacts of global change may largely depend on adaptive evolution.

The adaptive evolution of any natural population requires that individuals vary in fitness, and that this variation has a genetic basis. Phenotypic variation in fitness constrains the evolution of fitness itself and the intensity of selection that acts on any trait (Crow 1958, Arnold and Wade 1984). The genetic component of this variation in turn constrains the rate at which fitness increases from generation to generation: the greater the genetic variance in fitness, the faster the evolution of fitness and of traits that are correlated with it (Fisher 1930). For the most part, studies that have explored the capacity for populations to adapt to future scenarios of global change have focused on the additive genetic variance in fitness (Merilä and Hendry 2014), which predicts the phenotypic effects of alleles independently of their specific genetic background (Falconer and Mackay 1996). Thus, additive genetic effects account for the fraction of fitness variation that is known to be inherited stably from one generation to the next, forming the basis of evolutionary responses to selection. Individual phenotypes, however, are also the products of nonadditive genetic effects due to allele interactions within loci and between loci (i.e., dominance and epistasis, respectively), combined with sources of environmental variation (e.g.,

maternal influences on offspring beyond gene transmission) introduced in early development (Lynch and Walsh 1998).

Unlike additive genetic effects, nonadditive genetic effects on phenotype depend on genetic backgrounds that are continuously reshuffled by sex and recombination (Wolak and Keller 2014). Their lack of heritability in the usual sense has made their evolutionary role uncertain, despite considerable theoretical attention (e.g., Keightley 1996, Wade and Goodnight 1998, Barton and Turelli 2004), and underpinned Fisher's (1930) argument that they are irrelevant if populations are assumed to be infinitely large and randomly mating (Wade and Goodnight 1998). Natural populations, however, often violate these assumptions. In such cases, nonadditive genetic effects can have important effects on evolutionary processes, for example, by creating peaks and valleys on the adaptive landscapes that populations traverse (Wright 1931, Peck et al. 1998, Wade and Goodnight 1998), by contributing to inbreeding depression (Fenster et al. 1997), or by converting to additive genetic variance during population bottlenecks (Goodnight 1988, Wang et al. 1998, Cheverud et al. 1999). Nonadditive genetic effects may especially influence adaptive divergence in response to environmental change (Roff and Emerson 2006, Carroll 2007, Hendry 2013). For instance, a diverse range of dominance and epistatic effects were the basis of rapid divergence between soapberry-bug populations following a change in host plant (Carroll et al. 2001, Carroll et al. 2003; see other examples in Bernatchez et al. 2010, and Berner et al. 2011). Indeed, nonadditive genetic effects have been shown to contribute significantly to population differentiation in various aspects of life-history and morphology (Roff and Emerson 2006). In contrast, their contribution to traits of evolutionary interest, and fitness especially, within natural populations remains poorly understood (Sztepanacz and Blows 2015).

Similarly, the role of maternal environmental effects in evolutionary processes has often been overlooked due to the difficulty of estimating these effects reliably, or because they were traditionally viewed as little more than a nuisance source of variance. Nonetheless, they are now recognised as key influences on offspring fitness (Marshall and Uller 2007, Rasanen and Kruuk 2007). Several studies have shown that mothers exposed to a particular type of environmental stress go on to produce

offspring with enhanced performance under that stress (Agrawal et al. 1999, Johnsen et al. 2005, Parker et al. 2012). Other studies, however, have found that stressed mothers go on to produce lower-quality offspring relative to unstressed mothers (Huxman et al. 1998, Moran et al. 2010, Shama and Wegner 2014). These conflicting results may reflect the degrees to which mothers can predict the environments of offspring (Uller et al. 2013, Burgess and Marshall 2014). In the longer term, Kirkpatrick and Lande (1989) showed that the evolution of maternally-influenced traits is facilitated when the selective environments of parents and offspring match, but retarded when they do not (Kirkpatrick and Lande 1989). Consequently, predicting the evolutionary consequences of maternal effects remains an ongoing challenge, requiring a clearer understanding of how maternal environmental effects, partitioned from genetic effects, contribute to offspring fitness.

The limited evidence available suggests that nonadditive genetic and maternal environmental effects can be sensitive to environmental stress (Blows and Sokolowski 1995, Rasanen and Kruuk 2007, Kelly et al. 2013), which creates the ecological context (e.g., smaller, subdivided populations, stronger selection, reduced gene flow) that give the former, especially, greater evolutionary relevance (Wade and Goodnight 1998, Wade 2002). For instance, thermal stress altered the expression of nonadditive genetic variance for morphological traits in the field cricket, *Teleogryllus oceanicus* (Nystrand et al. 2011) and for larval hatching success in the sea-urchin, *Heliocidaris erythrogramma* (Lymbery and Evans 2013). Conversely, Foo et al. (2012) found no effects of temperature or pH on nonadditive genetic effects on embryonic development in the sea-urchin, *Centrostephanus rodgersii*, nor were maternal effects on development sensitive to CO_2 in another sea-urchin, *Strongylocentrotus franciscanus*, or the mussel, *Mytilus trossulus* (Sunday et al. 2011). Furthermore, nonadditive genetic and maternal effects on fitness in one environment can have fitness effects in other environments, which may constrain or accelerate adaptive divergence across them (e.g., Wade 2000). For example, maternal effects in the marine bryozoan, *Bugula neritina*, increased offspring fitness in high-pollution and high-predation environments but decreased fitness under salinity stress (Moran et al. 2010). To our knowledge, however, no study has formally evaluated cross-environment covariation in nonadditive genetic and maternal environmental effects. Hence, we still know very little about their

contributions to fitness, the stability of these contributions under environmental change, and whether their covariation across environments could mitigate or exacerbate the fitness consequences of environmental change.

Here, applying a quantitative genetic breeding design to the ecologically-important marine tubeworm, *Galeolaria caespitosa* (henceforth referred to by genus name), we evaluated the contributions of nonadditive genetic effects, and maternal environmental effects, to phenotypic variation in fitness (measured as larval survival) across multiple thermal environments. Typical of free-spawning marine invertebrates, which shed sperm and eggs into the sea to fuse externally, *Galeolaria*'s lifecycle includes a free swimming larval stage and a sessile adult stage (Jackson and Strathmann 1981, Marshall and Evans 2005). We focused on larval survival as our fitness measure because free-spawned larvae are more vulnerable than adults to environmental stress, especially relative to species that brood their young (Jackson and Strathmann 1981, Byrne 2011, Marshall and Morgan 2011). For many marine organisms, therefore, survival at this early stage of the lifecycle will be a critical bottleneck in the persistence of future populations. *Galeolaria*'s free-spawning nature also makes it ideal for exploring larval vulnerability to environmental stress using cross-classified breeding designs, whereby the subdivision of ejaculates and egg-clutches allows males to be mated with multiple females and vice versa (Galletly et al. 2007, Munday et al. 2013). Using such a design, we decomposed phenotypic variance in larval survival within and across thermal environments into its nonadditive genetic and maternal environmental components (partitioned from additive genetic effects; see Chirgwin et al 2015). Our goal was to understand the relative magnitudes of these often-neglected sources of fitness variation and their potential consequences for population and evolutionary dynamics under global change.

Methods

Study species and collection site

Galeolaria is an intertidal tubeworm common to South Eastern Australia. The adult stage plays an important ecological role in intertidal areas, forming high-density colonies that provide habitat for

unique endemic communities (Edgar 2000, Bulleri et al. 2005). We sampled adult *Galeolaria* from an intertidal population at Brighton Marina, Victoria (37°540S 144°590E). The population spawns year-round and experiences water temperatures ranging from 8°C to 25°C, with a mean of ~17°C and a typical maximum of ~22°C. In the previous 15 years, temperatures exceeded 24.5 °C for only 6 days (3 days each in January 2013 and 2014). As an intertidal species, however, adults and larvae may experience more extreme temperatures in rockpools at low tide. We sampled the population across two periods (May-August 2013 and February-April 2014). Adults were transported in insulated aquaria to a controlled temperature room at Monash University, Clayton, where they were housed in separate aquaria according to collection date. To reduce the effect of variation in parental environment among collection dates, all adults were acclimatised for 2–3 weeks at ~16°C before their gametes were collected.

Gamete collection and fertilisation protocol

Each mature adult was extracted from its calcareous tube and placed into a petri dish of fresh seawater. Individuals began spawning eggs or sperm within 10 seconds of extraction, at which point gametes were collected. All seawater used during gamete collection and subsequent fertilisations was 17 °C, filtered to 0.22 μm and pasteurised.

Following gamete collection, we diluted sperm with seawater to a concentration of 4×10^6 cells per mL (pilot studies showed that fertilisation success was maximized at this concentration, before declining at higher concentrations due to polyspermy). As the less abundant gamete, egg concentration has little influence on fertilisation success (Levin et al. 1991), so we simply extracted all available eggs per female and diluted them to 1.2 mL in seawater. We subsequently added 0.1 mL of the dilute sperm solution to 0.1 mL of the egg solution, doing so three times at 10 minute intervals. This gradual addition of sperm was done to reduce the likelihood of multiple sperm fertilising the same egg (polyspermy), and to maximise the total fertilisation success of each male–female cross (Styan 1998). The resulting gamete solution was left for 1 hour, then rinsed twice through 0.25 μm Nitex mesh to remove excess sperm.

Cross-classified (North Carolina II) breeding design and survival assays

Using the fertilisation protocol above, sperm and eggs of *Galeolaria* were crossed according to the North Carolina II (NCII) breeding design (Lynch and Walsh 1998). Our design consisted of 51 replicate NCII blocks. Each block was the product of sperm from two sires crossed with eggs from two dams, yielding four parental combinations per block (Fig. 1). Each parental combination was replicated six times per block, with each of the 24 replicates comprising an independent fertilisation (Fig. 1).

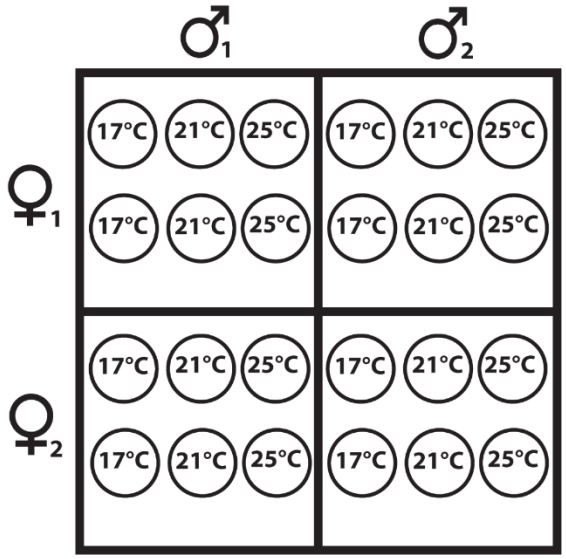

Figure 1. A single block of the North Carolina II breeding design used to estimate parental effects across thermal environments. For each block, eggs from two individual dams were crossed with sperm from two individual sires. Each cross was replicated by six separate fertilisations. Fertilised eggs were then assigned to one of three temperature treatments (17, 21 or 25 °C) so that each sire–dam combination was replicated twice per treatment.

Approximately 2 hours post-fertilisation, ~25 normally-cleaving embryos were collected from each of the 24 replicates per block and placed in a 1.5 mL test tube with filtered and pasteurised seawater. The percentage of normally-cleaving embryos was ~70-80% per replicate. Each test tube of embryos was randomly assigned to one of three thermal environments (17, 21 or 25°C), such that each parental combination was replicated twice per temperature (Fig. 1). Note that thermal environment was not manipulated during fertilisation because gamete environment is known to influence larval

performance in other externally-fertilising species, including another *Galeolaria* species (Crean et al. 2012, Ritchie and Marshall 2013, White et al. 2014). Instead, we manipulated temperature post-fertilisation to isolate sire and dam effects (which are used to estimate the importance of non-genetic and maternal environmental effects) on larval survival from environmental effects on gametes. After a 48 hour incubation period, we scored whether larvae had successfully survived to the trochophore stage, which previous ecotoxicological studies have identified as the most sensitive and reliable indicator of larval tolerance to stress (Ross and Bidwell 2001). Hence, larval survival, quantified as the number of normally-developing trochophores, was the ecologically-relevant measure of fitness used in our study. Overall, we scored over 30 000 embryos from 204 families.

Manipulation of thermal environment

Thermal environments were chosen to represent 1) the mean annual sea-surface temperature at our collection site (17°C); 2) a low-to-moderate rise from the mean annual sea-surface temperature (21°C); and 3) the highest sea-surface temperature recorded in the past 12 months at our collection site (25°C; CSIRO, 2014). Ecologically, our elevated temperatures represent the typical summer conditions (21°C), plus a temperature that is currently rare but likely to become more common in future years (25°C). As such, they are likely to be a good reflection of near-future thermal stress for our study population. All thermal environments were implemented by incubating test tubes of embryos in mini heating dry-baths. For the two elevated temperatures, test tubes of embryos were gradually heated to the desired temperature over ~20 minutes. Each thermal environment was maintained within 0.2 °C of its nominal temperature throughout the 48 hour incubation period.

Statistical analyses

We used a multivariate linear mixed model to investigate how temperature influenced the expression of nonadditive genetic and maternal environmental effects (partitioned from additive genetic variance; see Chirgwin et al. 2015) on larval survival. The model was fitted with restricted maximum likelihood (REML) in the MIXED procedure of SAS 9.3 (SAS Institute, Cary, NC). Specifically, the model was:

$$\text{larval survival} = \mathbf{XB} + \mathbf{Z}_s\sigma^2_s + \mathbf{Z}_d\sigma^2_d + \mathbf{Z}_{sd}\sigma^2_{sd} + \boldsymbol{\varepsilon}$$

where **X** was the design matrix for the fixed effects (**B**) of temperature and block, and $\mathbf{Z}_s$, $\mathbf{Z}_d$, and $\mathbf{Z}_{sd}$ were design matrices for the random effects of sire (σ^2_s), dam (σ^2_d) and sire x dam interaction (σ^2_{sd}), respectively. Each random effect (and the residual term, $\boldsymbol{\varepsilon}$) was an unstructured matrix containing the variances within, and covariances across, the three thermal environments. The model also included sampling period (May-August 2013 vs February-April 2014) as another fixed factor, plus a separate residual matrix for each period. Note, however, that sampling period did not alter the expression of parental effects (i.e., the contributions of sires, dams and sire x dam interactions to larval survival) across temperatures, so is not considered further here.

We then converted the observational (co)variance components (σ^2_s, σ^2_d and σ^2_{sd}) obtained from this model into causal components of additive genetic variance (σ^2_A), nonadditive genetic variance (σ^2_I), and maternal environmental variance (σ^2_M), using the following standard equations (Fry 2004):

$$\sigma^2_A = 4\sigma^2_s$$

$$\sigma^2_I = 4\sigma^2_{sd}$$

$$\sigma^2_M = \sigma^2_d - \sigma^2_s.$$

Note that σ^2_I represents the combined effects of dominance and epistasis, which the experimental design did not allow us to disentangle. Note also that the estimate of σ^2_M assumes that dams and sires have the same additive genetic contribution to their offspring (Fry 2004).

We used an *F*-test to examine temperature effects on larval survival, and used standard log-likelihood ratio tests to examine the significance of all random effects. We tested the overall contribution of nonadditive genetic effects to larval survival by comparing the full model to a reduced model that constrained all sire x dam (co)variances to be zero. We tested the overall contribution of maternal environmental effects by comparing the full model to a reduced model that constrained dam (co)variances and sire (co)variances to be equal (Fry 2004). For each set of effects, we also tested whether individual (co)variance components differed to zero. To visualise the total contribution of parental effects to variance in larval survival at each temperature, we plotted the sum of each additive genetic, nonadditive genetic, and maternal environmental variance against the residual variance. Next,

to visualise the relative contributions of parental effects at each temperature, we plotted the proportional effects of additive genetic, nonadditive genetic, and maternal environmental variance against each other.

Readers should note that the data set analysed here was also the source for Chirgwin et al. (2015), which nonetheless has limited overlap with the present study. That paper focused on the distribution of additive genetic variance in multivariate space, reporting only the percentages of variance in larval survival contributed by dams (combining genetic and environmental effects) and sire x dam interactions within single environments. Here, we focus explicitly on maternal environmental effects (partitioned from genetic effects) and present new multivariate analyses that offer novel insights into cross-environment covariation in both those and nonadditive genetic effects. Sire effects from Chirgwin et al. (2015) are re-included here (converted from σ^2_s to σ^2_A) as a benchmark for evaluating the magnitudes of other parental influences on fitness.

Results

Larval survival across thermal environments

Larval survival declined across thermal environments ($F_{2,153} = 104.76$, $P < 0.001$; Fig. 2). Note that this test differs to Chirgwin et al. (2015) because it is conditioned on a different specification of random effects (biological inferences are unchanged). Post-hoc tests confirmed that survival differed significantly between each pair of temperatures, with survival at the highest temperature approximately two thirds of that at the lowest temperature.

Nonadditive genetic and maternal environmental effects within thermal environments

Overall, the total variance in larval survival was similar at the two coolest temperatures (17°C and 21°C), but was amplified four-fold by the warmest temperature (25°C; Fig. 2). Since the unexplained (residual) variance in survival remained more-or-less similar among environments, accounting for no more than 20% of the total variance in any single one, this four-fold increase represents a temperature-

induced change in the expression of parental effects (summed across additive genetic, nonadditive genetic and maternal environmental effects).

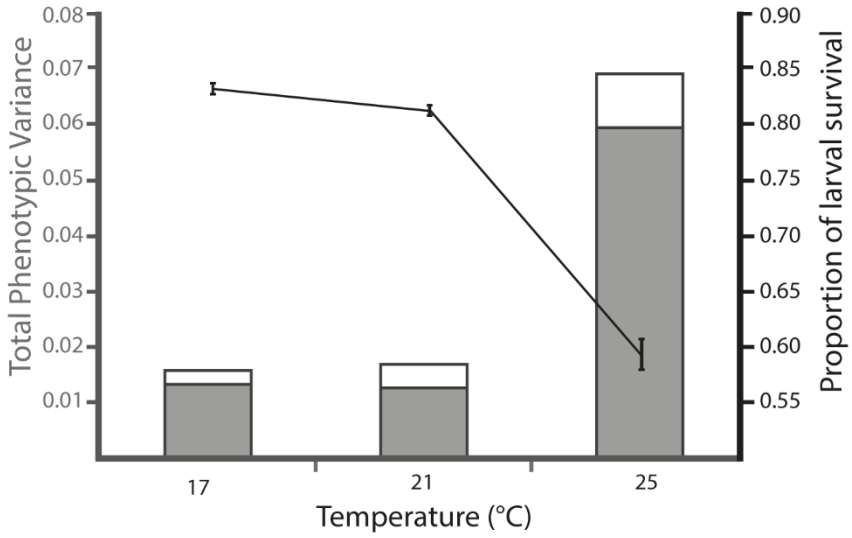

Figure 2. The left axis and columns show the amount of phenotypic variance in larval survival explained by parental effects (summed across additive genetic, nonadditive genetic and maternal environmental effects, in grey) relative to unexplained variance (white) in at each temperature. The right axis and black line show the mean survival (± SE) of larvae at each temperature.

A closer look at the relative contributions of each parental influence (Fig. 3) revealed that nonadditive genetic and maternal environmental effects drove the greater variability of larval survival at 25°C. Indeed, they explained 96% of all parental effects on survival at this temperature (rising from 58% at 17°C and 44% at 21°C), whereas additive genetic effects were similar in magnitude, or relatively greater, at the less extreme temperatures. Specifically, nonadditive genetic effects on survival were significant in all environments (Table 1), accounting for roughly two-thirds of the parental effects expressed at 17°C and 25°C, and nearly half of them expressed at 21°C (Fig. 3). In contrast, maternal environmental effects on survival were significant at 25°C only (Table 1), accounting for roughly a third of the parental effects expressed in that environment (Fig. 3), but contributing relatively little to their expression at 17°C and 21°C (Fig. 3).

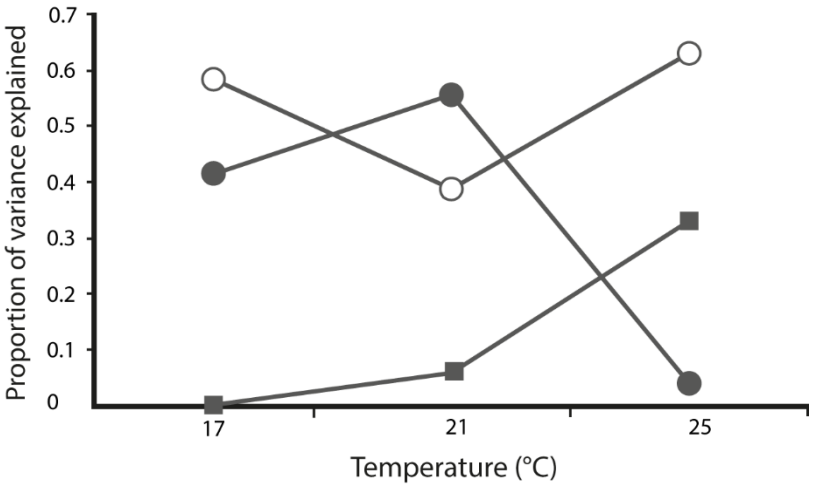

Figure 3. The relative proportions of the total phenotypic variance in larval survival explained by each source of parental effect: additive genetic effects (grey circles), nonadditive genetic effects (white circles), and maternal environmental effects (grey squares).

Cross-environment covariation in nonadditive genetic and maternal environmental effects

Nonadditive genetic effects on larval survival covaried significantly across adjacent thermal environments (i.e., between 17°C and 21°C, and between 21°C and 25°C, but not between 17 °C to 25 °C; Table 1a). Such covariation was positive in both cases (Table 1), indicating that parental combinations that performed relatively well (or poorly) at one temperature tended to also do so at the next warmest temperature. In contrast, maternal environmental effects on survival were decoupled across environments (Table 1b). Hence, whether or not a given maternal environment was beneficial to offspring at one temperature had no bearing on offspring survival at other temperatures (though this lack of covariation might also reflect the overall weakness of maternal environmental effects in our study).

Table 1. The variances and covariances of parental effects on larval survival within and across thermal environments: a) nonadditive genetic effects, b) maternal effects (reported as 0 when the dam variance was less than the corresponding sire variance; see text for details) and c) additive genetic effects. Within-environment variances are in bold on the diagonal and cross-environment covariances are in italics below the diagonal (* $P < 0.05$).

a) Nonadditive genetic effects			
	17°C	***21°C***	***25°C***
17°C	**0.0078***		
21°C	*0.0048**	**0.0049***	
25°C	*-0.0010*	*0.0107**	**0.0375***
b) Maternal effects			
	17°C	***21°C***	***25°C***
17°C	**0**		
21°C	*0*	**0.0007**	
25°C	*0*	*0.0010*	**0.0196***
c) Additive genetic effects†			
	17°C	***21°C***	***25°C***
17°C	**0.0055***		
21°C	*0.0050**	**0.0071***	
25°C	*0.0081**	*0.0022*	**0.0024**

† Reproduced from Table 2 in Chirgwin et al., 2015, converted to causal components (see text for details).

Discussion

Additive genetic variance is critical for evolutionary responses to global change, yet is not the only source of fitness variation available for selection in natural populations. While the evolutionary roles of nonadditive genetic and maternal environmental effects remain controversial, theory and data argue that they can substantially alter evolutionary trajectories, as well as magnitudes and effects of gene flow (Kirkpatrick and Lande 1989, Wang et al. 1998, Wade 2002, Rasanen and Kruuk 2007, Hendry 2013, Dey et al. 2016). Little is known, however, of their relative contributions to fitness variation in natural populations, and even less of their multivariate, multi-environment impacts that might exacerbate or ameliorate global-change stressors. We found that nonadditive genetic and maternal environmental effects on larval survival in *Galeolaria* are non-trivial and environment-dependent,

explaining no less than 44% of parental effects on survival in any environment, and 96% of parental effects in the most stressful one. In Chirgwin et al. (2015), we examined the fraction of variance in larval survival explained by additive genetic effects; here, we consider the possible fitness consequences of the other 96%.

Our results imply that nonadditive genetic and maternal environmental effects may increasingly influence the population and evolutionary dynamics of marine free-spawners, like *Galeolaria*, as water temperatures rise with global change. Nonadditive genetic effects accounted for large proportions (39-63%) of parental effects on larval survival across thermal environments ranging from present-day conditions to those predicted in the future, while maternal environmental effects accounted for considerable variance (33%) in the warmest one. Previously, we showed that *Galeolaria* harbors significant levels of additive genetic variance in larval survival across these environments that may facilitate adaptation to future warming (Chirgwin et al. 2015). However, adaptation to environmental change requires more than additive genetic variance alone: that populations must also persist while they accumulate alleles that are beneficial in the changed conditions (Gomulkiewicz and Holt 1995, Bell 2013) warrants attention to other sources of fitness variation that may aid persistence and contribute to evolutionary processes (Merilä and Sheldon 1999). The adaptive value of nonadditive genetic and maternal environmental effects is often discounted on grounds (i.e., that they are small and transient in nature) that are increasingly disputed (Hansen 2013, Uller et al. 2013). Here, their effects on larval survival in *Galeolaria* give them the potential to aid persistence in the face of future warming and thermal variability, and lead to evolutionary dynamics that differ to those predicted by additive genetic variance alone.

Nonadditive genetic effects on fitness were strongly temperature-dependent, being similar in magnitude to additive genetic effects at 17°C and 21°C, but explaining the majority of fitness variation at 25 °C. Previous studies have detected similar patterns, finding that environmental stress reduces additive genetic variance (Bubliy and Loeschcke 2002, Galletly et al. 2007) and increases nonadditive genetic variance (Jinks et al. 1973, Blows and Sokolowski 1995). However, other studies have found

stress to have the opposite effect, or little effect at all (Hoffmann and Parsons 1991, Pakkasmaa et al. 2003). One reason for this discrepancy could be that different stress levels impose different strengths of selection on focal traits. Crnokrak and Roff (1995), for example, reported that traits under stronger selection harbor higher levels of nonadditive genetic variance relative to weakly-selected traits (see also Hoffmann and Parsons 1991). Currently, however, empirical tests remain too few to allow for broad generalisations about the environment-dependence of nonadditive genetic effects. Their evaluation across a greater range of traits and stressors would greatly enhance our understanding of this issue.

That nonadditive genetic effects were amplified at the highest temperature implies that they may become progressively important to population and evolutionary dynamics under future warming. This is essentially because such effects are most influential in small, subdivided populations incurring strong selection (Wade 2002), which are increasingly associated with global change (Jump and Penuelas 2005, Gienapp et al. 2008, Moller et al. 2008). Warming-driven declines in population size, for example, could see greater conversion of nonadditive variance into additive variance (Goodnight 1988, Wang et al. 1998, Cheverud et al. 1999, Barton and Turelli 2004), although van Heerwaarden et al. (2008) showed that increases in the latter during population bottlenecks do not necessarily improve adaptive capacity in *Drosophila*. Alternatively, greater expression of nonadditive genetic effects under warming might hinder adaptive capacity by masking favourable or unfavourable alleles from selection, but also hinder the erosion of additive genetic variance in doing so (Crnokrak and Roff 1995). Regardless, the presence of substantial nonadditive genetic effects on fitness has implications for how managers use genetic translocations to maintain population genetic diversity (Tallmon et al. 2004, Edmands 2007). If nonadditive genetic effects rely on allele interactions that have evolved within specific populations, then translocations between populations may in principle cause outbreeding depression due to hybrid breakdown (Fenster et al. 1997, Edmands 1999), though in practice there is little evidence of this phenomenon (Frankham 2015). Further work exploring how nonadditive genetic effects on fitness influence the efficacy of genetic translocations could provide managers with crucial information for protecting populations from future environmental change.

While maternal environmental effects had little impact on the survival of *Galeolaria* larvae at lower temperatures, their greater expression at the highest temperature suggests that they may also influence how marine ectotherms respond to warming waters. There is growing awareness that such effects can contribute to adaptation in natural populations, especially when maternal and offspring environments are positively correlated (Salinas and Munch 2012, Uller et al. 2013, Burgess and Marshall 2014, Shama 2015, Dey et al. 2016). For instance, Donelson et al. (2012) found that damselfish (*Acanthochromis polyacanthus*) exposed to thermal stress produce offspring with superior thermal tolerance relative to offspring of unexposed parents. Other studies, however, have shown that stressful parental environments can lower offspring quality (Huxman et al. 1998, Shama and Wegner 2014, Lane et al. 2015, Guillaume et al. 2016). In our study, maternal environmental effects on survival were unlikely to have been caused by past environmental conditions, since all mothers came from the same collection site and were acclimatised before use. Although the mechanism remains unclear, our results nonetheless indicate that maternal environmental effects can potentially influence the viability of marine populations in warming waters, and should therefore be considered in future management strategies.

As global change is predicted to increase both the mean and variability of water temperatures, it is important to understand the capacity for populations to withstand and adapt to multiple temperatures simultaneously. To explore how nonadditive genetic and maternal environmental effects on larval survival may affect *Galeolaria*'s persistence in variable thermal environments, we estimated covariation in these effects across all environments in which survival was assayed. Encouragingly, we found that nonadditive genetic effects on survival covaried positively across environments, in contrast to recent suggestions that the exposure of unfavourable nonadditive effects by thermal stress (Eads et al. 2012, Lymbery and Evans 2013) may lead to fitness trade-offs across stress levels. Here, however, we found no evidence of such trade-offs. Instead, parental combinations that produce a selective advantage in one thermal environment may also do so in other environments, thereby buffering *Galeolaria* against temperature variation.

The question remains of whether cross-environment covariation in nonadditive genetic effects can influence thermal adaptation beyond such buffering — for example, if parental combinations that perform well under ambient heat stress are primed to exploit more extreme environments (e.g., higher in the intertidal), or contribute disproportionately to the gene pool after warming-driven declines in population size. These scenarios, of course, assume that nonadditive genetic effects are to an extent stable across generations. However, growing evidence of their effects on population differentiation following environmental change (Carroll 2007, Hendry 2013) suggests some capacity for this to occur, particularly when populations undergo decline or subdivision (Wade 2002, Roff and Emerson 2006). If such is the case for *Galeolaria*, then cross-environment covariation in nonadditive genetic effects on fitness could potentially influence the evolutionary dynamics of our study population under global change. Given how little is currently known about the generality of this phenomenon, we suggest that estimates of such covariation warrant better characterisation and be reported whenever possible in future.

Surprisingly, we found no evidence of that maternal environmental effects on survival covaried across temperatures. Thus, maternal environmental effects that conferred either a benefit or burden to offspring survival in one environment had no bearing on offspring performance in any other environment. Consequently, the ability of our study population to withstand greater temperature variability appears unlikely to be facilitated or constrained by cross-environment correlations in maternal environmental effects on fitness. Nevertheless, such correlations may potentially influence population responses to other global-change stressors, such as water pH and oxygen concentration (Byrne 2011, Reusch 2014), and are worthy of further investigation.

Despite ongoing debate over the evolutionary relevance of nonadditive genetic and maternal environmental effects (Rasanen and Kruuk 2007, Hill et al. 2008, Uller et al. 2013, Wolak and Keller 2014), the rapid rate of global change, and its impacts on population size and structure, makes understanding their fitness consequences increasingly important. Overall, we argue that nonadditive

genetic and maternal environmental effects may play important roles in population and evolutionary responses of marine species to rising water temperatures. While our goal here was to draw attention to the size and environmental sensitivity of these effects, our work now highlights the need to better incorporate them into predictions of population persistence in changing environments. In particular, there is pressing need for studies that examine the stability of nonadditive genetic and maternal environmental effects across multiple generations (e.g., van Heerwaarden et al. 2008, Dey et al. 2016), that incorporate them into projections of population dynamics (e.g., Coulson et al. 2010) and that consider their effects in multiple or fluctuating environments. Such work is currently rare, but will enhance our ability to forecast the adaptive capacity of populations exposed to global change so they can be managed more efficiently.

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Chapter 3

How does parental environment influence the potential for adaptation to global change?

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Abstract

Parental environments are regularly shown to alter the mean fitness of offspring, but their impacts on the genetic variation for fitness, which predicts adaptive capacity and is also measured on offspring, are unclear. Consequently, how parental environments mediate adaptation to environmental stressors, like those accompanying global change, is largely unknown. Here, using an ecologically-important marine tubeworm in a quantitative-genetic breeding design, we tested how parental exposure to projected ocean warming alters the mean survival, and genetic variation for survival, of offspring during their most vulnerable life stage under current and projected temperatures. Offspring survival was higher when parent and offspring temperatures matched. Across offspring temperatures, parental exposure to warming altered the distribution of additive genetic variance for survival, making it covary across current and projected temperatures in a way that may aid adaptation to future warming. Parental exposure to warming also amplified nonadditive genetic variance for survival, suggesting that compatibilities between parental genomes may grow increasingly important under future warming. Our study shows that parental environments potentially have broader-ranging effects on adaptive capacity than currently appreciated, not only mitigating the negative impacts of global change, but also reshaping the raw fuel for evolutionary responses to it.

Introduction

As environments become adversely affected by global change, populations can maintain or recover fitness *in situ* via plasticity in the short term (one or a few generations), and adaptation in the longer term (Gienapp et al. 2008). Understanding how plasticity and adaptation potentially mitigate the negative impacts of global change, both separately and in tandem, is essential for predicting biodiversity loss and directing conservation strategies (Chevin et al. 2013).

Individuals faced with environmental stress may respond through plastic changes in morphology, physiology, and/or behavior that help maintain fitness under that stress (Chevin et al. 2013). As environmental stressors such as temperature rise steadily due to global change, individuals may increasingly need to respond plastically during their lifespans (Sgrò et al. 2016). Plasticity can also be transgenerational if the effects of stressors on parents carry over to their offspring (Agrawal et al. 1999). To the extent that offspring face similar environmental conditions, transgenerational plasticity can potentially buffer offspring fitness against environmental stress (Burgess and Marshall 2014). Such benefits of transgenerational plasticity are documented in several plant and animal taxa (Agrawal et al. 1999, Sgrò et al. 2016). In other cases, however, parental exposure to environmental stress has only weak, or even detrimental, effects on offspring (Uller et al. 2013). Thus, understanding population vulnerability to global change may hinge on understanding how exposure to environmental stress in one generation affects fitness in the next (Donelson et al. 2018).

Nevertheless, plasticity alone is unlikely to buffer populations against sustained increases in environmental stress (Gienapp et al. 2008). Population persistence in the longer term will often require adaptive evolution, which rests on the availability of adequate genetic variation in fitness and related traits (Bell 2013, Chevin et al. 2013). Predictions of adaptive capacity focus on the additive component of this variation (i.e., the average effects of alleles summed across loci; Lynch and Walsh 1998), which in principle constrains the rate that fitness increases from generation to generation. Nonadditive genetic variation can also arise due to allele interactions within (dominance) and among (epistasis) loci, which may reflect how well the genes of parents function together in offspring

(Puurtinen et al. 2009). However, speculation about its adaptive value (Carroll et al. 2003, Hansen 2015) is often dismissed because such interactions are deemed unlikely to contribute much to trait variation in natural populations (Hill et al. 2008).

Crucially, levels of genetic variance for fitness and related traits are often environment-dependent (Rowinski and Rogell 2017), and should therefore be estimated under future levels of environmental stress to predict adaptive capacity under global change. However, past work on this front has shown mixed results (Rowinski and Rogell 2017), with additive genetic variance being abundant under some environmental stressors (Kelly et al. 2013, Chirgwin et al. 2015, Munday et al. 2017), but limited under others (Kellermann et al. 2009).

Adaptation to global change will not only depend on genetic variation within environments, but also on genetic correlations across environments if similar alleles affect the phenotypes expressed in different environments (Lynch and Walsh 1998). In some cases, genetic correlations across environments can slow adaptation, or constrain it altogether, if alleles that are beneficial under current conditions become detrimental once those conditions change (Teplitsky et al. 2014). Alternatively, such correlations may accelerate adaptation if the alleles favoured in current environments are also favoured in future environments (Bell 2013, Chirgwin et al. 2015). Overall, however, we still have little idea of how genetic correlations across traits or environments affect adaptation, and reviews of the existing literature have identified few general trends in their effects (Agrawal and Stinchcombe 2009). Nonetheless, genetic correlations are expected to influence the ability of populations to keep pace, in an evolutionary sense, with the rate of environmental change (Via and Lande 1985, Bell 2013), meaning further research is needed to measure the strength and direction of genetic correlations across current and projected future environments.

It is clear that population persistence under global change will rely on plasticity and/or adaptation, yet it remains unclear how these two key processes interact (Chevin et al. 2013). In particular, the effects of parental environment on mean phenotypes of offspring are well-acknowledged (Uller et al. 2013),

but genetic variation is typically assumed to be unaffected. Curiously, this assumption has rarely been tested (but see Sgrò and Hoffmann 1998b, a, Munday et al. 2017), and was recently highlighted as key knowledge gap (Donelson et al. 2018). Furthermore, studies have yet to consider how parental environment affects the multivariate structure of genetic variation, taking into account the potential for variances within environments and correlations across them to respond simultaneously. If parental environment does indeed alter genetic expression in offspring, failing to account for its effects could lead us to misjudge adaptive potential, and thereby misjudge the vulnerability of populations facing rising environmental stress under global change.

Here, we used the native marine tubeworm, *Galeolaria caespitosa* (henceforth described by genus name), to examine how parental exposure to projected ocean warming affects adaptive potential, in terms of genetic variation for survival during the most vulnerable life stage. *Galeolaria* is a habitat-forming ecosystem engineer on rocky shores of southeast Australia, where its dense colonies of calcareous tubes support endemic communities that cannot otherwise persist there (Cole et al. 2017). Typical of marine invertebrates that are sessile as adults but have planktonic gametes and larvae, the viability of early life stages is the main bottleneck in the lifecycle, dictating adult abundances. Our past work on *Galeolaria* showed that thermal environments of parents alter mean offspring survival (Guillaume et al. 2016), and that thermal environments of offspring alter genetic variation for offspring survival (Chirgwin et al. 2015). Here, we ask whether parental exposure to projected ocean warming alters levels of additive and nonadditive genetic variation for offspring survival at current and projected temperatures, plus genetic correlations for survival across temperatures. Our findings provide novel insights into the interplay of plasticity and adaptation in the probability of population persistence under global change.

Methods

Study species and collection site

Galeolaria is an external fertiliser that spawns large numbers of gametes continuously year-round with no defined breeding season: at any time, populations contain spawned adults, ripe adults, and adults

with gametocytes at different stages of development (Kupriyanova 2006). Duration of gametogenesis is unknown, but spawned adults ripen new gametes in 7-10 days in closely-related species (Kupriyanova et al. 2001). Such reproductive biology gives ample scope for parental environments, and temperature especially, to influence offspring fitness through effects on developing gametes.

We collected adult *Galeolaria* (the parents in our study) from an intertidal population on pier pylons at Brighton Marina, Victoria, Australia. Here, sea surface temperature has ranged from 9°C to 25°C over the last decade, averaging ~16.5°C annually and ~20.5°C in summer (CSIRO 2018). Since our breeding design consisted of replicate superblocks (see Fig. 1 and below), we collected adults for one superblock at a time from January to April 2016, when seasonal variation in temperature is minimized (CSIRO 2018). Nonetheless, temperature ranged from 18°C to 23°C across the period, which we accounted for by including superblock in statistical analyses (see below). Adults were transferred in insulated aquaria to Monash University, held for 2 hours at temperatures matching those in nature on the day of collection, then ramped to the temperature treatments detailed below over another 24 hours.

Temperature treatment of parents

Parent *Galeolaria* were either non-warmed or warmed to an elevated temperature representing future conditions in coastal waters. To do so, non-warmed parents were held in seawater tanks at the annual mean sea-surface temperature (16.5°C), while warmed parents were held at a relatively stressful temperature (22.5°C) based on projected regional increases of ~2°C above the current summer mean by 2050, and ~3°C higher by 2070 (Hobday and Lough 2011, Mills et al. 2013). This temperature is already exceeded during rare, extreme events, but is above the normal conditions where the vast majority of *Galeolaria*'s breeding occurs (Kupriyanova 2006) and is projected to persist long enough (weeks or months) in future to act as a reliable cue for transgenerational plasticity (Burgess and Marshall 2014).

We held adults at ~1°C of their nominal temperatures (checked daily) for 14 days, reflecting thermal predictability at our collection site based on data loggers recently deployed there (Guillaume et al.

2016). Water temperatures are uncorrelated beyond this period (Guillaume et al. 2016), limiting the capacity of parents to predict the thermal environments of offspring and influence offspring fitness through plasticity (Burgess and Marshall 2014). Nonetheless, a 14-day change in parental temperature can affect continuously-developing gametes, and adults in our study were likely at similar stages of gametogenesis based on similar ripeness at the time of use. We did not simulate diurnal temperature variation because it is relatively small (~0.7°C SD of the daily mean at our collection site) and too transient for parents to respond to (Burgess and Marshall 2014). To disentangle parental and tank effects, we split the parents used for each superblock across two tanks per temperature treatment and cross-fertilised individuals from different tanks.

Gamete collection and fertilisation protocol

After the treatment period, we collected parents' gametes by extracting each individual from its tube into a dish of seawater. *Galeolaria* release eggs or sperm soon after extraction, allowing gametes to be collected for use in fertilisation crosses. All seawater for gamete collection was filtered and pasteurised.

To ensure a constant temperature for all crosses, we brought vials of gametes to an intermediate temperature of 19.5°C (± 0.2°C) over ~30 minutes using mini dry-bath heaters (MajorScience 2015). This required ramping gametes from non-warmed parents up 3°C, and ramping those from warmed parents down 3°C, but we previously showed this did not affect *Galeolaria*'s fertilisation success under the same conditions (Guillaume et al. 2016). We used this protocol because variation in fertilisation environment can influence offspring traits in external fertilizers (Parker et al. 2009, Ritchie and Marshall 2013) and other small ectotherms (Hoffmann and Sgrò 2017, Kellermann et al. 2017). Controling this variation was therefore necessary to isolate the effects of parental environment on offspring survival here, but meant that other effects of fertilisation environment could not be assessed. Once at 19.5°C, we diluted each male's sperm to ~5 x 10^5 cells per mL, which optimised fertilisation success in a pilot study. Since density of the limiting gamete has little influence on this success (Levitan et al. 1991), we simply diluted each female's eggs to 1.0 mL, yielding densities of

~700-1500 eggs per mL. Pilot data showed that fertilisation success is unaffected by variation in egg density within this range (test of success across 100–4000 eggs per mL: $F_{1, 16} = 0.51$, $P = 0.49$).

We initiated each cross by adding 0.3 mL of sperm solution to 0.1 mL of egg solution, doing so 3 times at 5-minute intervals to increase fertilisation success and limit the risk of polyspermy (Hollows et al. 2007). Gamete solutions were left for 50 minutes, then rinsed twice through 0.25 μm Nitex mesh to remove sperm.

Quantitative genetic breeding design

Using the protocol above, we subdivided and crossed the gametes of parents in a cross-classified North Carolina II (NCII) breeding design (Lynch and Walsh 1998), with sperm from each male (sire) crossed with eggs from multiple females (dams) and *vice versa*. Such designs differ from the nested (NCI) ones often used to estimate genetic parameters by separating additive genetic variance (based on the covariance of paternal half-siblings) and nonadditive genetic variance (the covariance of full-siblings less those of paternal and maternal half-siblings) from maternal and residual variances (see analyses). In turn, the precision of genetic estimates always increases with more sires (which we therefore maximized), but NCII designs can double this precision relative to NCI designs, making the former more powerful (Pederson 1972, Lynch and Walsh 1998).

We conducted crosses in replicate superblocks, each comprising a block of crosses using non-warmed parents and a block of crosses using warmed parents (Fig. 1). Parents were not crossed between temperature treatments due to logistic constraints and because mates are likely to have similar thermal histories in nature, with previous studies suggesting most fertilisations occur between individuals within centimeters to meters of each other (Pennington 1985, Marshall 2002, Hollows et al. 2007). Within each block, sperm from two sires were crossed with eggs from two dams, yielding 4 families per block and 8 families per superblock (Fig. 1). Each cross was replicated four times (using embryos from an independent fertilisation), allowing two replicates per cross to be subsequently assigned to each of two offspring temperatures (see below). Each superblock therefore comprised 32 independent

fertilisations (Fig. 1) and our experiment had 25 superblocks overall. Due to a small number of inviable crosses, this yielded offspring from 87 families with non-warmed parents (44 sires and 44 dams), and 86 families with warmed parents (47 sires and 49 dams).

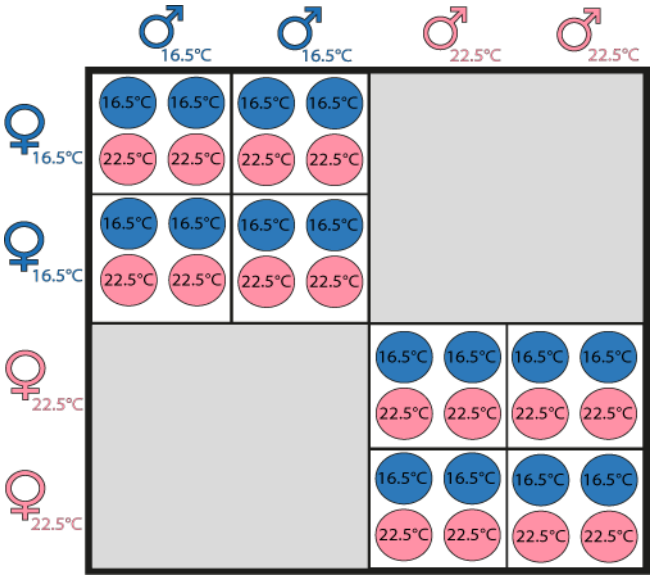

Figure 1. North Carolina II breeding design. Gametes collected from two sires and two dams per parental temperature (16.5°C or 22.5°C) were crossed as shown, with each cross replicated four times. Embryos were then reared at either 16.5°C or 22.5°C, such that each cross was replicated twice per rearing temperature.

Assays of offspring survival at parental treatment temperatures

Next, we reared offspring at the same temperatures as parents (16.5°C or 22.5°C; Fig. 1). Embryos began cleaving ~90 minutes after fertilisation, at which point we pipetted ~30 cleaved embryos per replicate cross (photographed beforehand on a glass slide at 40x magnification to recover their exact number) into a 1.5 mL vial of seawater. The tiny size of *Galeolaria* embryos (~60µm in diameter (Monro and Marshall 2016)) meant they were unlikely to become oxygen-limited at this volume. Pilot work also found embryos had negligible effect on oxygen concentrations under our experimental conditions (test of dissolved oxygen with and without embryos present; $F_{1, 8} = 0.07$, $P = 0.80$).

Two vials per family were haphazardly assigned to each rearing temperature, and ramped to that

temperature over 30 minutes using mini dry-bath heaters. Embryos were held within 0.2°C of nominal temperatures (per heater specifications; MajorScience 2015) throughout a 48 hour development period, before 0.1mL of Lugol's solution was added to each vial to fix and stain the contents for counting. This period allowed sufficient time to assess whether embryos survived development and successfully hatched into trochophore larvae capable of swimming and feeding independently in the plankton, identified by previous studies as the most sensitive and reliable indicator of stress tolerance in *Galeolaria* (Ross and Bidwell 2001). More broadly, embryos and larvae are the life stages most sensitive to stress in marine invertebrates, and are key for assessing vulnerability to ocean warming (Ross and Bidwell 2001, Byrne 2011). Our measure of survival to this point is thus an ecologically-relevant measure of fitness in *Galeolaria*, and was ultimately scored for greater than 20,000 offspring (~30 x 2 vials x 2 rearing temperatures for 173 families).

Statistical Analyses

We used restricted maximum likelihood (REML) and Bayesian approaches to estimate genetic matrices containing the variances of, and covariance between, offspring survival across rearing temperatures, and compare matrices across parental temperatures. Survival data were standardized to a variance of 1 by parental treatment, giving them a common scale for analysis.

Genetic matrices for offspring from warmed and non-warmed parents. We started by analysing offspring survival in a multivariate animal model, fitted via REML in ASReml-R 3.0 (Butler et al. 2007). An animal model is a linear mixed-effects model that estimates additive genetic effects on quantitative traits from pedigree information (Kruuk 2004), constructed here from parental identities in NCII crosses. Our model also included family (sire x dam interaction) and dam identities to estimate nonadditive genetic and maternal effects on survival, respectively. Sire x dam interaction was assumed to estimate nonadditive genetic effects only, since replicate vials of siblings never shared a common environment (Kruuk and Hadfield 2007). Specifically, the model was:

$$\text{offspring survival} = \mathbf{XB} + \mathbf{Z}_a\sigma^2_a + \mathbf{Z}_{na}\sigma^2_{na} + \mathbf{Z}_m\sigma^2_m + \mathbf{Z}_b\sigma^2_b + \boldsymbol{\varepsilon}$$

where **X** was the design matrix for the fixed effects (**B**) of offspring and parental temperatures, while

$\mathbf{Z}_a$, $\mathbf{Z}_{na}$, $\mathbf{Z}_m$, and $\mathbf{Z}_b$ were design matrices for the random effects estimating additive genetic variance (σ^2_a), nonadditive genetic variance (σ^2_{na}), maternal variance (σ^2_m), and superblock variance (σ^2_b). The latter was modeled as a single variance (Table S1), while each of the other random effects (and the residual, $\boldsymbol{\varepsilon}$) was initially modeled as a block-diagonal covariance matrix, containing a separate matrix per parental temperature. Each parental matrix contained the variances of, and covariance between, offspring survival across rearing temperatures. We tested the significance of all variance components using likelihood ratio tests, and removed maternal covariances from the final model without significant loss of fit (see Table S1 for maternal variances).

Comparisons of genetic matrices between parental temperatures. Next, we used two complementary approaches to explore the multivariate structures of genetic matrices and their responses to parental temperature (see Fig. 2 for a conceptual overview). First, we used a form of eigenanalysis, termed factor-analytic modeling, to identify how many independent dimensions (eigenvectors) occurred in each matrix, and how their variances (eigenvalues) were distributed (see details in Hine and Blows 2006). By fitting these dimensions within our animal model (Butler et al. 2007), we could directly test how many were required to explain genetic effects on survival across offspring rearing temperatures, and do so separately by parental temperature.

Second, we used genetic covariance tensors to explore how parental temperature affected aspects of matrix structure other than dimensionality (Fig. 2b-e). Tensors compare any number of matrices (see (Hine et al. 2009) and (Aguirre et al. 2014)) but here, with only two parental temperatures, the comparison amounts to an eigenanalysis of **E**, the matrix of pairwise differences between genetic estimates. Eigenvectors ($\boldsymbol{e}_1$ and $\boldsymbol{e}_2$) of **E** are the dimensions that differentiate the original matrices, and corresponding eigenvalues are the amounts of genetic variation for those dimensions. For example, if matrices differ in size (total variation), both eigenvalues of $\boldsymbol{e}_1$ and $\boldsymbol{e}_2$ may be non-zero and similar in sign, meaning variation increased or decreased for both matrix dimensions (Fig. 2b). If matrices differ in shape (distribution of variation), both eigenvalues may be non-zero and different in sign, meaning variation increased for one matrix dimension and decreased for the other (Fig 2c). If matrices differ in

orientation, survival at each rearing temperature may contribute differently to $\boldsymbol{e}_1$ and/or $\mathbf{e}_2$, meaning a change in trait relationships across temperatures (Fig. 2e).

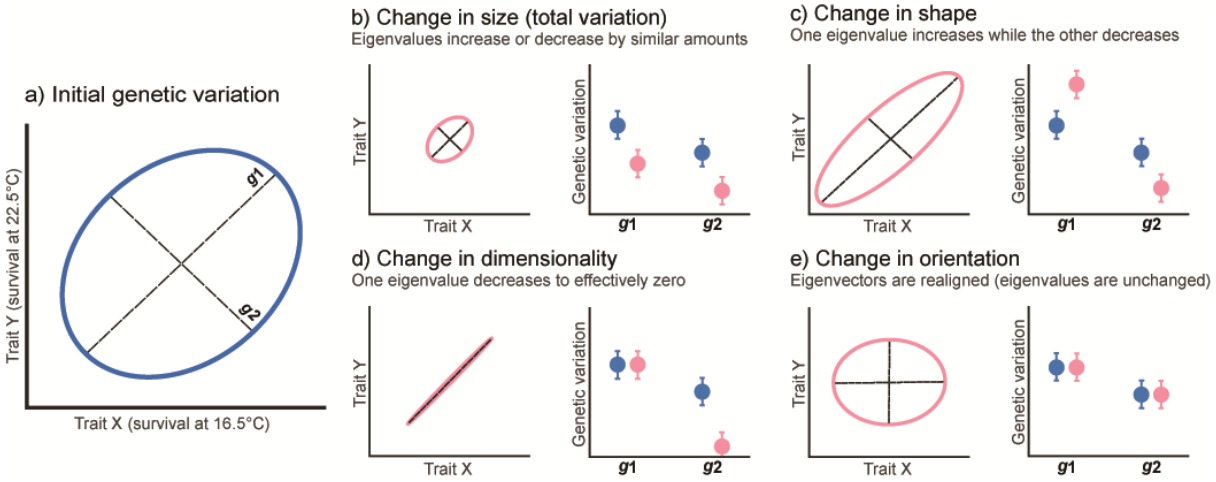

Figure 2a-e. Changes in the multivariate structure of genetic variation. a) The initial structure for two traits, X and Y (survival at 16.5°C and survival at 22.5°C). Genetic variation has two independent dimensions, ***g*1** and ***g*2**, which are diagonal to plot axes because X and Y are genetically correlated. Each dimension has a direction (or eigenvector, representing a linear combination of X and Y) and a length (or eigenvalue, representing the amount of variation for that direction). If different parental temperature (with non-warmed parents in blue and warmed parents in pink) causes different alleles to affect X and Y, or the same alleles to affect them differently, this structure could change in several ways (b-e, with right-hand panels indicating what tensor analyses would show if initial and changed structures were compared). b) The total variation changes due to similar decreases in both eigenvalues (e.g., if alleles affecting both traits are lost, or allele effects become more uniform, with parental warming). c) Variation is re-distributed toward ***g*1** and away from ***g*2** (e.g., if the genetic correlation between traits grows stronger with parental warming). d) Variation for ***g*2** declines to the point where it is effectively zero. e) The eigenvectors are reoriented due to changes in the relationship between underlying traits (e.g., if parental warming changes allele effects more for one trait than the other), but eigenvalues are unchanged.

To facilitate tensor analyses, we re-fitted our animal model in a Bayesian framework, using the *MCMCglmm* package of *R* (Hadfield 2010) to sample the marginal posterior distributions of genetic

parameters. We used weakly-informative inverse-Wishart priors (parameter-expanded priors were explored but gave similar results), with scale parameters defined as diagonal matrices containing values of one-fifth of the total variance in offspring survival per rearing temperature, and distribution parameters set to 0.001 for degrees of freedom (Hadfield 2010). Posterior distributions came from 220,000 MCMC iterations sampled every 200 iterations after an initial burn-in of 20,000 iterations. We checked convergence from plots of traces and posterior distributions, and autocorrelations between samples (all were below the recommended level of 0.1, yielding effective sample sizes close to 1,000 for all parameters).

We applied the tensor analyses to our 1,000 MCMC samples using the *R* routine in (Aguirre et al. 2014), modified to also compare nonadditive genetic matrices. Briefly, this routine compared the observed **E** to a null model constructed by randomising additive (or nonadditive) genetic effects on offspring survival across parental temperatures. We then inferred whether parental temperature significantly altered these genetic effects from the posterior probability (the proportion of samples where differences in the observed **E** exceeded those under the null model; Hadfield et al. 2010) and 95% HPD intervals. Finally, to visualise *how* parental temperature altered these variances, we projected the eigenvectors ($\boldsymbol{e}_1$ and $\boldsymbol{e}_2$) of the observed **E** onto original matrices to find the relative amounts of variation in those directions.

Results

Effects of parental temperature on mean offspring survival across rearing temperatures

We detected interactive effects of parental and offspring temperatures on mean offspring survival ($F_{1, 68} = 50.7$, $P < 0.05$), which was ~6% higher when offspring were reared at the temperature of their parents than when they were not (Fig. 3).

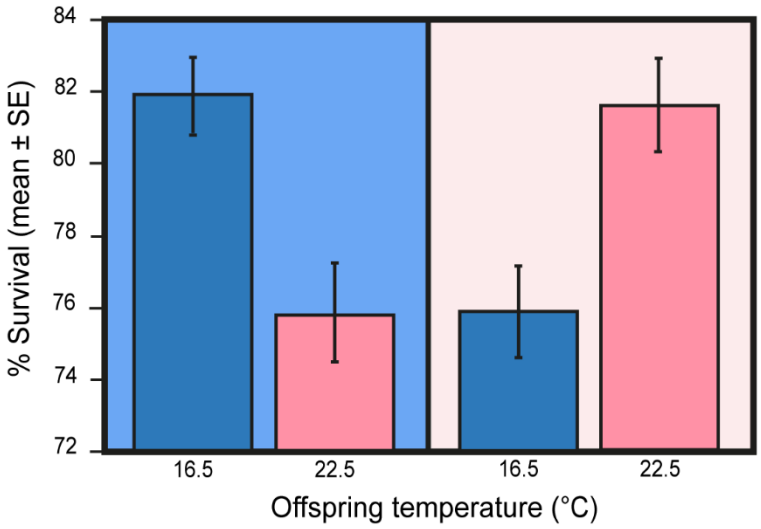

Figure 3. Mean (±SE) survival of offspring from non-warmed parents (blue background, LEFT) and warmed parents (pink background, RIGHT) at each offspring rearing temperature.

Effects of parental temperature on genetic variation for offspring survival across rearing temperatures

Inspecting each genetic matrix implied that additive genetic variance was lower when parents were exposed to higher temperature (Table 1a), whereas offspring rearing temperature had little effect on this variance (Table 1a). Notably, additive genetic covariance across rearing temperatures was non-significant for offspring of non-warmed parents, but became significantly positive for offspring of warmed parents (Table 1a).

Parental exposure to warming substantially increased the nonadditive genetic variance for offspring survival. At both rearing temperatures, this variance was roughly four-fold greater for offspring of warmed parents relative to offspring of non-warmed parents (Table 1b). It was also marginally greater when offspring were reared at warmer temperature (Table 1b). Nonadditive genetic covariance across rearing temperatures was positive regardless of parental treatment, but increased six-fold when parents were exposed to warming (Table 1b).

Table 1. Matrices of additive and nonadditive genetic variances (diagonal elements, in bold) and cross-environment covariances (below-diagonal elements) for survival of offspring reared at current (16.5°C) and projected (22.5°C) water temperatures. Offspring of non-warmed parents are in blue (LEFT) and offspring of warmed parents are in pink (RIGHT). The standard error of each estimate is in brackets; *P < 0.05.

a) Additive genetic variation					
	16.5°C	*22.5°C*		*16.5°C*	*22.5°C*
16.5°C	**0.116 (0.020)***		*16.5°C*	**0.081 (0.014)***	
22.5°C	-0.003 (0.015)	**0.138 (0.023)***	*22.5°C*	0.022 (0.010)*	**0.0863 (0.015)***
b) Nonadditive genetic variation					
	16.5°C	*22.5°C*		*16.5°C*	*22.5°C*
16.5°C	**0.183 (0.056)***		*16.5°C*	**0.857 (0.180)***	
22.5°C	0.127 (0.046)*	**0.224 (0.066)***	*22.5°C*	0.816 (0.169)*	**0.968 (0.201)***

Comparisons of genetic matrices between parental temperatures

Our comparisons of these genetic matrices showed that parental temperature altered their multivariate structures. Since offspring were reared at two (current and projected) temperatures, each matrix could have up to two dimensions, depending on the amounts of genetic variance for survival at each temperature and covariance for survival across temperatures. For offspring of non-warmed parents, additive genetic variance was distributed quite evenly across two statistically-supported dimensions that each associated strongly with survival at one rearing temperature (per Fig. 2e) — the first dimension with survival at 22.5°C, and the second dimension with survival at 16.5°C (Table 2a). In contrast, offspring from warmed parents had effectively only one dimension of additive genetic variance (per Fig. 2d), with positive contributions from survival at both temperatures (Table 2a). Dimensionality is reduced by lower variance or stronger covariance (Monro and Marshall 2014), and parental exposure to warming did both these things (Table 1a), lowering additive genetic variance for survival at each rearing temperature, while strengthening covariance for survival across them. Hence, parental exposure to elevated temperatures may affect adaptive capacity by reducing the additive genetic variance on which it depends, but compensate by making adaptation to current temperatures facilitate adaptation to future ones.

Parental exposure to warming also re-distributed the nonadditive genetic variance for offspring survival across rearing temperatures toward one dimension (per Fig. 2c), but without loss of dimensionality (since factor-analytic modeling also offered statistical support for the other, lesser dimension; Table 2b). For offspring of non-warmed parents, 75% of nonadditive variance lay in a dimension with positive, evenly-weighted contributions from survival at both rearing temperatures, while the other 25% lay in a dimension with opposing contributions. For offspring of warmed parents, nonadditive genetic variance remained associated with two dimensions that were very similar to those above (Table 2b), but variance for the largest dimension rose to 95%. Hence, parental exposure to warming reshaped nonadditive genetic variance to enhance positive covariation between the compatibilities of parental genomes across current and future temperatures.

Table 2. Eigenanalyses of additive and nonadditive genetic matrices for survival of offspring at current (16.5°C) and projected (22.5°C) water temperatures. Offspring from non-warmed parents are in blue (LEFT) and offspring from warmed parents are in pink (RIGHT). Offspring from warmed parents had only one dimension (eigenvector) of additive genetic variance, while all other matrices had two dimensions of genetic variance (likelihood ratio tests identified significant loss of model fit if dimensionality was further reduced). Loadings on each eigenvector show the contribution from offspring survival at each rearing temperature, while eigenvalues show the amount of genetic variance for each eigenvector.

a) Additive genetic variation				
	Eigenvector 1	Eigenvector 2	Eigenvector 1	
16.5°C	-0.111	0.994	0.244	
22.5°C	0.994	0.111	0.970	
Eigenvalue	0.075	0.068	0.092	
% Variance	52.7	47.3	100	
b) Nonadditive genetic variation				
	Eigenvector 1	Eigenvector 2	Eigenvector 1	Eigenvector 2
16.5°C	0.667	-0.745	0.684	-0.730
22.5°C	0.745	0.667	0.730	0.684
Eigenvalue	0.304	0.104	1.925	0.100
% Variance	74.6	25.4	95.1	4.9

When genetic matrices were compared using tensors, parental temperature had no detectable effect on the additive genetic variance for offspring survival across rearing temperatures (observed differences between additive matrices often overlapped differences under the null model: P = 0.124, Fig. S1a). This result in no way opposes our results from factor-analytic modeling, since Bayesian comparisons constrain matrices to be full rank (Aguirre et al. 2014) and cannot test changes in dimensionality. The tensor comparison did, however, detect a significant effect of parental temperature on the nonadditive genetic variance for survival (observed and null differences between nonadditive matrices did not overlap: P < 0.001; Fig. S1b). The leading dimension of the tensor ($\boldsymbol{e}_1$, explaining 99.8% of differences between original matrices; Table 3) had positive, even contributions from survival at both offspring temperatures. Projecting this dimension (and the lesser one, $\boldsymbol{e}_2$) onto each original matrix showed that parental exposure to warming significantly increased nonadditive genetic variance for $\boldsymbol{e}_1$ alone (Fig. 4). Overall, this supports the impression from factor-analytic modeling that warming re-distributed the nonadditive genetic variance for survival in line with Fig. 2c.

Table 3. Dimensions (or eigenvectors, $\boldsymbol{e}_1$ and $\boldsymbol{e}_2$) of the tensor capturing the effects of parental temperature on the nonadditive genetic variation for offspring survival. Loadings on each dimension show the contributions from survival when reared at current (16.5°C) and projected (22.5°C) water temperatures.

	$\boldsymbol{e}_1$	$\boldsymbol{e}_2$
16.5°C	0.686	-0.727
22.5°C	0.727	0.686

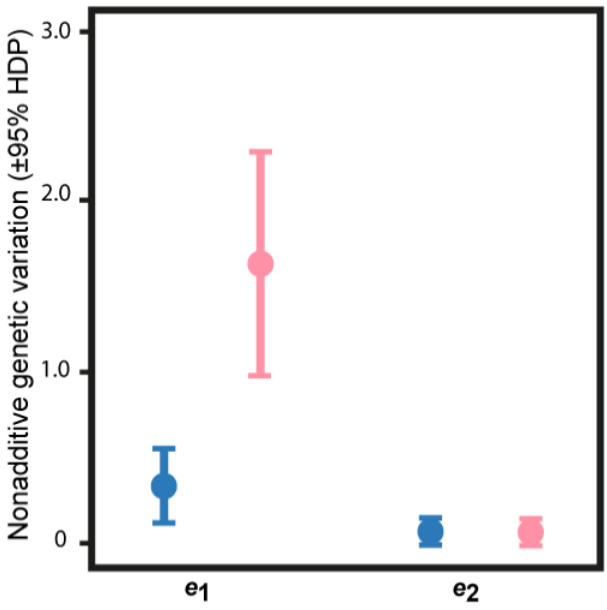

Figure 4. Effect of parental temperature on the nonadditive genetic variation for survival. Variation for $\boldsymbol{e}_1$ (the leading dimension of the tensor, capturing 99.8% of differences between original matrices) is greater for offspring from warmed parents (pink, RIGHT) than offspring from non-warmed parents (blue, LEFT; note the lack of overlap between HPD intervals). Variation for $\boldsymbol{e}_2$ was unaffected by parental temperature.

Discussion

Populations may respond to environmental change via plasticity and/or adaptation (Gienapp et al. 2008). While these processes are predicted to interact (Donelson et al. 2018), few experimental studies have tested how they may do so. Here, we examined how exposing parents to projected ocean warming altered the survival, and genetic variance for survival, of offspring during their most vulnerable life stage under current and projected temperatures. Offspring survival was higher when parent and offspring temperatures matched. Across offspring temperatures, moreover, parental exposure to warming altered the distribution of additive genetic variance, and increased levels of nonadditive genetic variance linked to the compatibilities of parental haplotypes. These results show that parental environments may potentially have broader-ranging effects on adaptive capacity under global change than currently appreciated.

Despite long-standing interest in the idea that parental environments may prime offspring to cope with similar conditions, evidence remains equivocal. Notable cases reporting adaptive parental effects of this kind (Agrawal et al. 1999, Parker et al. 2012), are countered by others where stressed parents simply yield poor-quality offspring with impaired tolerance of the same stressor (Shama and Wegner 2014, Guillaume et al. 2016). When they occur, moreover, adaptive parental effects are often subtle (Uller et al. 2013). Our results for *Galeolaria* support this observation, with parental exposure to projected warming buffering offspring against this stress (i.e., offspring from warmed parents survived just as well at 22.5°C as offspring from non-warmed parents at 16.5°C), but this stress only reduced absolute survival by 6%. Notably, our results differ from previous work on *Galeolaria* (Guillaume et al. 2016) where parental temperature had inconsistent effects on offspring survival. However, our sample sizes doubled those of the previous study (Guillaume et al. 2016), suggesting that evidence for adaptive parental effects may remain equivocal because previous tests lacked sufficient power to detect them (Uller et al. 2013).

While adaptive parental effects were subtle here, even this modest buffering may help natural populations maintain large enough sizes to persist under rising thermal stress, buying time for evolutionary rescue (which lowers extinction risk by restoring absolute fitness) to take effect (Bell 2017). Alternatively, such buffering could eliminate the need for rescue altogether, so long as the loss of absolute fitness due to thermal stress is fully restored (Bell 2013). This is a subtle, but important, distinction to the idea that plasticity in traits underlying relative fitness may limit the evolution of those traits by weakening selection on them (Ancel 2000, Price et al. 2003), which ignores absolute fitness and takes persistence for granted (Bell 2017). The key issue now is whether transgenerational buffering will be equally effective under more extreme conditions, or even disadvantageous in the longer term — for instance, if conditions that induce it are not met, suddenly exposing buffered populations to severe stress (Donelson et al. 2018).

Transgenerational effects on the genetic variation required for adaptation remain poorly understood (Donelson et al. 2018). Past work indicates that additive genetic variance for single traits is amplified

by parental exposure to stress (Sgrò and Hoffmann 1998a, Munday et al. 2017), or by mismatches between parental and offspring environments (Sgrò and Hoffmann 1998b). Here, if anything, parental exposure to thermal stress weakly reduced additive genetic variance for offspring survival at current and future temperatures, regardless of whether parental and offspring environments matched. We also build on past work by analysing parental effects on the multivariate structure of additive genetic variation (Hine and Blows 2006). These analyses revealed that exposing parents to warmer temperature not only reduced additive genetic variation for survival, but limited its distribution to a single dimension defined by positive genetic covariation for survival across current and future temperatures. Thus, loss of variance to fuel thermal adaptation may be offset by greater tendency for alleles that benefit survival at current temperatures to also do so at future ones. As such, our study population may progressively adapt to future warming using standing stocks of alleles with positive effects on survival under current-day conditions (Bell 2013).

To our knowledge, ours is also the first study to show that parental environment not only alters, but increases, nonadditive genetic variance for any component of fitness. While nonadditive gene action is seemingly common in nature, its contribution to nonadditive genetic *variance* is assumed to be small and of limited evolutionary value (Hill et al. 2008). Yet in breeding designs like ours, such variance manifests as sire x dam interaction (signaling variation in the compatibilities of parental haplotypes; Puurtinen et al. 2009) and often increases with stress (Jinks et al. 1973, Chirgwin et al. 2017). Here, exposing parents to thermal stress increased this variance more than four-fold, while stress at rearing increased it by another ~20%. However, the consequences of such increases are uncertain in the absence of clear links to theory (Hansen 2015, but see Hill 2017). At the very least, future warming may make evolutionary dynamics less predictable, if fitness increasingly depends on allele combinations shuffled by random segregation and recombination in parents (Puurtinen et al. 2009). Given growing evidence of nonadditive genetic effects on adaptation (Carroll et al. 2003, Forsberg et al. 2017, Hill 2017), and new or underused ways of linking them to the variances measured here (Le Rouzic 2014), understanding their role in a warming world warrants further attention.

Adaptation requires genes to stably affect phenotypes from one generation to the next (Lynch and Walsh 1998). While individuals in our study population will likely experience persistent warming in future years (Mills et al. 2013), they may also experience periodic rises in water temperatures lasting weeks to months. In such cases, the population-wide impacts of parental effects will depend on how long they last within and across generations, which is unknown for *Galeolaria*. Past work has shown that plastic responses to stress affect some traits irreversibly, whereas others eventually revert to their original states once that stress eases (Beaman et al. 2016). Whether our manipulation of parents captured the full extent of their effects on offspring is also unclear, since environmental exposure at earlier life stages, or repeated across stages, has important phenotypic consequences in other species (Kellermann et al. 2017). Hence, testing the duration and extent of environmental exposure in parents and offspring (including effects on other fitness components, like reproduction, later in the lifecycle) is a key future step. Regardless, the transgenerational effects of parental environment may not need to be entirely stable to have evolutionary consequences. In theory, at least, even transient effects on offspring can produce major shifts in evolutionary dynamics and outcomes (Day and Bonduriansky 2011).

A key question unanswered by our study is how temperature at fertilisation may affect adaptive capacity through genetic effects on offspring. Here, we had to control this temperature in order to isolate parental effects on offspring, but previous work indicates that phenotypically at least, offspring can be sensitive to environmental conditions at this life stage (Parker et al. 2009, Byrne 2011, Ritchie and Marshall 2013). In the oyster *Saccostrea glomerata*, for instance, thermal stress was more detrimental to embryos when applied before fertilisation than afterwards (Parker et al. 2009), and gamete exposure to stress could affect *Galeolaria* embryos in a similar way. To our knowledge, however, genetic effects of fertilisation environment on offspring remain untested and are a key step for future studies.

In conclusion, we show that parental environment can potentially influence adaptation to projected future warming, not only by altering mean offspring fitness but also by altering the genetic variance

for fitness. Yet phenomenological approaches like ours cannot identify the mechanistic basis of parental effects like those seen here. Recently, how various forms of non-genetic inheritance (e.g., epigenetic mechanisms including DNA methylation and microRNAs) contribute to plasticity within and across generations has been much-debated (Futuyma 2017), and resolving this debate remains a priority. More work is also needed to dissect the thermal sensitivity of genetic effects on fitness, potentially involving interactions between nuclear and cytoplasmic genes (Dowling et al. 2007) or, in external fertilisers like *Galeolaria*, effects on gametogenesis and/or fertilization dynamics (Guillaume et al. 2016, Alavioon et al. 2017). Regardless of the underlying mechanisms, however, our study represents an important step toward understanding how plasticity and adaptation jointly shape population dynamics and extinction risk under global change.

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Supplementary material

Table S1. a) Maternal variation for survival of offspring reared at current (16.5°C) and projected (22.5°C) water temperatures. Offspring of non-warmed parents are in blue (LEFT) and offspring of warm parents are in pink (RIGHT) at current and projected water temperatures; b) Variation for offspring survival among superblocks (see Fig. 1). The standard error of each estimate is in brackets.*P < 0.05.

d) Maternal variation			
16.5°C	*22.5°C*	*16.5°C*	*22.5°C*
0.079 (0.059)	0.049 (0.061)	0.146 (0.095)	0.108 (0.099)
e) Superblock variation	0.435 (0.151)*		

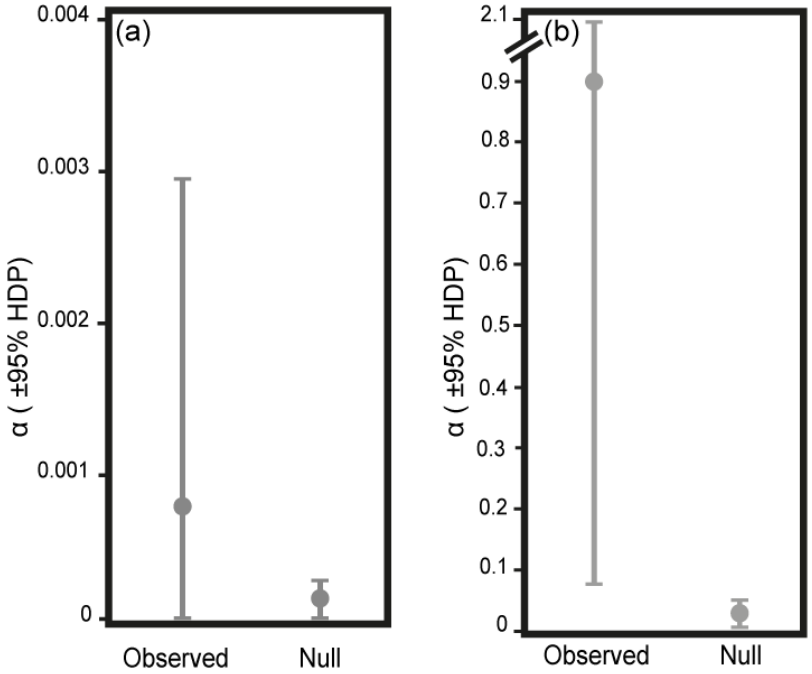

Figure S1. Tensor comparisons of a) additive and b) nonadditive genetic matrices for the survival of offspring from warmed parents versus non-warmed parents. Observed differences between matrices captured by the tensor (α) are contrasted with the randomized (null) levels expected by chance alone.

Chapter 4

Fertilisation under projected ocean warming decreases adaptive potential in an external fertiliser

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Abstract

Populations require additive genetic variation in components of fitness (survival and reproduction) to fuel their adaptive evolution under the environmental stressors of global change. Past work has shown that levels of additive genetic variation for components of fitness can depend on current and past environments, including those experienced by preceding stages in the life cycle, or even of previous generations. Fertilisation links one generation to the next for all sexual populations, yet how fertilisation under different environmental conditions impacts the genetic variation expressed amongst the resulting offspring has rarely been considered. Here, using an externally fertilising marine tubeworm, we combine a quantitative genetic breeding design with a factorial manipulation of fertilisation and development temperatures under current conditions and projected warming. We find that fertilisation and development under projected warming both reduce offspring survival, but fertilisation under projected warming also alters the additive genetic variation for survival, virtually eliminating it at both development temperatures, and also eliminating the positive covariation in offspring survival across development temperatures. In turn, we argue that fertilisation environment may have a far greater impact on the adaptive potential of populations than currently acknowledged, and therefore may be a key factor in assessing the vulnerability of populations to global change.

Introduction

Rapid global warming is exposing populations to increasing environmental stress (Scheffers et al. 2016). Populations can move to escape the stress, use phenotypic plasticity to buffer against the stress, or evolve to adapt to the stress (Hoffmann and Sgrò 2011). However, change is happening so fast, and is so widespread, that many populations cannot escape or cope *in situ* using plasticity alone (Gienapp et al. 2008). Their long term persistence may therefore depend on their potential for adaptation (Bell 2013). For any population, this potential relies on the presence of genetic variants for components of fitness (reproduction and survival) that help individuals to cope under new conditions. The frequency and distribution of these variants (genetic variation) is potentially altered by the environmental experiences of previous life stages, and even of previous generations (Via and Lande 1985, Chirgwin et al. 2018). Fertilisation links one generation to the next; consequently, stressful environmental conditions during this key point in the life cycle can impact fitness in both the parental and offspring generations (Walsh et al. 2019). For instance, fertilisation under environmental stress can reduce not only the number of offspring that parents produce, but also the survival and performance of those offspring (Parker et al. 2012). Yet the impacts on the genetic variation for offspring survival and performance, and therefore on the potential for adaptation, have gone virtually unconsidered. Since fertilisation is required for the persistence of all sexual populations, understanding how the fertilisation environment influences adaptive potential is a crucial step in forecasting the vulnerability of biodiversity to projected warming.

The maximum rate of adaptation from one generation to the next is set by the additive genetic variation for fitness, which is the proportion of the total variation for fitness that is consistently heritable (Lynch and Walsh 1998). Genetic variation for fitness (as for any trait) will be environmentally dependent whenever fitness is affected by different alleles in different environments, or the same alleles affect fitness differently in different environments (Via and Lande 1985). Accordingly, considerable research has explored the adaptive potential of populations under projected climate scenarios by measuring additive genetic variation for the expression of key traits under such conditions. Populations have been found to harbour abundant levels of additive genetic variation for

adaptation to future climates in some cases (Kelly et al. 2013, Chirgwin et al. 2015, Munday et al. 2017), but not others (Kellermann et al. 2009, Martins et al. 2019). Less explored, however, is the potential for adaptation to be shaped by genetic covariation between measures of fitness expressed in different environments — whether environments reflecting different types of stress (e.g., ocean warming and acidification; Foo et al. 2012), or reflecting current and future levels of the same stress (Chirgwin et al. 2015). In either case, genetic covariation between the same alleles or sets of alleles underpinning fitness in different environments can prevent independent adaptation to those environments. This can slow adaptation if alleles have antagonistic effects on fitness in different environments, or speed up adaptation if alleles are consistently beneficial across those environments (Bell 2013). Nevertheless, the extent to which genetic covariances across environments are likely to help or hinder adaptation to future environmental conditions remains broadly unclear.

Populations will usually be more susceptible to environmental stress at some life stages than others (Pandori and Sorte 2019). In turn, when assessing the vulnerability of populations to environmental stress, it is critical to focus on adaptive potential at the most susceptible life stages because they are when population bottlenecks are most likely to occur (Byrne 2011). However, levels of genetic variation for key traits expressed at these life stages may still depend on episodes of selection experienced earlier in the life cycle, and be mediated by past environmental conditions (Schluter et al. 1991, Marshall et al. 2016, Postma and Ågren 2016). For instance, early life stages (e.g., embryos, larvae, or seeds) are usually periods of high mortality (Morgan 1995, Donohue 2014), allowing natural selection during these stages to potentially modify the frequency and distribution of alleles expressed at subsequent life stages (Marshall and Morgan 2011, Donohue 2014). Additionally, the environment experienced during one life stage can induce epigenetic or other plastic effects that alter how alleles are expressed later on (Nijhout 2003, Beaman et al. 2016, Shama et al. 2016). Despite the potential for such environmental carry-over effects on genetic variation, empirical tests of how environmental conditions during one life stage impact the genetic variation expressed at later stages or generations remain rare (Donelson et al. 2018).

Populations rely on the successful collision and fusion of gametes to produce offspring, but how the environments experienced by gametes during this process shape patterns of genetic variation in offspring has hardly been considered. Growing evidence suggests that temperature stress arising from global change will impair the function and performance of gametes across a range of taxa, with the strongest impacts likely to occur in external fertilisers (Walsh et al. 2019). Unlike internal fertilisers, whose sperm and eggs interact entirely within the female reproductive tract, many aquatic species (including most fishes, amphibians, and marine invertebrates) spawn sperm and eggs into the external environment (Monro and Marshall 2015). Consequently, spawned gametes are directly exposed to environmental stress, and will likely incur rising temperature stress under global change (Walsh et al. 2019). Further, gamete exposure to stress can have important carry-over effects on later life stages, with stressed gametes going on to produce offspring with heightened susceptibility to the same stressor (Parker et al. 2009, White et al. 2014). Yet to the best of our knowledge, no study has explicitly tested how the external fertilisation environments of spawned gametes impact the genetic variation for key traits in offspring. Crucially, if such impacts are taking place, continuing to overlook them may lead us to misjudge the potential of populations to adapt to global change.

Here, using an externally-fertilising marine tubeworm, *Galeolaria caespitosa* (henceforth described by genus), we test how fertilisation under projected ocean warming impacts the genetic variation for offspring survival. Typical of marine invertebrates, *Galeolaria* has sessile adults but planktonic gametes, embryos and larvae (Monro and Marshall 2016). Embryo and larval stages are most susceptible to environmental stress, and are thus the main bottleneck for most marine populations under global change (Foo and Byrne 2016, Pandori and Sorte 2019). Our past work on *Galeolaria* shows that temperatures experienced by parents, as well as by developing embryos and larvae, impact the genetic variation for early survival under current and projected water temperatures, and the genetic covariation for survival across those temperatures (Chirgwin et al. 2015, 2018). Here, we complete the missing link between parental and early offspring stages by testing if patterns of genetic variation and covariation depend also on the temperatures experienced by gametes. To do so, we combine a quantitative genetic breeding design with a factorial manipulation of fertilisation and development

temperatures. We show that fertilisation temperature profoundly alters the expression of genetic variation for survival, to the point of virtually eliminating the additive genetic variation required for adaption to projected warming. Our findings suggest that accounting for the environments experienced by gametes during fertilisation will be a vital step in forecasting the vulnerability of natural populations to global change.

Methods

Study species and collection site

Galeolaria is a calcareous tubeworm native to rocky shores of southeastern Australia, where it acts as an ecosystem engineer by forming dense colonies of adult tubes that provide habitat for endemic communities (Cole et al. 2017, Wright and Gribben 2017). Adults reproduce year-round by spawning gametes into the external water column (Kupriyanova et al. 2001), where eggs and sperm must fuse to achieve fertilisation (Monro and Marshall 2016). After fertilisation, embryos and larvae also develop in the water column before eventually settling and recruiting into sessile adult populations (Ross and Bidwell 2001). Surviving development to the trochophore stage, when larvae swim and feed independently, is the most sensitive and reliable indicator of stress tolerance in *Galeolaria*, and thus a key component of fitness in this species (Ross and Bidwell 2001, Chirgwin et al. 2015). Indeed, in marine invertebrates more broadly, embryo and early larval life stages are the most sensitive to stress, and hence vital for evaluating population vulnerability to future warming (Byrne 2011, Pandori and Sorte 2019).

We sampled a population from the intertidal zone at Chelsea (Victoria, Australia) from April to June 2018, transferring individuals to Monash University in insulated aquaria. To reduce any effect of environmental variation among adults sampled at different times, we acclimatised adults for 14-17 days at ~16.5 °C before extracting their gametes. To extract gametes, we induced spawning by removing adults from their tubes and placing them in petri dishes with filtered seawater. Sperm or eggs were collected immediately for use in experimental trials.

Factorial manipulation of fertilisation and development environments

Fertilisation and development (to the trochophore stage) were trialled in a factorial design, crossed with two temperatures that represent current and projected future conditions at our study site. Here, sea-surface temperature has ranged from 9 to 25°C over the previous decade, averaging ~16.5°C annually and ~20.5°C in summer (CSIRO 2018). Temperature is projected to rise ~2°C by 2050, and ~3°C by 2070 (Hobday and Lough 2011, Mills et al. 2013). Given these projections, we conducted trials at 16.5°C (the current annual mean) and 24°C (currently rare during summer months, but projected to become increasingly common). To disentangle the effects of temperature at different life stages, our trials involved four treatments: fertilisation at 16.5°C with development at 16.5°C, fertilisation at 16.5°C with development at 24°C, fertilisation at 24°C with development at 16.5°C, and fertilisation at 24°C with development at 24°C. Admittedly, gametes that experience 16.5°C are unlikely to produce offspring that experience 24°C during embryonic development (or *vice versa*) under natural conditions. However, these temperature manipulations were nonetheless necessary to achieve our primary goal of disentangling how fertilisation and offspring environment affects offspring survival. All treatments were implemented and maintained within 0.2°C of the nominal temperature using drybath incubators (MajorScience 2015).

Quantitative genetic breeding design

Within the factorial design above, we crossed gametes of males (sires) and females (dams) in a cross-classified North Carolina II (NCII) breeding design (Lynch and Walsh 1998). This design allows phenotypic variation among offspring to be partitioned into additive genetic variation, nonadditive genetic variation due to allele interactions within (dominance) and among (epistasis) loci, maternal environmental variation and residual variation. In each experimental block, we crossed sperm from two sires with eggs from two dams, yielding four families per block (Fig. 1). Each sire-dam cross was replicated in eight independent fertilisations, providing two replicates per cross for each of the four temperature treatments. Consequently, each block comprised 32 independent fertilisations (Fig. 1). Our experiment had 28 blocks overall, yielding offspring from 56 sires, 56 dams, and 112 families.

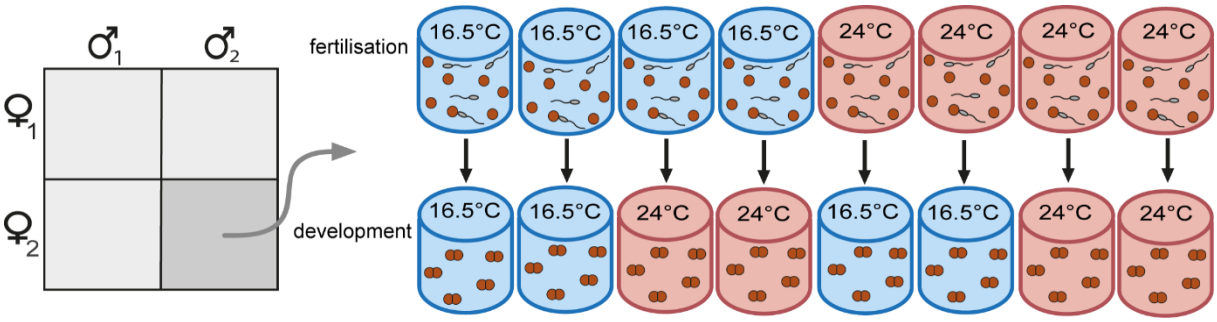

Figure 1. North Carolina II breeding design embedded in a factorial manipulation of life history stage (fertilisation and development to the trochophore larval stage) and temperature (16.5°C and 24°C). Each sire-dam cross was replicated in eight independent fertilisation trials, with four conducted at 16.5°C and four conducted at 24°C. Embryos resulting from each fertilisation trial developed at either the same temperature or the alternative temperature, such that each sire-dam cross was replicated twice in each combination of fertilisation and development temperatures.

Fertilisation and development protocol

Fertilisation was initiated by adding ~900 of a dam's eggs in 0.1 ml of filtered seawater to a vial containing ~5 x 10^5 of a sire's sperm in 1 ml of filtered seawater. For *Galeolaria*, these gamete concentrations have been shown to maximise fertilization success while minimizing lethal polyspermy (Chirgwin et al. 2018). Before mixing, sperm and egg solutions were separately ramped to the desired temperature over 30 minutes. Since sperm are activated by dilution, we ramped them at high concentration (10^7 sperm ml^{-1}) to minimize ageing. At this concentration, the ramping period had no effect on male fertility (E. Chirgwin *unpubl. data*). Each fertilisation trial ran for 30 minutes, which maximised fertilisation success in pilot work. Vials were agitated every 10 minutes to reduce oxygen depletion. We ended each trial by syringing out ~95% of the sperm solution, then removing the remainder by rinsing embryos twice through 0.25 µm Nitex mesh.

Next, we transferred embryos from each fertilisation trial to their development temperatures (Fig. 1). Because different fertilisation temperatures led embryos to develop at different rates, we transferred them at the same development stage (2 to 8 cells, reached in ~60 minutes at 24°C, and ~90 minutes at

16.5°C). Doing so reduced any confounding effects of implementing the development temperature at different stages, while still capturing ~95% of embryonic development. For each replicate sire-dam cross, we pipetted ~30 embryos into a 1.5 ml vial of seawater, which was either maintained at its fertilisation temperature or ramped to the alternative temperature over ~30 minutes (Fig. 1). Embryos do not become oxygen-limited while developing at this density (Chirgwin et al 2018).

Offspring survival assays

All embryos were held at their nominal temperatures (±0.2°C) for a 48-hour development period (embryos start hatching into trochophore larvae after ~24 hours). The 48-hour development period was used because previous work on *Galeolaria* determined it to be the most appropriate time to visually assess whether embryos survived development to the larval stage (Ross and Bidwell 2001). At the end of the 48-hour development period, we added 0.1 ml of Lugol's solution to each vial to fix and stain the contents so the number of surviving larvae could be counted. We scored the survival of over 25,000 embryos (~30 embryos × 2 replicates × 4 temperature treatments x 112 families) overall.

Statistical analyses

We used a multivariate linear mixed model, fitted via restricted maximum likelihood (REML) in ASReml-R 3.0 (Butler et al. 2007), to analyse additive genetic, nonadditive genetic, and maternal environmental effects on survival across fertilisation and development temperatures. Specifically, the model was:

$$\text{Offspring survival} = \mathbf{XB} + \mathbf{Z}_s\sigma^2_s + \mathbf{Z}_d\sigma^2_d + \mathbf{Z}_{sd}\sigma^2_{sd} + \mathbf{Z}_b\sigma^2_b + \boldsymbol{\varepsilon}$$

where $\mathbf{X}$ was the design matrix for the fixed effects ($\mathbf{B}$) of fertilisation and development temperatures, while $\mathbf{Z}_s$, $\mathbf{Z}_d$, $\mathbf{Z}_{sd}$, and $\mathbf{Z}_b$ were design matrices for the random effects estimating the sire variance (σ^2_s), dam variance (σ^2_d), sire × dam variance (σ^2_{sd}), and block variance (σ^2_b), respectively. The latter was modelled as a single variance, while each of the other random effects (and the residual, $\boldsymbol{\varepsilon}$) was modelled as a block-diagonal covariance matrix, containing a separate matrix for each of the two fertilisation temperatures. Each of the latter matrices contained the variances of, and covariance between, survival at the two development temperatures. We multiplied sire and sire × dam variances

by four to calculate additive genetic and nonadditive genetic variances respectively, and subtracted dam variances from sire ones to calculate maternal environmental variances (Fry, 2004). We tested the significance of each variance by constraining it to 0 (or constraining sire and dam variances to be equal, in the case of maternal environmental variances), and evaluating the loss of fit relative to the original model using a likelihood ratio test.

To test if fertilisation and development temperatures significantly impacted additive genetic, nonadditive genetic, and maternal environmental effects on offspring survival, we again used likelihood ratio tests to compare pairs of nested models. For fertilisation, we tested if modelling a single covariance matrix with each type of effect pooled across fertilisation temperatures resulted in poorer fit than the original model with temperature-dependent matrices. For development, we tested if constraining each covariance matrix to have the same variance at both development temperatures resulted in poorer fit than the original model. Operationally, these tests are equivalent to classic tests of genotype (or dam) x environment interactions.

Readers should note that our estimates of genetic variation in this current chapter differ by an order of magnitude compared to Chapter 3 simply because of how we standardised larval survival before each variance was estimated. In this current chapter we analysed larval survival as unstandardized, whereas in Chapter 3 we standardised larval survival to a variance of 1. How, or whether, to standardise proportional estimates of fitness (as larval survival was measured here) is still an ongoing debate, and we direct readers to Stinchcombe 2005 and Hansen et al. 2011 for a compressive discussion of the issue. Regardless of this debate, we found that standardising larval survival to a variance of 1 does not alter the key findings presented below.

Results

Effects of fertilisation and development temperatures on offspring survival

Warmer temperatures at fertilisation and development significantly reduced offspring survival, and did not interact in their effects (χ^2= 0.99, d.f.=1, p=0.32). Specifically, warmer temperature at fertilisation

reduced survival by ~7% (χ^2= 128.25, d.f.=1, p<0.01; Fig. 2), while warmer temperature during development reduced survival by another ~6% (χ^2= 66.99, d.f.=1, p<0.01; Fig. 2).

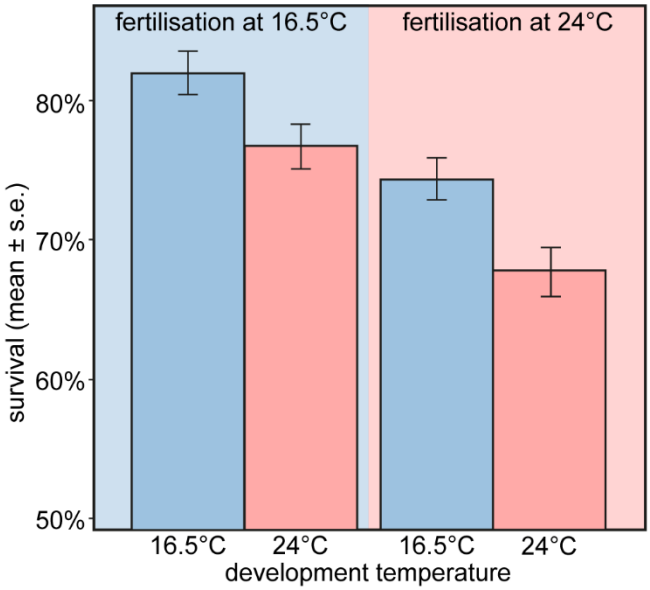

Figure 2. The effects of fertilisation and development temperatures on offspring survival in *Galeolaria* (mean ± s.e.).

Effects of fertilisation and development temperatures on genetic variation for offspring survival

Additive genetic effects on offspring survival depended significantly on fertilisation temperature (χ^2 =9.02, d.f.=3, p=0.03; Table 1a), but not the subsequent development temperature (fertilisation at 16.5°C: χ^2 =0.02 d.f.=1, p=0.87; fertilisation at 24°C: χ^2 =0.01, d.f.=1, p=0.95; Table 1a). When fertilisation occurred at 16.5°C, survival showed significant levels of additive genetic variation at both development temperatures, and significantly positive additive genetic covariation between development temperatures (Table 1a). However, all additive genetic effects on survival became non-significant when fertilisation occurred at 24°C (Table 1a).

Nonadditive genetic effects on offspring survival also depended significantly on fertilisation temperature (χ^2 =23.22, d.f.=3, p<0.01; Table 1b), but not the subsequent development temperature (fertilisation at 16.5°C: χ^2 =1.81 d.f.=1, p=0.18; fertilisation at 24°C: χ^2 =0.13, d.f.=1, p=0.72; Table 1b). While nonadditive genetic variation for survival was consistently significant, it was generally

higher when fertilisation occurred at 24°C than at 16.5°C (it was also higher when development occurred at the warmer temperature, but not significantly so; Table 1b). Nonadditive genetic covariation was also consistently significant and positive across development temperatures, but declined marginally when fertilisation occurred at 24°C.

Last, maternal environmental effects on offspring survival (Table S1) again depended significantly on fertilisation temperature (χ^2 =16.04, d.f.=3, p<0.01), but not development temperature (fertilisation at 16.5°C: χ^2 =0.85 d.f.=1, p=0.35; fertilisation at 24°C: χ^2 =3.63, d.f.=1, p=0.06). This latter result was likely driven by the fact that such effects were weak overall, and only contributed to variation in survival when fertilisation occurred at 24°C (Table S1).

Table 1. Matrices of additive and nonadditive genetic effects on offspring survival after fertilisation and development at current (16.5°C) and projected (24°C) water temperatures. Results for fertilisation at 16.5°C are shown in blue, and results for fertilisation at 24°C are shown in pink. Estimates are ± 1 standard error, with significant estimates in bold text.

(a) Additive genetic variance and covariance					
	16.5°C	*24°C*		*16.5°C*	*24°C*
16.5°C	**0.014 ± 0.005**		16.5°C	0.004 ± 0.004	
24°C	**0.008 ± 0.005**	**0.013 ± 0.006**	24°C	0.003 ± 0.003	0.003 ± 0.005
(b) Nonadditive genetic variance and covariance					
	16.5°C	*24°C*		*16.5°C*	*24°C*
16.5°C	**0.010 ± 0.004**		*16.5°C*	**0.015 ± 0.005**	
24°C	**0.011 ± 0.004**	**0.017 ± 0.006**	*24°C*	**0.009 ± 0.005**	**0.018 ± 0.007**

Discussion

Populations need additive genetic variation in components of fitness (survival and reproduction) to adapt to global change (Hoffmann and Sgrò 2011). Crucially, levels of additive genetic variation for components of fitness can depend on current and past environments, including those experienced by preceding stages in the life cycle (Via and Lande 1985, Munday et al. 2017, Chirgwin et al. 2018). Yet how, or even whether, fertilisation under different environmental conditions affects the genetic

variation expressed by resulting offspring is rarely considered. Here, using an externally-fertilising marine tubeworm, we show that the fertilisation environment not only influences offspring survival, but also the genetic variation expressed for survival. Specifically, fertilisation and development under projected warming both reduce offspring survival, but fertilisation under projected warming also affects the additive genetic variation for survival, virtually eliminating it at both development temperatures, and also eliminating the positive genetic covariation in offspring survival across development temperatures. Our findings indicate that fertilisation environments may have far greater impacts on the adaptive potential of populations than currently acknowledged, and will be a key factor in assessing the vulnerability of populations to global change.

Our findings add to mounting evidence that gametes exposed to projected levels of environmental stress will go on to produce poorer performing offspring (Parker et al. 2009, Byrne 2011, White et al. 2014, Kekäläinen et al. 2018, but see Ritchie and Marshall 2013). Notably, this contrasts with studies on diploid life stages, which often respond to environmental stress by undergoing plastic changes that buffer later life stages (and sometimes generations) to the same or even other stressors (Donelson et al. 2012, Sgrò et al. 2016, Kellermann et al. 2017, Chirgwin et al. 2018). One potential explanation for contrasting stress responses between haploid and diploid stages is that the latter can sometimes undergo plastic changes through environmentally-induced epigenetic alterations (e.g., DNA methylation and mRNAs) (Beaman et al. 2016, Sgrò et al. 2016). Gametes seem to have little capacity to undergo similar epigenetic alteration after gametogenesis (Reinhardt et al. 2015, Donkin and Barrès 2018), which is complete by the time they experience stress in the external environment. In addition, gametes may be more susceptible than diploid stages to environmental stress because of their smaller size, which is often associated with lower heat tolerance (Terblanche et al. 2011, Klockmann et al. 2017), or their lower ploidy, which growing evidence (especially in plants, where polyploidy is common) suggests can also lower stress resistance (Scholes and Paige 2015, Godfree et al. 2017).

Fertilisation under projected warming profoundly reduced the expression of additive genetic variation for offspring survival, virtually eliminating the genetic variation required for adaptation to projected

warming. This implies that fertilisation under warmer conditions altered the expression of alleles in offspring, or the frequencies of alleles present in offspring (Via and Lande 1985). For instance, heat stress may have caused DNA damage in gametes via mechanisms such as oxidative stress or aberrant apoptosis (Lewis and Aitken 2005), which could directly disrupt the expression of alleles underpinning offspring survival. Heat stress may have also altered selection among gametes within ejaculates or egg clutches after spawning, thereby altering alleles frequencies in offspring. An increasing number of studies indicate that selection on gametes can potentially have important implications for phenotypes expressed in the resulting offspring (Crean et al. 2012, Immler and Otto 2018, Alavioon et al. 2019), but how such selection impacts genetic variation in offspring phenotypes remains to be tested. Selection among gametes may also act on variants caused by epigenetic alterations (e.g., to sperm mRNA profiles) during gametogenesis, which could affect later sperm function or gene expression in offspring (Dadoune 2009, Evans et al. 2019), although evidence for such phenomena remains scarce.

Failing to account for effects of fertilisation temperature on the additive genetic variation in offspring survival would have led us to overestimate the adaptive potential of our study population under projected warming. Since fertilisation will likely occur under similar conditions to those experienced during early life stages, our findings potentially raise concerns for several past studies, including our own, that conducted fertilisation under benign conditions before assessing the adaptive potential of early stages under projected levels of environmental stress (e.g.,Pakkasmaa et al. 2003, Merila et al. 2004, Galletly et al. 2007, Sunday et al. 2011, Clark et al. 2013, Lymbery and Evans 2013, Chirgwin et al. 2018, Rudin-Bitterli et al. 2018, Tasoff and Johnson 2019). While such studies usually conducted fertilisation under benign conditions to isolate the effects of stress on other life stages, our findings here nonetheless suggest that also accounting for the effects of stress during fertilisation is necessary to accurately assess adaptive potential.

Building evidence suggests that nonadditive genetic effects on fitness and related traits will become more important to evolutionary trajectories under future global change (Lymbery and Evans 2013,

Chirgwin et al. 2017, 2018, Rudin-Bitterli et al. 2018). Here, the nonadditive genetic variation for offspring survival was significant regardless of fertilisation or development environment, but was also increased by fertilisation under projected warming. The patterns of nonadditive genetic variation detected here suggests that the compatibilities of parental haplotypes have important effects on offspring survival (Puurtinen et al. 2009), and that warmer fertilisation environments increase the magnitude of those effects. The broader implications for adaptation to projected warming remain unclear, however, due to gaps in available theory (Hansen 2015, but see Hill 2017). At a minimum, the presence of nonadditive effects may make demographic and evolutionary dynamics less predictable, since a substantial amount of fitness variation depends on allele combinations that are shuffled by random segregation and recombination from one generation to the next (Falconer and Mackay 1996, Puurtinen et al. 2005). Declines in population size under projected warming can also result in nonadditive variation being converted into additive variation as a product of allele frequencies being changed by genetic drift (Goodnight 1988, Willis and Orr 1993, van Heerwaarden et al. 2008). Although much of this converted additive variation is expected to be deleterious and quickly removed by selection, some of it may have a lasting impact if it allows the population to move to a new evolutionarily stable state (e.g., adaptation rather than extinction; Barton and Turelli 2004). On longer-term scales, nonadditive genetic effects are implicated in population divergence and speciation (Carroll et al. 2003, Roff and Emerson 2006, Rego et al. 2007), and thus increases in nonadditive genetic variation could potentially facilitate these processes under future warming.

The evolutionary consequences of exposure to environmental stress at different life stages remains poorly understood (Beaman et al. 2016, Marshall et al. 2016, Donelson et al. 2018). Here, fertilisation and development under projected warming both imposed an equal amount of stress on offspring survival, but only fertilisation under projected warming impacted genetic variation for survival. Thus, in *Galeolaria* at least, genetic variation for offspring survival seems to be more sensitive to the life stage at which environmental stress occurs than to the amount of stress itself. While offspring fitness may also be sensitive to parental environments that were not considered here (Sgrò and Hoffmann 1998, Munday et al. 2017), our previous work on *Galeolaria* found that exposing parents to projected

warming improved offspring survival, and only weakly reduced additive genetic variation for survival (Chirgwin et al. 2018). Therefore, it is possible that parental exposure to warming might buffer offspring survival against the negative impacts of warming during fertilisation and development detected here, but is less likely to compensate for the added effects on genetic variation for survival. However, if parental and fertilisation environments have interactive effects on additive genetic variation in offspring traits is currently unknown. Indeed, a potential limitation of our current study is that fertilisation under projected warming may have reduced larval survival and genetic variation because of the *change* in temperature between gametogenesis in parents (~16.5°C) and fertilisation in the external environment, rather than due only to the stress of fertilisation under projected warming. Under natural conditions, parents will usually undergo gametogenesis at a similar temperature that their gametes or offspring (during embryonic development) will experience. Therefore, future work that quantifies the combined effects of experiencing projected warming during parental stages and fertilisation on additive genetic variation in offspring survival is needed to obtain the most ecologically-realistic estimates of adaptive potential. Admittedly, an experiment capable of testing this three-way interaction (i.e., parental × fertilisation × offspring environment) will require a huge logistical undertaking, but sits as the key next step. Future work is also needed to consider if the environmental conditions during fertilisation and development can affect fitness beyond the larval stage measured here, as how long the effects of the fertilisation environment persist into the life cycle remains untested.

In conclusion, our findings suggest that environmental conditions during fertilisation warrant greater attention in a rapidly warming world. Here in *Galeolaria*, fertilisation under projected ocean warming not only reduced a key component of fitness, but also the potential for fitness to be recovered through adaptation. Whilst global change is likely have the greatest impact on the key process of fertilisation in external fertilisers like *Galeolaria*, internal fertilisers — particularly ectothermic ones — are also expected to experience fertilisation under novel levels of environmental stress in coming years (Walsh et al. 2019). Given that fertilisation is necessary for the persistence of all sexual populations, continuing to overlook the effects of fertilisation environment on genetic variation for key traits may

lead us to misjudge the adaptive potential of those populations, and thus misjudge their vulnerability to global change.

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Supplementary material

Table S1. Matrices of maternal environmental effects on offspring survival after fertilisation and development at current (16.5°C) and projected (24°C) water temperatures. Results for fertilisation at 16.5°C are shown in blue, and results for fertilisation at 24°C are shown in pink. Estimates are ± 1 standard error with significant estimates in bold.

Maternal environmental variance and covariance					
	16.5°C	*24°C*		*16.5°C*	*24°C*
16.5°C	0.000 ± 0.001		*16.5°C*	0.001 ± 0.001	
24°C	0.000 ± 0.001	0.000 ± 0.001	*24°C*	0.001 ± 0.001	**0.005** ± **0.002**

Chapter 5

Development time is targeted by selection, independent of initial size but not subsequent size

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Abstract

Body size and development time (the time between distinct life stages) are fundamentally important traits for all organism. Evolutionary patterns in development time are often viewed as allometric and physiological by-products of size. In contrast, life history theory predicts that size and development time are both targets of selection and may thus evolve adaptively, outside of allometric and physiological constraints, through opposing effects on fitness. Surprisingly, this prediction has rarely been tested by studies that disentangle the direct and indirect effects of size and development time on fitness, as necessary to understand how selection targets these traits. Here we use an externally-fertilising marine invertebrate that provides a novel opportunity to measure selection, acting via survival in the field, on size and development time at key early life stages. We show that development time (fertilisation to hatching) is not only a direct target of selection, but also affects how indirect selection acts on initial size before development, and contributes to nonlinear selection on size after development. Furthermore, we found initial size and development time are positively correlated, but this correlation is selectively neutral, suggesting the relationship between these traits has resulted from physiological or allometric constraints. In turn, our finds suggest that appreciating how selection act alongside the allometric and physiological constraints is crucial to understanding the evolution of size and development time.

Introduction

Whether microevolutionary processes (e.g. selection, inheritance) within populations can explain macroevolutionary patterns at larger scales remains a key problem in evolutionary biology (Arnold et al. 2001, Futuyma 2010, Uyeda et al. 2011). Among species, size is often positively correlated with biological rates and times at a given temperature, including development time from one life stage to the next (Pauly and Pullin 1988, Gillooly 2000, Gillooly et al. 2002). Consequently, variation in species' development times has been declared the evolutionary by-product of allometric and physiological constraints arising from variation in size (Gillooly 2000, Gillooly et al. 2002). Within populations, however, correlations between size and development time range from positive (McLaren 1965, Ficetola and De Bernardi 2006, Marshall and Bolton 2007) to negative (Hinegardner 1975, Sinervo and McEdward 1988, Rius et al. 2010) or non-existent (Jones et al. 1996, Kingsolver et al. 2012, Pettersen et al. 2018). This inconsistency at the population scale suggests that development time is more than a simple function of size and temperature, and evolves in response to additional factors.

Life-history theory assumes that size and development time evolve in response to selection, within the constraints set by a trade-off in their effects on fitness (survival and reproduction of individuals). On one hand, selection is expected to target larger sizes at every life stage because larger individuals tend to have higher survival, fecundity, and mating success (Blanckenhorn 2000, Marshall and Keough 2008, Dmitriew 2011). On the other hand, selection is expected to target shorter development times because individuals that reach maturity sooner tend to have less predation risk, better access to resources (including mates), and prolonged breeding due to earlier reproduction (Sibly et al. 1985, Kingsolver and Pfennig 2004). Unless selection shapes both traits independently, however, the optimal value of each one will be a compromise between these conflicting pressures — for instance, the optimal development time may be one that maximizes the benefits of reaching large size while minimizing the costs of delaying maturation. From this perspective, evolutionary patterns in size and development time should reflect the specific patterns of selection acting on those traits, as well as the specific characteristics of the organism (e.g., phenotypic or genetic correlations between traits) shaped by its evolutionary history and local environmental conditions (Dmitriew 2011).

Understanding how selection acts on multiple, interacting traits requires disentangling its direct effects on targeted traits from its indirect effects on other traits that are correlated with those targets. For example, if traits under direct selection are unaccounted for when measuring selection on other, correlated traits, the latter will appear to be causally linked to fitness even if they are selectively neutral (Lande and Arnold 1983, Phillips and Arnold 1989). Measuring multiple traits on individuals, and using Lande and Arnold's (1983) classic regression framework to tease apart the direct and indirect effects of selection on them, offers a solution to this problem. In the case of size and development time, however, selection has usually been measured for each trait in isolation (Kingsolver and Huey 2008, Marshall and Keough 2008, Dmitriew 2011), leaving it unclear whether selection targets one or the other, or both of them at once. To our knowledge, selection on both size and development time has only been measured for two populations (Crean et al. 2011, Kingsolver et al. 2012), with conflicting results. Directional selection, which acts to shift a trait's mean by selecting against phenotypes at one extreme of the trait distribution, favoured larger sizes and shorter development times in the tobacco hornworm, *Manduca sexta* (Kingsolver et al. 2012), but was absent on size and favoured longer development times in the ascidian, *Styela plicata* (Crean et al. 2011).

Even less is known about nonlinear (i.e., quadratic and correlational) selection on these traits, which was measured in the latter study only. This is problematic because trait evolution is unlikely to depend on linear fitness effects in isolation (Lande and Arnold 1983, Brodie 1992). Quadratic selection acts to increase (or decrease) trait variation by favouring extreme (or intermediate) phenotypes, or phenotypes that make fitness approach some asymptotic value, whereas correlational selection acts to weaken or strengthen phenotypic correlations by targeting one trait based on its interaction with others (Brodie 1992). However, the distinction between quadratic and correlational selection is often arbitrary, allowing nonlinear selection to be visualised more generally as curvature in multivariate relationships between traits and fitness (Phillips and Arnold 1989, Blows 2007).

Selection is also unlikely to act independently on traits expressed across life stages (Schluter et al. 1991, Monro and Marshall 2014). Correlations among traits at different ages or stages are common (Chippindale et al. 1997, Rius et al. 2010, Marshall and Morgan 2011), and can influence both direct and indirect components of selection on sequential traits like size and development time (Kingsolver et al. 2012). If, for instance, development time depends on initial (e.g. embryo) size, and in turn affects subsequent (e.g., hatchling) size, then such correlations can ultimately determine how selection shapes developmental trajectories, or early life stages as a whole, to maximise survival to reproduction. Thus, measuring traits across multiple life stages can provide a more compete view of not only *how* but also *when* selection is acting on key traits of interest over the lifecycle. We address the knowledge gaps outlined above by measuring how size and development time are targeted by linear and nonlinear forms of selection across multiple life stages.

Marine invertebrates offer a novel opportunity to measure selection on size and development time, especially at early life stages (e.g. embryos and larvae). Trait expression during these stages is not only important for an individual's immediate survival, but also carries over to impact the performance and dynamics of adult populations (Marshall and Morgan 2011, Foo and Byrne 2016). Furthermore, most marine invertebrates reproduce via external fertilisation (Monro and Marshall 2015), whereby fertilisation and development outside of the parent's body allows size and development time during early life stages to be measured more easily and precisely than in most other taxa. Here, using a natural population of the ascidian, *Pyura dalbyi*, we assessed how initial (embryo) size, development time (from fertilisation to hatching), and subsequent size (larval size at hatching) impacted fitness (in terms of juvenile survival) in the field. We then implemented Lande and Arnold's (1983) multiple regression framework to disentangle the direct and indirect forces of selection on each trait. We found that selection directly targets development time and larval size through their independent effects on survival, but embryo size incurs only indirect selection through correlations with those traits. Hence, in *Pyura* at least, development time may be more than just an evolutionary by-product of initial size. Rather, it may evolve adaptively as a direct target of selection, and shape the evolution of initial size in doing so.

Methods

Study species

Pyura dalbyi (henceforth referred to by genus) is a solitary, habitat-forming ascidian native to the southern Australian coast (Rius et al. 2017). Adults are hermaphroditic, but self-sterile, and reproduce via broadcast spawning with external fertilization (Lambert 2005). After fertilisation, embryos hatch into non-feeding tadpole larvae, which settle and undergo metamorphosis to form sessile juveniles. Juveniles must then survive for ~3 months to reach reproductive maturity (Rius et al. 2017). We haphazardly collected adult *Pyura* for our study from a population at Blairgowrie, Victoria, Australia, between September and October 2016.

Fertilization protocol

We produced focal individuals in five replicate blocks of *in vitro* fertilizations between collected adults. All steps in producing and phenotyping focal individuals were done at 15°C, which was within ~1°C of natural conditions at our field site during the study (CSIRO 2018). Each adult was used solely as a sire or dam (i.e., no individual contributed both sperm and eggs to fertilizations). To maintain genetic variation within each block and reduce any effects of sire-dam genetic compatibility (Chirgwin et al. 2017), we crossed sperm from seven sires with eggs from seven dams, producing up to 49 families per block.

We collected gametes for fertilizations by dissecting adults to expose their gonads, which we carefully dissected to liberate ripe eggs and sperm. To isolate each dam's eggs, eggs were placed in a 100μm filter pot and gently rinsed 3 times with 1.5L of filtered seawater, washing unwanted sperm (which were not retained by this mesh size) into a beaker below. To isolate each sire's sperm, macerated testes were placed in a 50μm filter pot and gently rinsed with 5mL of filtered seawater, concentrating sperm in a beaker below whilst retaining unwanted eggs in the filter pot.

Once gametes were collected, we pooled 0.1mL of sperm from each of the seven sires per block in a single vial before diluting the solution threefold (which maximized fertilization success in pilot

studies). Egg concentration has little influence on fertilization success (Levitan et al. 1991, Chirgwin et al. 2018), so we simply pooled eggs from all seven dams, before pipetting ~ 300 eggs into a 5mL vial with filtered seawater and adding 1 mL of the diluted sperm solution. The resulting gamete solution was agitated every 5 minutes (to prevent oxygen limitation) for 15 minutes before being rinsed through a 100μm filter to remove excess sperm (as above). Viable embryos began cleaving ~2 hours after fertilization, and were haphazardly selected as the focal individuals for our study.

Measurements of traits in focal individuals

For each focal individual, we measured embryo size, development time (from fertilization until hatching into a free-swimming tadpole larva), and larval size. To measure these traits, we pipetted each individual embryo into its own individual well of a 96-well plate, placed on a microscope with a motorized stage. To track each embryo's development, a digital camera mounted on the stage photographed it every 6 minutes for the next 21 hours (based on pilot data, embryos that were unhatched after this time never hatched). These photographs allowed us to measure embryo size (as area at the 16-cell stage) and development time, along with larval size (as area) after hatching. After hatching, focal individuals were pipetted into their own 60mm petri dishes of fresh seawater, lined with acetate covered by natural biofilm from our field site, and left in darkness for 25 hours to settle and metamorphose into sessile juveniles. Settlement was checked every 5 hours.

Field measurements of survival of focal individuals

After settlement, we held individuals in new petri dishes of fresh seawater for another 12 hours to ensure they were securely attached to the acetate before being deployed in the field. Each piece of acetate with a settled individual was then glued onto a labelled PVC plate (55 × 55 × 3 mm), which was randomly arranged on a larger PVC backing panel (570 × 570 × 6 mm). Each experimental block was deployed on a separate backing panel. Backing panels were then suspended 2m below the water surface, with focal individuals facing down to match *Pyura's* natural environment and orientation. Their survival was scored twice a week for 4 weeks, then weekly for another 7 weeks. We ceased scoring survival at 11 weeks, since >90% of juveniles had died and visible signs of fish predation

meant that variation in survival could no longer be reasonably attributed to variation in measured traits. We tracked the survival of 186 focal individuals in total.

Data analyses

First, we estimated individual-level correlations between our three measured traits to explore the potential for indirect selection on them. We fitted a multivariate linear mixed model using restricted maximum likelihood, with traits as response variables, focal individuals as a random effect and experimental blocks as a fixed effect. We tested if each correlation differed significantly to 0 using log-likelihood ratio tests based on χ^2 distributions.

Next, to test for selection acting on the three measured traits through their impacts on juvenile survival, we fitted mixed-effect logistic regression models using maximum likelihood with Laplace approximation, and tested the significance of regression coefficients using χ^2 tests (Bolker et al. 2009). We modelled survival as a binomial response, standardised traits (expressed in units of standard deviation) as continuous effects, time when survival was measured and block as categorical fixed effects, and individual as a random effect on which survival was measured repeatedly over time. All P-values for time × trait interactions on survival exceeded 0.5, indicating that selection on traits was temporally constant. We disentangled direct and indirect selection using a two-step approach. Our first step was to fit first- and second-order simple regressions to each of our three traits separately, thereby testing the total effects of directional and quadratic selection (direct selection plus indirect selection acting through trait correlations) on each trait (Lande and Arnold 1983). Our second step was to fit a first- and second-order multiple regression (with the latter model including all squared and cross-product terms) to all three traits at once, thereby testing the significance of partial regression coefficients that describe only the direct effects of directional and nonlinear (quadratic and correlational) selection on each trait, corrected for correlations with other traits (Lande and Arnold 1983).

Note that the logistic regression framework above simply modifies the classic linear regression framework of Lande and Arnold (1983) to accommodate binomial fitness measures, as required for valid significance tests of selection acting on standardised traits (Janzen and Stern 1998). Nonetheless, fitting equivalent models using linear regression is still necessary for numerical estimates of selection, and also the curve fits needed to fully interpret its form (Janzen and Stern 1998). We therefore fitted the equivalent linear regression models to our standardised traits after making survival relative (i.e., dividing each absolute value by the experiment-wide mean). In the absence of random effects, each regression coefficient would usually estimate selection as the change in relative fitness resulting from 1 standard deviation of change in a trait (Kingsolver and Pfennig 2007). In our case, however, models included individual as a random effect to account for repeated measures of survival. This affects the scales of our selection coefficients (which should not be interpreted on the usual scale), but not their relative magnitudes, nor the form of selection inferred by visualising the regression curve (or surface) they describe. We visualised this surface using a thin plate spline (Brodie et al. 1995), after doubling quadratic coefficients to obtain the appropriate estimates of quadratic selection relative to other forms (Stinchcombe et al. 2008).

Results

Phenotypic correlations between size and development time

Larger embryos took longer to develop ($r = 0.17$, $p=0.03$) and hatched into larger larvae ($r = 0.31$, $p<0.01$), but larval size and development time were not significantly correlated ($r = 0.08$, $p=0.29$).

Disentangling direct and indirect selection on size and development time

Phenotypic selection analyses of these traits indicated that development time and larval size are directly targeted by directional and nonlinear selection through their impacts on survival, but embryo size is not (Table 1). A positive directional selection coefficient for embryo size, coupled with a negative quadratic (i.e., convex) one, implied that larger embryos survive better than smaller ones, at least up to a point (Table 1a). However, this apparent selection on embryo size was the product of indirect selection generated by correlations with other traits, given the non-significant directional and

quadratic selection coefficients for embryo size after correcting for trait correlations (Table 1b). Indirect selection contributed less to selection on development time and larval size, since estimates of total and direct selection on these traits were consistently significant and roughly similar in size (Table 1).

Direct effects of selection on development time and larval size

Directional selection acting directly on development time and larval size independently targets increases in both traits (Table 1b), but is not open-ended for either one. Rather, a negative quadratic coefficient for development time implies that survival is maximized at an intermediate trait value (potentially indicating stabilizing selection; Table 1b), while a positive quadratic coefficient for larval size implies that survival is maximized at extreme trait values (potentially indicating disruptive selection; Table 1b). Moreover, development time and larval size do not affect survival independently, given a significantly negative correlational selection coefficient for these traits (Table 1b). This coefficient is in the opposite direction to the correlation between traits (which is non-significant but weakly positive), implying that selection acts to completely decouple them completely, or to shape a negative association between them.

We also visualized the overall selection surface for development time and larval size, to evaluate whether the quadratic coefficients for these traits coincided with intermediate maxima or minima within the current ranges of trait values (as necessary to classify selection as stabilizing or disruptive; Mitchell-Olds and Shaw 1987), and to gain a clearer picture of the correlational selection acting on them. This surface (Fig. 1) showed that survival was maximised at an intermediate development time (implying stabilizing selection on development) and at extremely small or large larval sizes (implying disruptive selection on size). Taken for both traits together, the selection surface is a saddle shape, implying that multiple fitness peaks and troughs exist within the range of trait combinations sampled. More specifically, selection actively disfavours larvae that hatch at slightly above the average size after extremely long development, or slightly below the average size after extremely short development (Fig. 1). Such phenotypes dramatically reduce the odds of survival, causing individuals

to fall below the central part of the surface where fitness initially plateaus (Fig. 1). Above this plateau, larvae gain a further advantage by hatching at extremely large sizes after developing for slightly less than the average time, or extremely small sizes after developing for slightly more than the average time (Fig. 1).

Table 1. Coefficients of directional, quadratic, and correlational (nonlinear) selection on embryo size, development time, and larval size in *Pyura*. (a) Total selection, combining direct selection on traits with indirect selection acting through trait correlations. (b) Direct selection on traits, corrected for trait correlations. All estimates are ± 1 standard error, with bold text indicating $P < 0.05$.

	(a) Total selection		***(b) Direct selection***			
	directional	*quadratic*	*directional*	*quadratic and correlational*		
				ES	DT	LS
embryo size (ES)	**0.06 ± 0.02**	**-0.08 ± 0.02**	0.02 ± 0.02	-0.06 ± 0.04		
development time (DT)	**0.05 ± 0.01**	**-0.06 ± 0.02**	**0.04 ± 0.02**	0.02 ± 0.02	**-0.06 ± 0.02**	
larval size (LS)	**0.10 ± 0.02**	**0.06 ± 0.02**	**0.09 ± 0.02**	0.02 ± 0.02	**-0.12 ± 0.02**	**0.10 ± 0.04**

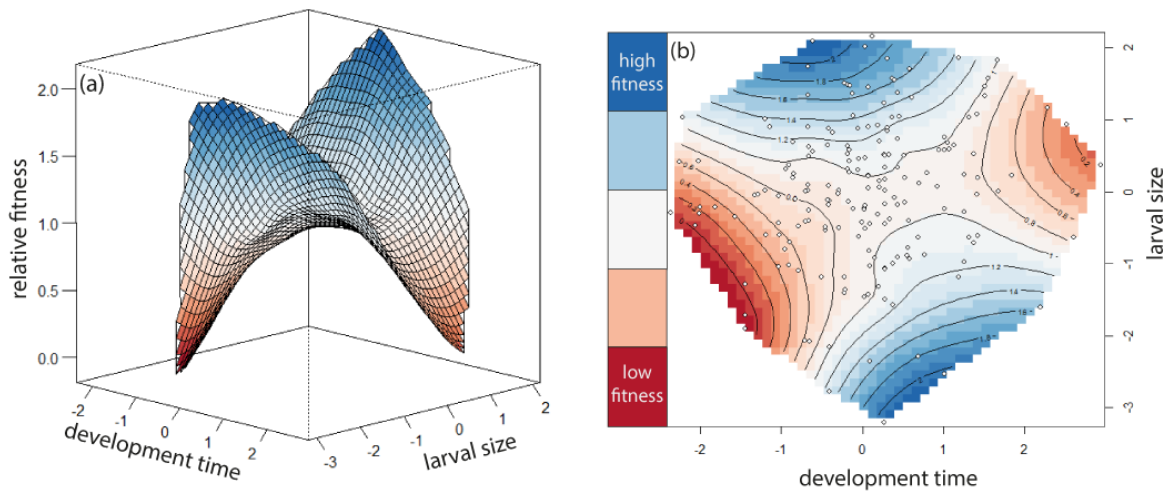

Figure 1. The selection surface for development time and larval size in *Pyura*. The three-dimensional surface on the left (a) was fitted using a thin-plate spline. The contour plot on the right (b) is the same surface viewed from above. Plotted points are predicted values for individuals. Traits are mean-centred and in units of standard deviation, so that 0 marks the mean trait value on each axis.

Discussion

Evolutionary trends in development time are often regarded as allometric and physiological by-products of initial size (Gillooly et al. 2002 and references therein). In contrast, life history theory predicts that size and development time are both targets of selection and may thus evolve adaptively, outside of allometric or physiological constraints, through opposing effects on individual fitness (Stearns 1992). Surprisingly, this prediction has rarely been tested in empirical studies that explicitly disentangle the direct and indirect effects of size and development time on fitness, as necessary to understand how selection acts on these traits. Here we used *Pyura*, an externally-fertilising marine invertebrate that provides a novel opportunity to measure how selection targets such traits at key early life stages. We show that selection, acting through juvenile survival in the field, directly targets longer development time (fertilisation to hatching) and larger (subsequent) size at hatching, while initial (embryo) size incurs only indirect selection from correlations with those traits. Selection also targets combinations of development time and subsequent size, whereas a positive correlation between initial size and development time is selectively neutral. Therefore, development time in *Pyura* may evolve as a direct target of selection to enhance juvenile survival, independently of initial size but in combination with subsequent size. Further, that selection does not target initial size and development time as life history theory predicts, suggests that their positive correlation could well be due to allometric and physiological constraints or to additional episodes of selection earlier in the life cycle.

Disentangling direct and indirect selection, acting here through juvenile survival, revealed that directional selection on subsequent size and development time is driving indirect selection on initial size. While life history theory predicts selection on size should also drive indirect selection on development time (Roff 2000, Kingsolver and Huey 2008), we found selection on subsequent size does not indirectly affect selection on development time, and initial size is not directly targeted by selection. As such, if patterns of directional selection remain stable and trait correlations have a heritable basis, we would predict initial size to only increase as a correlated response to adaptive increases in subsequent size and development time. However, it is worth noting that initial and

subsequent size may also experience indirect selection due to selection on egg size, which was not measured here. Egg size in marine invertebrates usually correlates with size during early offspring stages (Marshall and Keough 2008), and can be targeted by selection through its effects on the fertilisation success of females (Levitan 1993, Marshall et al. 2002). Hence, episodes of selection during fertilisation may act on egg size, which could have some indirect influence on the evolution of the two size traits measured here.

The directional selection detected here show there are independent benefits of taking longer to develop, and of being larger afterwards. While selection for longer development is contrary to several previous studies (Kingsolver and Pfennig 2004), these predominantly focused on development from juvenile stages to maturity, where shorter development provides fitness benefits via earlier mating. Nevertheless, longer development time may benefit juvenile survival by reducing errors in DNA replication and repair (Karr and Mittenthal. 1992), or developmental defects (Arendt 1997). Further, that longer development is beneficial to juvenile survival, may also explain why variation in development time is maintained in marine species, such as *Pyura*, with a planktonic phase in their life cycle. Here, we insulated individuals from mortality during planktonic phases (fertilisation to settlement) to measure their traits, but these phases are naturally periods of intense mortality due to predation and currents carrying individuals away from settlement habitats (Morgan 1995). In turn, episodes of selection during planktonic phases are expected to favour a rapid development that allows individuals to escape this period of high mortality sooner (Hirst and Lopez-Urrutia 2006). Despite this, marine populations still maintain abundant variation in planktonic development time, indicating a rapid development has counterbalancing costs during post-planktonic stages (Strathmann et al. 2002). Here we found evidence of such costs, since shorter development leads to lower post-planktonic (i.e. juvenile) survival. Consequently, variation in development time is potentially maintained by opposing selection pressures acting on planktonic and juvenile stages.

Selection for larger subsequent size at the end of embryonic development matches expectations. In many taxa, including mammals, birds, reptiles, fish, and invertebrates, individuals that are larger at the

end of a developmental period go on to survive, grow, or reproduce better (Baker and Fowler 1992, Janzen 1993, Jarrett and Pechenik 1997, Naef-Daenzer et al. 2001, Vigliola and Meekan 2002). In *Pyura*, whose larvae are non-feeding and rely on maternally-derived energy resources to survive early stages, individuals that are larger at the end of embryonic development are likely to incur two key benefits: more resources and more efficient use of those resources (Marshall et al. 2018). Both mechanisms were recently demonstrated in other marine invertebrates with non-feeding larvae, whereby larger individuals of *Bugula neritina* and *Watersipora subtorquata* began larval stages with more energy resources and used proportionally less of their resources by the end of these stages (Pettersen et al. 2015).

We also found that development time and subsequent size have direct, nonlinear effects on juvenile survival, creating a saddle-shaped fitness surface that promotes the evolution of alternative combinations of these traits. Curiously, the saddle indicates that hatching at larger sizes after extremely long development lowers survival, which is at odds with the pattern of directional selection on each trait alone. This trait combination could possibly reduce survival because extremely long embryonic development times often deplete the energetic reserves (i.e., yolk) available for larvae to continue development after hatching (Semmens and Swearer 2011, Trabelsi et al. 2016, Pettersen et al. 2019). Regardless of the underlying mechanisms, the two fitness peaks on the saddle promote two alternative trait combinations: large size after an average development time, or small size after a long development time. Interestingly, both peaks are only attainable if the two traits are independent or negatively correlated, meaning that selection may be acting to decouple the positive correlations between size and development time expected from allometric or physiological constraints alone (Gillooly et al. 2002). Thus, if selection remains stable and constraints are absent, the bivariate mean for development time and size should gradually evolve up one of the two peaks (Lande 1976, Wright 1977, Brodie 1992). Conversely (and perhaps less likely here), saddle-shaped fitness surfaces may facilitate population divergence and even speciation, if other factors cause evolution towards both fitness peaks simultaneously (Sinervo 2001, Calsbeek and Irschick 2007).

Given we detected no sign of selection on combinations of initial size and development time, the positive correlation between them could be a result of physiological and allometric constraints. Larger organisms typically have lower mass-specific metabolic rates, and may therefore be constrained to have longer development as a result (Gillooly et al. 2002). However, the correlation between initial size and development was also quite weak, implying that ample scope remains for the two traits to evolve independently of each other. Within populations, moreover, initial size and development time can covary positively (Ficetola and De Bernardi 2006, Marshall and Bolton 2007), negatively (Sinervo and McEdward 1988, Rius et al. 2010), or not at all (Kingsolver et al. 2012, Pettersen et al. 2018), implying that allometric or physiogical constraints do not ultimately constrain the relationship between these traits to be positive.

The question remains of what, exactly, drives the phenotypic correlation between size and development time to vary in strength and direction among populations (Pettersen et al. 2018). An important future step will be to assess how environment factors simultaneously affect the expression of these traits, and the patterns of selection acting on them. For instance, size and development time both show enormous phenotypic plasticity in response to temperature (Kingsolver and Huey 2008, Pettersen et al. 2019), yet the stability of selection on both traits across different temperatures, and whether thermal plasticity may itself be targeted, is unclear. Additionally, the phenotypic correlations measured here do not necessarily reflect heritable (i.e., additive genetic) correlations (Lynch and Walsh 1998). Thus, size and development time may be genetically constrained in their ability to evolve in response to the patterns of selection detected here (Walsh and Blows 2009, Chirgwin et al. 2015). While we are unaware of any study that has formally measured genetic correlations between size and development time, Chippindale et al. (1997) found that selection on development time over several generations in *Drosophila* produced a correlated response in size, suggesting a genetic correlation with development time.

Overall, our study shows that development time can directly impact fitness, as life history theory has long predicted (Stearns 1992, Dmitriew 2011). Here, we show that development time is not only a

direct target of selection in its own right, but affects how selection acts on size before development, and contributes to nonlinear selection on size after development. Therefore, appreciating how microevolutionary processes like selection act alongside the allometric or physiological constraints predicted from macroevolutionary patterns (Gillooly et al. 2002), is crucial to achieving the most complete understanding of the evolution of size and development time.

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Chapter 6

Dual physical and physiological impacts of ocean warming alter phenotypic selection on sperm morphology

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Abstract

Fertility is a key component of individual fitness and vital for population persistence. For males, fertility relies on the ability of their sperm to collide and fuse with eggs; consequently, sperm morphology is predicted to be a prime target of selection owing to its effects on male fertility. Projected ocean warming will expose the gametes of externally-fertilising marine species to the physiological effects of higher temperature and the physical effects of lower viscosity, but the consequences for fertility, and for selection acting on sperm traits to maximize fertility, are virtually unexplored. Here, by disentangling the effects of temperature and viscosity on the key process of fertilisation in an externally-fertilising marine tubeworm, we show that projected changes in both factors independently reduce male fertility, and act in combination to alter selection on sperm morphology. Specifically, the higher temperatures and lower viscosities of fertilisation environments under projected warming alter selection on the sperm midpiece, doing so in ways that suggest shifts in the energetic challenges of functioning under stressful conditions. Selection also targets sperm head dimensions and tail length, but irrespective of fertilisation environment. Our findings provide the first evidence that projected changes in ocean temperature and viscosity will not only impact the fertility of marine external fertilisers, but expose their gametes to novel selection pressures that may drive them to adapt in response.

Introduction

Rapid global warming is exposing natural populations to novel selection pressures on various phenotypes (Hoffmann and Sgrò 2011). Phenotypic selection occurs when individuals with different trait values (phenotypes) differ in survival, reproduction, or mating success (fitness), and is the driver of evolutionary adaptation in natural populations (Lande and Arnold 1983, Kingsolver and Pfennig 2007). The strength, direction, and form of phenotypic selection can be altered by environmental factors (agents of selection) that modify relationships between phenotypes and fitness (Wade and Kalisz 1990, Siepielski et al. 2017). For example, rising temperatures and altered patterns of rainfall have led several populations to experience novel selection for earlier times of flowering and mating (Franks et al. 2007, Gienapp et al. 2014). In many cases, however, stressful changes in environment may simply cause reductions in fitness without altering selection (Arbuthnott and Whitlock 2018). Beyond a few notable exceptions like those above, we still have a poor grasp on which traits are likely to experience novel selection pressures under projected warming, and therefore which traits will matter most for future adaptation.

Fertility, describing the ability of individuals to produce viable offspring, is a key component of fitness and vital for population persistence (Walsh et al. 2019). For males, fertility relies on the ability of their sperm to collide and fuse with eggs; hence, selection has ample scope to target sperm traits that are involved in this process of fertilisation (Simmons and Fitzpatrick 2012, Reinhardt et al. 2015). Indeed, in a range of taxa, aspects of sperm morphology (e.g., head and tail length) are known to have important effects on sperm performance through effects on sperm swimming speed and longevity (Malo et al. 2006, Humphries et al. 2008, Simpson et al. 2014), and ultimately on male fertility (Devigili et al. 2015, Monro and Marshall 2016, Lymbery et al. 2018). However, despite growing evidence that sperm performance will be susceptible to projected warming (Adriaenssens et al. 2012, Duarte et al. 2013, Sales et al. 2018, Walsh et al. 2019), how selection on sperm traits will be affected under such conditions has gone untested.

Global warming is likely to have especially strong impacts on how selection targets the free-

swimming sperm of many marine species (Walsh et al. 2019). Unlike most terrestrial species, whose sperm and eggs interact entirely within the female reproductive tract and are thereby buffered against external conditions, most marine species spawn both gametes into the aquatic environment (Crimaldi and Zimmer 2014, Monro and Marshall 2015). This means that free-swimming sperm must not only overcome the challenges of locating and fusing with eggs in the external environment, but must also do so while contending with the harshness of that environment (Levitan 1996). In turn, spawned eggs are often left unfertilised due to sperm limitation, which arises from the rapid diffusion of sperm by currents. This is in marked contrast to internal fertilisers, where sperm nearly always compete for a limited number of eggs and egg wastage is rare (Levitan 1993, Simmons and Fitzpatrick 2012, Liao et al. 2018). Notably, projected levels of ocean warming have already been found to reduce the motility of free-swimming sperm (Duarte et al. 2013), and also fertility itself in marine populations (Parker et al. 2009, Foo et al. 2016, but see Byrne 2011). Yet the potential for warming to alter how selection acts on traits of free-swimming sperm through their links to fertility is unknown.

Warming in marine environments will expose populations to not only the physiological effects of higher temperatures, but also the physical effects of lower viscosities. Viscosity describes a fluid's resistance to flow (it is what makes honey pour slower than water), and most liquids are less viscous under higher temperatures (Dorsey 1968). Viscosity has an immense impact on the movement of small objects like sperm, because they move through a liquid world where viscosity dominates over their own inertia (Purcell 1977, Podolsky and Emlet 1993). A sperm's motility at any given moment in time is therefore the net outcome of the thrust produced by its tail and the drag produced by viscous forces acting on its surface area (Humphries et al. 2008). Consequently, the physiological effects of warming might retard the ability of sperm to produce thrust, because their tails are powered by mitochondria that can only function within limited temperature ranges (Blier et al. 2013). However, the physical effects of warming might increase sperm motility by weakening drag under lower viscosities (Kupriyanova and Havenhand 2005), thereby countering the negative impacts of higher temperatures on sperm function (Byrne et al. 2010). Despite the importance of water viscosity for sperm motility, the potential for lower viscosity to mediate changes in selection on free-swimming sperm under

projected warming has gone virtually unconsidered.

Here, we tested how changes in water temperature and viscosity under projected ocean warming alter selection on the morphology of free-swimming sperm in an externally-fertilising marine tubeworm. To do so, we independently manipulated water temperature and viscosity to create multiple fertilisation environments reflecting current-day conditions plus progressive levels of near-term and longer-term warming. In a split-ejaculate design, we measured the fertility of individual males in each fertilisation environment, then used phenotypic selection analyses (Lande and Arnold 1983) to assess how selection targets sperm morphology owing to its impacts on male fertility, and whether this selection differs environmentally. Our study provides the first empirical evidence that projected ocean warming will alter phenotypic selection on free-swimming sperm through dual effects of higher temperature and lower viscosity. In doing so, we reveal new insights into how marine populations may undergo evolutionary adaptation to maintain fertility under future warming.

Methods

Study species and collection site

Galeolaria caespitosa (henceforth described by genus name) is a calcareous tubeworm native to rocky shores of southeastern Australia, where it acts as an ecosystem engineer by forming dense colonies of adult tubes that provide habitat for endemic communities (Cole et al. 2017, Wright and Gribben 2017). Adults are dioecious and remain fertile year-round, continually spawning their gametes to collide and fuse in the water column before being dispersed by currents (Kupriyanova et al. 2001, Chirgwin et al. 2018). This mode of fertilisation means that water around eggs might contain sperm from multiple males or only one male, and that eggs might often go unfertilised due to sperm limitation (Levitan 1993, Hollows et al. 2007, Liao et al. 2018). Consequently, selection can target gamete phenotypes, including egg size and sperm morphology, that increase fertility through their effects not only on gamete competition but also on gamete encounter rates (Monro and Marshall 2016, Liao et al. 2018).

We sampled a population of *Galeolaria* from the intertidal zone at Chelsea (Victoria, Australia) from

March to April 2017, transferring individuals to Monash University in insulated aquaria for use in experimental work. We induced adults to spawn by removing them from their tubes and placing them in petri dishes with ~1 mL of filtered seawater at ~16.5°C (matching natural conditions). Gametes were collected immediately after spawning. We reserved a 10uL aliquot of sperm from each male's ejaculate for phenotyping of morphological traits (see below), and to measure initial sperm concentration from replicate haemocytometer counts. The remaining portion was used in fertility trials.

Manipulation of fertilisation environment

To test if temperature and/or viscosity alter selection on sperm morphology, we manipulated each factor independently to create five experimental fertilisation environments (see below), and trialled the fertility of 157 focal males of known sperm phenotype in each environment (Fig. 1). Trials were conducted in 16 replicate blocks, with 7-11 males per block, and each male's sperm was used in two replicate trials per environment. Each trial involved a single male's sperm exposed to a replicate cohort of eggs, pooled across 20 females to minimize male-female compatibility effects on male fertility. Care was taken to ensure that females contributed similar numbers of eggs to the cohort. Within blocks, males were mated with the same pool of females so that trials had similar genetic backgrounds of eggs, and differed only in male identity and our manipulation of fertilisation environment.

(a) Temperature. Fertility was trialed at three temperatures representing current conditions, plus two progressive levels of projected warming at our study site. Here, sea-surface temperature has ranged from 9 to 25°C over the previous decade, averaging ~16.5°C annually and ~20.5°C in summer (CSIRO 2018). Temperature is projected to increase ~2°C by 2050, and ~3°C by 2070 (Hobday and Lough 2011, Mills et al. 2013). As such, we conducted fertilisation trials at three temperatures: 16.5°C (the current annual mean), 21°C (a moderate level of warming, already common during summer months and projected to become more frequent in the near term), and 25°C (an extreme level of warming, currently rare but projected to become more frequent in the longer term). Temperature

environments were created and maintained using dry-bath incubators (MajorScience 2015).

(b) Viscosity. We manipulated viscosity independently of temperature using the hydrophilic polymer, Ficoll 400 (Winet 1976), which permits warmer water to be adjusted to the same viscosity as cooler water. Importantly, Ficoll is biologically inert (Berg and Turner 1979), and non-toxic to *Galeolaria* and several other marine species (Bolton and Havenhand 1998, Kupriyanova and Havenhand 2005, Orchard et al. 2016). Therefore, Ficoll is highly unlikely to have had a direct physiological effect on sperm or the fertilisation process, beyond the desired affect via its changes to water viscosity. To find concentrations of Ficoll that adjusted seawater to the desired viscosities, we conducted a pilot study using an Ubbelodhe viscometer (Chirgwin, *unpubl data*). Based on this study, we supplemented the three temperature environments above with two viscosity-modified environments: 21°C$_{viscosity\ at\ 16.5°C}$ (seawater at 21°C adjusted to viscosity at 16.5°C using 0.64% w/v Ficoll), and 25°C$_{viscosity\ at\ 21°C}$ (seawater at 25°C adjusted to viscosity at 21°C using 0.58% w/v Ficoll).

Physical and logistical constraints prevented us from creating all combinations of temperature and viscosity, so we prioritised the most ecologically-relevant combinations. We could not manipulate temperature and viscosity reciprocally, since cooler water cannot be adjusted to the same viscosity as warmer water, and we did not create an environment where seawater had an extreme temperature adjusted to current viscosity, since populations will need to adapt to moderate warming before adapting to more extreme warming (Bell 2013). Therefore, we assessed the effects of temperature and viscosity in a stepwise manner over these two progressive levels of warming. Specifically, we contrasted the effects of current and moderate warming on fertility in terms of: 1) the combined effects of temperature and viscosity, done by comparing fertility trials at 16.5°C$_{normal\ viscosity}$ and 21°C$_{normal\ viscosity}$; 2) the effect of temperature in isolation, done by comparing trials at 16.5°C$_{normal\ viscosity}$ and 21°C$_{viscosity\ at\ 16.5°C}$; and 3) the effect of viscosity in isolation, done by comparing trials at 21°C$_{normal\ viscosity}$ and 21°C$_{viscosity\ at\ 16.5°C}$. Likewise, we contrasted the effects of moderate and extreme warming on fertility in terms of: 1) the combined effects of temperature and viscosity, done by comparing trials at 21°C$_{normal\ viscosity}$ and 25°C$_{normal\ viscosity}$; 2) the effect of temperature in isolation, done by comparing trials

at 21°C$_{normal\ viscosity}$ and 25°C$_{viscosity\ at\ 21°C}$; and 3) the effect of viscosity in isolation, done by comparing trials at 25°C$_{normal\ viscosity}$ and 25°C$_{viscosity\ at\ 21°C}$.

Measurement of sperm morphological traits

Sperm morphological traits were measured using the reserved aliquot of each male's sperm. We photographed at least 15 normal, mature cells per male at 1000× magnification, ensuring the full cell was in the plane of focus, and tracing the tail during live capture to account for any flexing that may have been difficult to interpret from stored images. We used the image processing software, ImageJ (http://imagej.nih.gov/ij), to measure five traits: length and width of the sperm head (comprised of an oval nucleus and cap-like acrosome), length and width of the sperm midpiece (a ring of four spherical mitochondria at the base of the head), and length of the sperm tail (Jamieson and Rouse 1989, Monro and Marshall 2016). We then calculated each trait's mean value per male for use in analyses. Using male trait means assumes that sperm phenotypes are effectively diploid (i.e., they express the diploid genomes of males rather than the haploid genomes of sperm), as is expected to be the case in most species (Joseph and Kirkpatrick 2004, Dapper and Wade 2016). Analytically, it assumes that trait means are fixed variables measured without error, which may bias estimates of selection coefficients towards zero, but does not bias the hypotheses tests of them that were our primary interest (Quinn and Keough 2002).

Fertility trials

We initiated each fertility trial by adding 0.1 mL of egg solution (~10^3 eggs) to a vial containing 1.0 mL of sperm solution (10^4 sperm). These densities of gametes fall within the continuum present in the field and yield fertility rates that are comparable to field estimates (Hollows et al. 2007). Before mixing, sperm and egg solutions were separately ramped to the desired temperature over ~15 minutes. Since sperm are activated by dilution, we ramped them undiluted (at > 10^7 sperm mL^{-1}) to minimize ageing (holding sperm undiluted over the ramping period had no effect on male fertility; E. Chirgwin *unpubl. data*). We then diluted each vial of sperm to the final volume and density with seawater, which contained Ficoll as necessary to set each trial to the desired viscosity once the egg solution was

added. Vials were agitated every 15 minutes to reduce oxygen depletion, although pilot work showed that gametes have negligible effect on dissolved oxygen under the conditions used here (E. Chirgwin *unpubl. data*).

Next, we scored fertility in each trial by counting the number of normally-cleaving embryos relative to unfertilised eggs. Since temperature affected cleavage rates, we assessed fertility based on developmental stage (when embryos had 2–8 cells), as opposed to time since fertilisation. To do so, we used Lugol's solution to fix each trial when appropriate (~60 mins at 25°C, ~80 mins at 21°C, and ~100 mins at 16.5°C) (Engell-Sørensen et al. 2012), photographed a sample of ~80 embryos and/or eggs per trial, then scored fertility from photographs.

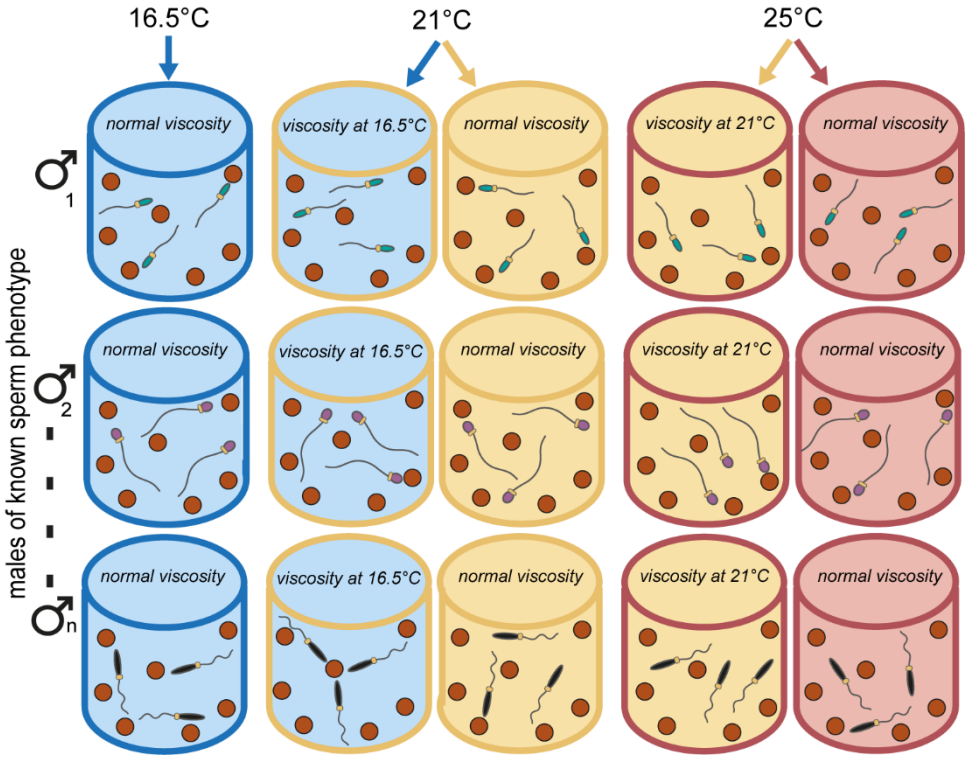

Figure 1. Experimental manipulation of fertilisation environment for trials of male fertility. Trials were conducted in each of five fertilisation environments that differed in temperature and/or viscosity, and crossed individual males of known sperm phenotype with cohorts of eggs pooled from multiple females. Each male had two replicate trials (not shown) per environment. Trials were conducted in 16 replicate blocks, with 7-11 males trialled against cohorts of eggs from the same set of females per block, and 157 males trialled overall.

Data analyses

First, we tested if male fertility was affected by overall warming (the combined effects of temperature and viscosity), plus each effect of temperature and viscosity in isolation. We did so by analysing fertility in a linear mixed-effects model fitted using maximum likelihood (model 1), with fertilisation environment as a fixed effect, plus block and male identity as random effects. The latter was modelled as a diagonal matrix, estimating the male variance in fertility within environments while accounting for the non-independence of trials from the same male. Note that we could not formally test for a temperature × viscosity interaction because we could not create all reciprocal combinations of temperature and viscosity (see above). We used a χ^2 test to determine if fertilisation environment had a significant effect on male fertility overall, and then used planned contrasts of pairwise means (Quinn and Keough 2002) to compare fertility between the specific pairs of environments specified above.

Next, to estimate selection on sperm traits owing to their impacts on male fertility, we implemented Lande and Arnold (1983)'s multiple regression approach in a mixed model framework. In this framework, we also tested whether selection coefficients (i.e., partial regression coefficients for trait effects on fertility) differed across fertilisation environments, using likelihood-ratio tests to compare the fits of models estimating coefficient × environment interactions to models with coefficients held constant across environments. Since we could not formally estimate coefficient × temperature × viscosity interactions (see above), we constructed a 2-step process to test whether coefficients were altered by overall warming, and then by temperature and viscosity in isolation.

In step 1, we tested if directional (i.e., linear) selection on sperm traits was altered by overall warming using data from fertility trials conducted with normal viscosities at 16.5°C, 21°C, and 25°C. First, we analysed relative fertility (absolute fertility scaled by the relevant environmental mean) in a model with the same random effects as model 1, but including a linear term for each of our five sperm traits. Next, we compared the fit of this model to one that also included trait × environment effects on relative fertility. Because this comparison showed that directional selection on sperm traits was

significantly altered by overall warming, we identified which selection coefficients differed among the three environments, then used planned contrasts of coefficients (akin to planned contrasts of means; Quinn and Keough 2002) to compare them between specific pairs of environments. To do so, we tested if model fit was significantly reduced by pooling selection coefficients across environments representing current and moderate warming (16.5°C$_{normal\ viscosity}$ and 21°C$_{normal\ viscosity}$), or across environments representing moderate and extreme warming (21°C$_{normal\ viscosity}$ and 25°C$_{normal\ viscosity}$).

In step 2, we tested if the differences in directional selection detected in step 1 were due to temperature and/or viscosity in isolation. If step 1 showed that directional selection on sperm traits differed between environments representing current and moderate warming, we took data from fertility trials conducted at 16.5°C$_{normal\ viscosity}$, 21°C$_{viscosity\ at\ 16.5°C}$, and 21°C$_{normal\ viscosity}$, then tested if pooling selection coefficients across temperatures (16.5°C$_{normal\ viscosity}$ vs 21°C$_{normal\ viscosity}$) or viscosities (21°C$_{viscosity\ at\ 16.5°C}$ vs 21°C$_{normal\ viscosity}$) resulted in significant loss of fit compared to modelling separate selection coefficients for all three environments. Likewise, if step 1 showed that directional selection on sperm traits differed between environments representing moderate to extreme warming, we used the same process with data from fertility trials conducted at 21°C$_{normal\ viscosity}$, 25°C$_{viscosity\ at\ 21°C}$, and 25°C$_{normal\ viscosity}$.

Finally, we repeated this 2-step process for quadratic and correlational (i.e., nonlinear) selection coefficients, added to the previous model of directional selection as squared and cross-product terms for sperm traits, respectively. Quadratic coefficients measure curvature in the relationship between each trait and fertility, indicating selection for intermediate or extreme phenotypes, while correlational coefficients measure the bivariate effects of sperm traits on fertility, indicating selection for specific trait combinations (Lande and Arnold 1983). Note that in the absence of random effects, each regression coefficient would usually estimate selection as the change in relative fertility resulting from one standard deviation of change in a trait (Kingsolver and Pfennig 2007). In our case, however, models included male as a random effect to account for repeated measures of fertility on the same male. This affects the scales of our selection coefficients, but not their relative magnitudes, nor the

form of selection inferred by visualising the regression curve they describe. We visualised directional selection using cubic splines and nonlinear selection using thin plate splines.

Results

Effects of temperature and viscosity on male fertility

We found a significant effect of fertilisation environment on male fertility (χ^2= 147.74, d.f.=4, p<0.01), which planned contrasts attributed to significant reductions under increasing temperature or decreasing viscosity (all p<0.01). Specifically, the isolated effect of either temperature or viscosity caused fertility to decline by ~5% from current to moderate warming, and by another 5% from moderate to extreme warming (Fig. 2).

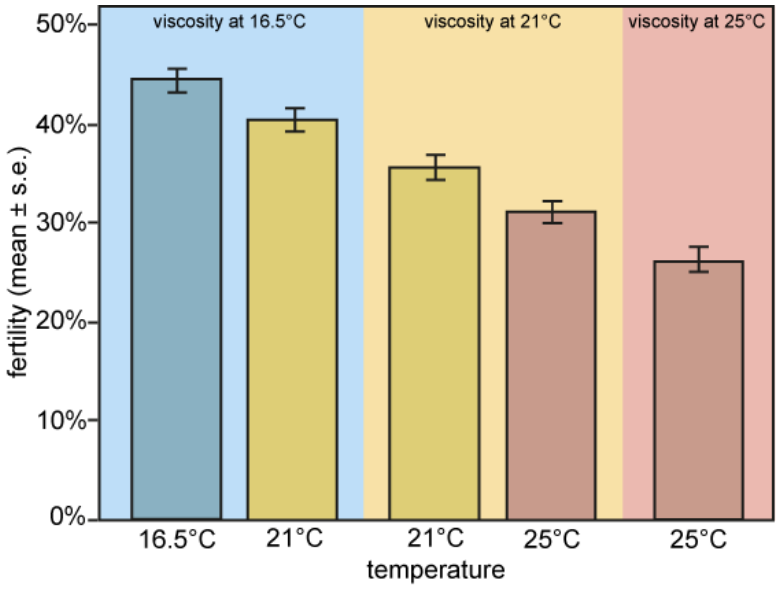

Figure 2. Higher temperature and lower viscosity independently reduce male fertility. Bars show male fertility (mean ± s.e.) in each fertilisation environment.

Directional selection on sperm morphological traits

a) Effects of overall warming on directional selection. Step 1 of our analyses showed that most sperm traits incur directional selection via their effects on male fertility (Table 1a, Fig. 3a-e), but directional selection also depends on overall warming (i.e., adding environment × linear terms significantly improved model fit; Table 1b). Inspecting tests of individual coefficients showed that this overall

result was driven solely by effects of the sperm midpiece on male fertility (Table 1b). For midpiece length, planned contrasts between specific pairs of environments showed that directional selection differed between current conditions and moderate warming, but not between moderate and extreme warming (Table 1c). Specifically, midpiece length had no directional effect on fertility at 16.5°C$_{normal\ viscosity}$ (Fig. 3d; p=0.67), but shorter midpieces enhanced fertility at 21°C$_{normal\ viscosity}$ (Fig. 3d, p=0.04) and 25°C$_{normal\ viscosity}$ (Fig. 3d, p=0.02). For midpiece width, these planned contrasts showed that directional selection did not differ between current conditions and moderate warming (Table 1c), but did differ between moderate and extreme warming. Specifically, narrower midpieces enhanced fertility at 16°C$_{normal\ viscosity}$ (Fig. 3e; p<0.01), but midpiece width had no directional effect on fertility at 21°C$_{normal\ viscosity}$ (Fig. 3e; p=0.57), while wider midpieces marginally enhanced fertility at 25°C$_{normal\ viscosity}$ (Fig. 3e; p= 0.10). The latter result indicates that increased warming from 21°C$_{normal\ viscosity}$ to 25°C$_{normal\ viscosity}$ strengthened directional selection on midpiece width, but not enough to generate significant selection on this trait. Independent of environment, sperm with longer tails and wider heads enhanced fertility (Table 1a, Fig. 3b-c) but sperm head length had no effect (Table 1a; Fig 3a).

b) Isolated effects of temperature and viscosity on directional selection. Step 2 of our analyses tested if environment-dependent selection on sperm midpiece was driven by the effects of temperature or viscosity in isolation. However, neither of these effects drove the change in directional selection on midpiece length from current conditions to moderate warming, or on midpiece width from moderate to extreme warming (Table 1d-e). Therefore, selection on both traits depended on the effects of temperature and viscosity acting in combination (i.e., overall warming).

Table 1. Tests of directional and nonlinear selection on sperm morphological traits, and the effects of fertilisation environment on selection. Bold text indicates $p < 0.05$.

(a) Directional selection coefficients	χ^2	d.f.	*p*
All sperm traits (overall test)	**12.18**	**5**	**0.03**
Head length	0.22	1	0.64
Head width	**4.92**	**1**	**0.03**
Tail length	**7.77**	**1**	**<0.01**
Midpiece length	0.19	1	0.67
Midpiece width	**10.23**	**1**	**<0.01**
(b) Effects of overall warming on directional coefficients			
All sperm traits × warming (overall test)	**26.11**	**10**	**<0.01**
Head length × warming	0.35	2	0.84
Head width × warming	3.27	2	0.20
Tail length × warming	1.39	2	0.50
Midpiece length × warming	**7.77**	**2**	**0.02**
Midpiece width × warming	**10.34**	**2**	**<0.01**
(c) Planned contrasts of effects of overall warming on directional coefficients			
Midpiece length × warming (16.5°C vs. 21°C)	**5.82**	**1**	**0.02**
Midpiece length × warming (21°C vs. 25°C)	0.23	1	0.63
Midpiece width × warming (16.5°C vs. 21°C)	2.21	1	0.14
Midpiece width × warming (21°C vs. 25°C)	**5.04**	**1**	**0.03**
(d) Isolated effects of temperature on directional coefficients			
Midpiece length × temperature (16.5°C vs. 21°C)	1.55	1	0.21
Midpiece width × temperature (21°C vs. 25°C)	2.19	1	0.14
(e) Isolated effects of viscosity on directional coefficients			
Midpiece length × viscosity (16.5°C vs. 21°C)	2.34	1	0.13
Midpiece width × viscosity (21°C vs. 25°C)	1.50	1	0.22
*(f) Nonlinear selection coefficients**			
All sperm traits (overall test)	**28.69**	**15**	**0.02**
Head length^2	0.79	1	0.38
Head width^2	2.63	1	0.11
Midpiece length^2	**10.19**	**1**	**<0.01**
Midpiece width^2	1.47	1	0.22
Tail length^2	**6.81**	**1**	**<0.01**
Head length × head width	0.06	1	0.81
Head length × midpiece length	1.46	1	0.22
Head length × midpiece width	0.44	1	0.51
Head length × tail length	0.01	1	0.95
Head width × midpiece length	**5.13**	**1**	**0.02**
Head width × midpiece width	0.43	1	0.52
Head width × tail length	**4.74**	**1**	**0.03**
Midpiece length × midpiece width	1.13	1	0.29
Midpiece length × tail length	**9.38**	**1**	**<0.01**
Midpiece width × tail length	**5.38**	**1**	**0.02**

** Effects of warming not significant ($\chi^2 = 28.11$, d.f. =30, p=0.57)*

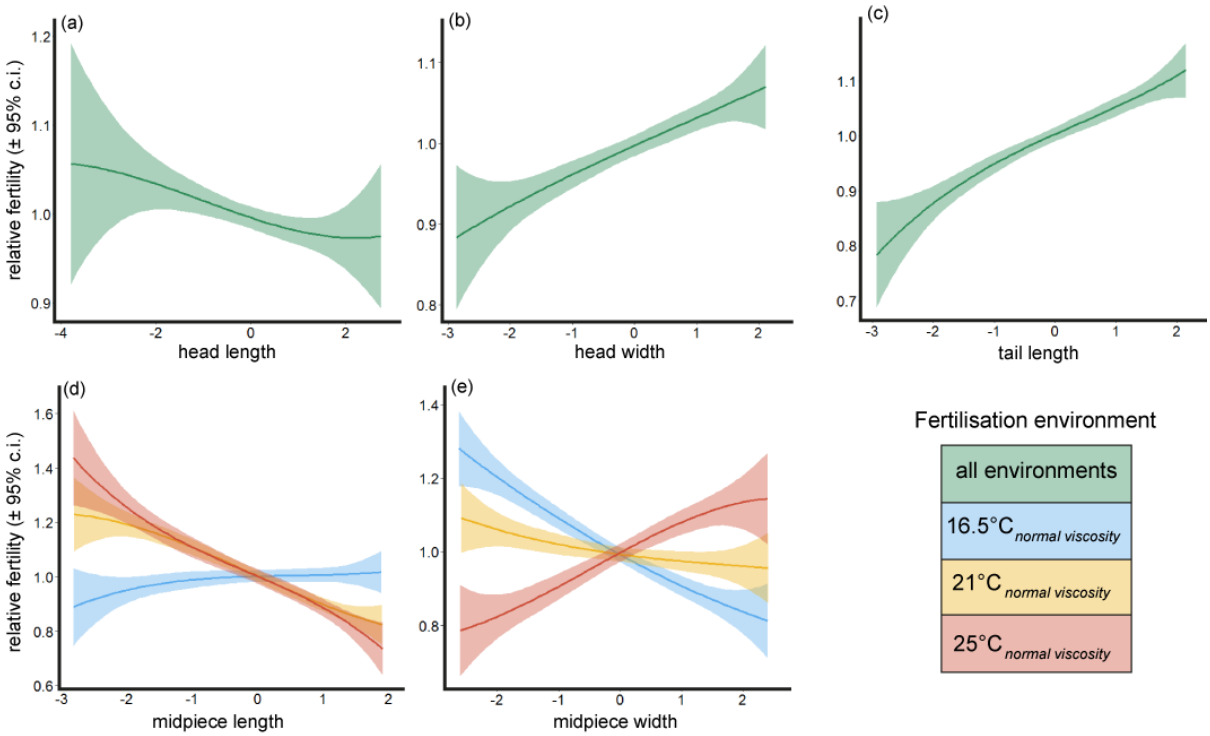

Figure 3. Spline visualisations of directional selection on sperm morphological traits. Splines are fitted separately for each fertilisation environment (current conditions and the two progressive levels of warming) where tests indicated that selection was environment-dependent, and pooled across all environments otherwise. Only the effects of overall warming are visualised here because selection was unaffected by temperature or viscosity in isolation.

Nonlinear selection on sperm morphological traits

Nonlinear selection on sperm traits was consistent across fertilisation environments (Table 1f). Correlational selection acting on several pairs of traits (Table 1f; Table S1) made it difficult to interpret the overall pattern of nonlinear selection from coefficients alone, so we visualized it using an eigenanalysis of those coefficients (Phillips and Arnold 1989, Blows and Brooks 2003). This *post-hoc* procedure complements the basic analysis by identifying linear combinations of sperm traits (eigenvectors) on which nonlinear selection is strongest, as indicated by the sizes of their eigenvalues (where positive values imply concave selection and negative values imply convex selection). We used Kaiser's rule to decide which eigenvalues to retain, since other ways of evaluating their significance (e.g., Reynolds et al. 2010) are not yet developed for mixed models like ours. Kaiser's rule is a standard heuristic that retains eigenvalues larger than 1, meaning the eigenvectors associated with

them explain more nonlinear selection on sperm traits than do single coefficients (Jolliffe 2002). One eigenvector ($\mathbf{m_5}$, explaining 64% of nonlinear selection; Table 2) met this rule, while another ($\mathbf{m_1}$, explaining 18% of nonlinear selection; Table 2) fell only marginally short. We therefore retained both eigenvectors to visualise a single fitness surface describing the vast majority of nonlinear selection. Overall, the combination of concave selection on $\mathbf{m_5}$ and convex selection on $\mathbf{m_1}$ resulted in a saddle-shaped fitness surface, with alternative peaks and troughs within the range of sperm phenotypes sampled (Fig. 4). Interpreting trait loadings on both eigenvectors at once suggests that selection favours males whose sperm combine intermediate tails and midpiece dimensions with unusually wide or narrow heads, since these combinations are associated with highest fertility (Table 2; Fig. 4).

Table 2. Combinations of sperm morphological traits (eigenvectors) under the strongest nonlinear selection. Loadings show the contributions of original traits to each combination. Eigenvalues show the strength of selection on each combination, with positive values meaning selection is concave and negative values meaning it is convex.

Eigenvector	*Eigenvalue*	*Head length*	*Head width*	*Tail length*	*Midpiece length*	*Midpiece width*
m1	0.88	-0.29	0.89	-0.09	-0.12	-0.31
m5	-3.17	0.33	0.03	0.55	0.49	-0.59

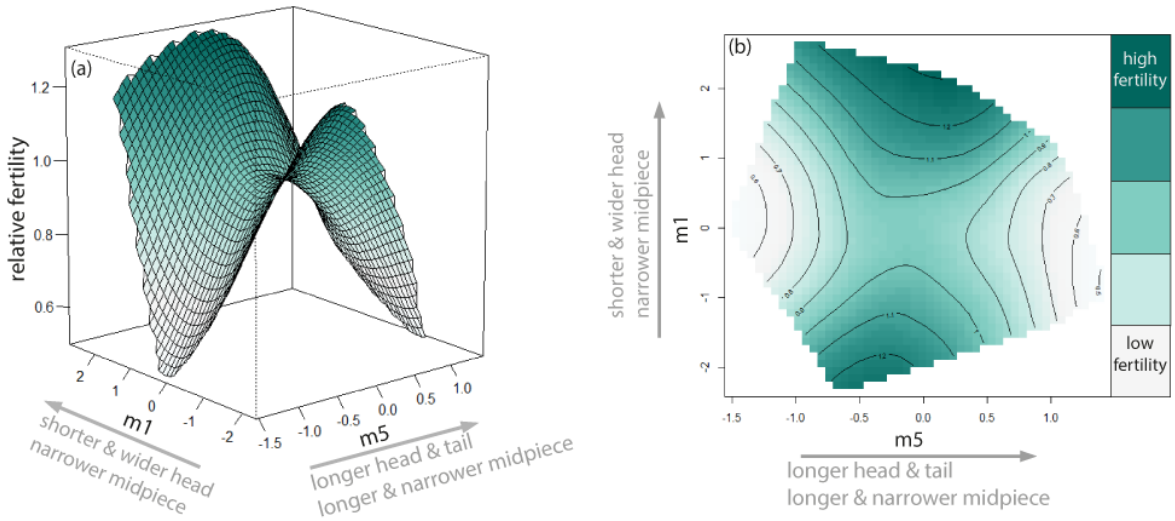

Figure 4. Visualizations of nonlinear selection on combinations of sperm morphological traits ($\mathbf{m_1}$ and $\mathbf{m_5}$). The three-dimensional surface on the left (a) was fitted using a thin-plate spline. The contour plot on the right (b) is the same surface viewed from above.

Discussion

Fertility is a key component of individual fitness and vital for population persistence (Walsh et al. 2019). For males, fertility relies on the ability of their sperm to collide and fuse with eggs, allowing selection to target sperm morphological traits via their effects on fertility (Devigili et al. 2015, Monro and Marshall 2016, Lymbery et al. 2018). Projected ocean warming will expose the sperm of externally-fertilising marine species to higher water temperatures and lower water viscosities (Kupriyanova and Havenhand 2005), but the impacts on fertility, and how those impacts modify selection on sperm traits that affect fertility, are virtually unexplored. Here, by disentangling the effects of temperature and viscosity on the key process of fertilisation in an externally-fertilising marine tubeworm, we found that projected changes in each factor independently reduce male fertility, but the dual effects of both factors together alter selection on sperm morphology. Specifically, narrower sperm midpieces enhanced fertility under current-day conditions, but the higher temperature and lower viscosity of fertilisation environments under projected warming shifted this selection in favour of shorter midpieces under moderate or near-term warming, and wider midpieces under more extreme or longer-term warming. Selection also targeted sperm head dimensions and tail length, but was consistent across fertilisation environments for these traits. Our findings provide the first evidence that projected ocean warming will impact not only male fertility in marine external fertilisers, but also how selection is shaping the evolutionary trajectories of their sperm to maximise fertility.

Past research on the impacts of projected warming for marine populations overwhelmingly focuses on the physiological consequences of temperature, while our broader understanding of the physical consequences of viscosity lags far behind. Here, we show that those physical consequences can be just as important as physiological ones. Our detection of reduced male fertility under higher temperatures was consistent with expectations, since exposure to temperatures above the usual range often disrupt physiological processes — including metabolic rate and enzyme activity — that are fundamental to cell function (Hofmann and Todgham 2010). However, reduced fertility under lower viscosities was in some ways surprising. On one hand, lower viscosity under warming is predicted to enhance male fertility by reducing the drag forces acting on sperm, allowing sperm to swim faster (Byrne et al.

2010). On the other hand, populations are predicted to be better adapted to current environmental conditions than to novel conditions not yet experienced, thereby making most environmental changes stressful (Bell 2013). Our results support the latter idea, suggesting that sperm may be poorer swimmers under novel levels of viscosity that they have had little prior exposure to. These results should apply more broadly, moreover, since many aquatic organisms are small enough to be dominated by viscous forces during key life stages (e.g., gametes, embryos, and larvae), if not their entire lifecycles (e.g., phytoplankton and prokaryotes; Podolsky and Emlet 1993, Humphries 2013). Consequently, the physical impacts of global change in aquatic ecosystems require greater attention.

Dual physical and physiological impacts of projected warming consistently shifted directional selection on sperm midpieces, favouring shorter midpieces under near-term warming and wider midpieces under more extreme warming in the longer term. Sperm midpieces contain the mitochondria that provide energy (i.e., ATP) for sperm function; consequently, shifts in selection on midpiece dimensions may reflect shifts in the energetic challenges of functioning under stressful conditions. For instance, sperm may require more ATP to successfully collide with eggs at low viscosities, and their mitochondria produce ATP less efficiently at high temperatures (Blier et al. 2013, Salin et al. 2015). Unfortunately, the exact energetic benefits of changes in midpiece morphology under warmer conditions are difficult to ascertain, since past studies have found it to have inconsistent relationships with sperm performance (Bennison et al. 2016) and have mostly focused on internal fertilisers with different reproductive biology to *Galeolaria* (but see Schlegel et al. 2015). Nonetheless, shorter or wider midpieces could potentially reflect beneficial rearrangements in the internal organisation of mitochondria (Mendonca et al. 2018). For instance, denser packing of mitochondrial membranes can allow ATP to be produced more efficiently (Demongeot et al. 2007, Mannella et al. 2013), and larger midpieces can store more energy to enhance sperm swimming speed or longevity (Lüpold et al. 2009, Firman and Simmons 2010, but see Malo et al 2006, Bennison et al. 2016). Warming might also relax current-day selection against wider midpieces because drag forces across sperm surfaces are reduced under warmer conditions. However, this explanation for our results seems unlikely, since warming does not affect selection on sperm head dimensions, which account for the majority of sperm surface

area and are therefore similarly exposed to drag. Irrespective of the underlying mechanisms, our findings suggest that the sperm midpiece is a prime target of selection acting via fertility under future warming.

Projected warming does not alter directional selection for sperm with longer tails and wider heads in *Galeolaria*. Selection for longer-tailed sperm supports previous studies suggesting that this phenotype increases male fertility by enhancing sperm swimming speed (Lüpold et al. 2009, Fitzpatrick et al. 2010), though longer tails can also reduce sperm longevity in other external fertilisers (Bakker et al. 2014). Selection for a wider head is less expected, however, since previous studies suggest that narrower heads enhance sperm swimming performance by reducing drag (Malo et al. 2006, Humphries et al. 2008), or that differences in head width do not impact sperm performance (Bakker et al. 2014). Yet one possible benefit of a wider sperm head is that it might coincide with a larger acrosome. The acrosome covers the head's anterior tip, and plays an essential role in fertilisation by secreting enzymes that help sperm attach to eggs and penetrate their outer membranes (Franklin 1970). As such, larger acrosomes could potentially provide sperm with more enzymes that enhance their ability to successfully collide and fuse with eggs (Menkveld et al. 2003).

Irrespective of warming-induced changes in fertilisation environment, nonlinear selection also favours specific combinations of sperm morphological traits — namely, extreme values of head width combined with intermediate values of other traits. Consequently, the benefits gained by males from producing sperm with shorter midpieces or longer tails are not open-ended, as patterns of directional selection alone would suggest, but may ultimately depend on other aspects of sperm phenotype. Moreover, selection acting on combinations of sperm traits is in line with predictions that selection is more likely to target the relative lengths of a sperm's constituent parts than their absolute lengths (overall size; Humphries et al. 2008). Indeed, our findings add to a growing number of studies that uphold this expectation (Fitzpatrick et al. 2012, Johnson et al. 2013, Monro and Marshall 2016, Lymbery et al. 2018). As such, measuring nonlinear selection on multivariate sets of sperm traits is

likely to be a necessary (though sometimes complicated) step in assessing patterns of selection on sperm morphologies and predicting their adaptive evolution in a changing world.

Our study represents a key step towards understanding the evolutionary impacts of projected ocean warming on fertilisation dynamics in marine external fertilisers, and the selection pressures likely to be imposed on free-spawned gametes. Whether gametes can evolve and adapt in response to those pressures will depend on the genetic basis of targeted traits (Lynch and Walsh 1998). As such, assessing the genetic basis of sperm morphology in *Galeolaria* is an important next step towards understand such adaptive capacity. Notably, populations may respond to environmental change not only through evolution, but also through phenotypic plasticity (Chevin et al. 2010). For instance, males of another marine tubeworm, *Hydroides diramphus*, respond to salinity stress by plastically adjusting sperm morphology to maximize sperm performance and male fertility under those conditions (Jensen et al. 2014). However, recent work on *Galeolaria* suggests that males have limited capacity to respond to projected warming in a similar way (Guillaume et al. 2016). Also, we only examined warming-mediated selection on sperm traits here, when fertilization dynamics of marine external fertilisers will also depend strongly on egg phenotypes (Vance 1973, Levitan 2006). In particular, changes in temperature and viscosity resulting from ocean warming will likely impact egg sinking speeds and egg rotation rates (Crimaldi and Zimmer 2014). Hence, more work is needed to understand how changes in fertilisation environments under projected warming will alter selection on eggs, as well as the mutual selection pressures exerted by free-spawned gametes at fertilisation.

To our knowledge, we provide the first evidence that projected ocean warming will not only impact the fertility of marine external fertilisers, but expose their gametes to novel selection pressures during the critical process of fertilisation that may drive gametes to adapt in response. We also highlight the unappreciated role of changes in water viscosity, likely to be hugely important for the tiny organisms and life stages ubiquitous in aquatic environments, in shaping the potential impacts of ocean warming on marine populations. In turn, a broader appreciation of the dual impacts of viscosity and temperature

on ecological and evolutionary processes in marine populations is needed to better predict how those populations will respond to future warming.

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Supplementary material

Table S1. Coefficients of directional and nonlinear selection on sperm morphological traits. Directional selection depended on overall warming (the combined effects of temperature and viscosity), so coefficients were estimated separately for current conditions and the two progressive levels of warming. Nonlinear selection on sperm traits was consistent across environments, so a single set of pooled coefficients was estimated. All estimates are ± 1 standard error, with bold text indicating $P < 0.05$.

	Directional selection			*Nonlinear selection*				
	16.5°C	*21°C*	*25°C*	*HL*	*HW*	*ML*	*MW*	*TL*
Head length (HL)	-0.01 ±0.02	-0.02 ±0.03	-0.04 ±0.04	0.02 ±0.04				
Head width (HW)	**0.06** **±0.03**	0.00 ±0.04	-0.01 ±0.04	-0.01 ±0.02	-0.06 ±0.04			
Midpiece length (ML)	0.02 ±0.04	**-0.10** **±0.05**	**-0.12** **±0.05**	-0.04 ±0.03	**0.07** **±0.03**	**-0.19** **±0.06**		
Midpiece width (MW)	**-0.09** **±0.03**	-0.02 ±0.04	0.08 ±0.05	0.02 ±0.03	-0.02 ±0.03	0.04 ±0.04	0.06 ±0.05	
Tail length (TL)	**0.07** **±0.02**	0.02 ±0.04	0.03 ±0.04	0.00 ±0.02	**-0.06** **±0.03**	**0.12** **±0.04**	**-0.07** **±0.03**	**-0.11** **±0.04**

Chapter 7

General Discussion

Improving our understanding of how, and to what extent, natural populations will adapt in response to projected warming can help guide management strategies to improve conservation outcomes (Bush et al. 2016, Cook and Sgrò 2017). So far, our understanding has been largely skewed towards terrestrial populations, while similar insights for marine populations remain relatively lacking (Sotka 2012, Munday et al. 2013, Donelson et al. 2019). Marine populations are expected to be particularly susceptible to projected warming during fertilisation and early development, which are likely to act as bottlenecks for population persistence (Byrne 2011, Pandori and Sorte 2019). My overarching goal in this thesis was to understand how projected warming will impact the adaptive potential of life stages involved in fertilisation (free-spawned gametes) and early development (embryos and larvae) in marine populations.

Early survival & adaptive potential depend on the environments experienced by past life stages

My work on *Galeolaria* in Chapters 2, 3, and 4 shows that larval survival depends not only on the environments of developing larvae, but also the environments of preceding life stages or generations. Larval survival was consistently lower when development occurred under projected warming, relative to when development occurred under current-day conditions (Chapters 2, 3, and 4). Encouragingly, more larvae survived development under projected warming when parents had also experienced the same conditions (Chapter 3). In contrast, fewer larvae survived development under projected warming when fertilisation also occurred under the same conditions (Chapter 4). As such, parental and fertilisation environments seemingly have important and contrasting effects on larval survival, and potentially the future persistence of *Galeolaria* populations under projected warming. Yet, future work is still need to test how, or whether, parental and fertilisation environments have an interactive effect on offspring survival under projected warming. Given parents will usually experience similar temperatures during gametogenesis that their gametes experience during fertilisation, I strongly encourage future work that combines the environmental manipulations conducted in Chapters 3 and 4 to assess how experiencing projected warming consecutively during parental stages and fertilisation

impacts larval survival. Such work will be an important step towards achieving the most ecologically realistic measures of how susceptible marine populations are to projected warming.

Current efforts to understand whether parental environmental effects are adaptive have yielded inconsistent results. Some studies have found that parents can respond to environmental stress via adaptive transgenerational plasticity, priming their offspring to cope better with that stressor (as seen in Chapter 3), but others have found that parental exposure to environmental stress has little, or even a detrimental, effect on offspring (Uller et al. 2013, Donelson et al. 2018). Current theory suggests a key reason for these inconsistent findings is that selection can only favour the evolution of adaptive transgenerational plasticity in populations where the environment experienced by parents reliably predicts the subsequent environments of their offspring (Donaldson-Matasci et al. 2013, Burgess and Marshall 2014, Leimar and McNamara 2015). Yet this theoretical prediction have rarely been tested empirically (but see Shama 2015, Dey et al. 2016) and should be a priority for future research, as understanding the capacity (or lack thereof) of populations to respond to projected warming via adaptive transgenerational plasticity can help evaluate their future vulnerability.

The extent to which populations will adaptively evolve under future conditions will depend on their additive genetic variation and across-environment correlations in components of fitness (Hoffmann and Sgrò 2011). Here, I provide some of the first evidence that the additive genetic variation and across-environment correlations in offspring fitness components can be altered by the environments experienced by parents (Chapter 3), and by gametes at fertilisation (Chapter 4). Parental exposure to projected warming weakly reduced additive genetic variation for larval survival, but also strengthened the positive genetic correlation for survival across current and future temperatures. The beneficial effect of this genetic correlation could therefore counterbalance the impact of reduced additive genetic variation on future adaptation. In contrast, fertilisation under projected warming profoundly reduced both additive genetic variation and the across-environment correlation for offspring survival under current-day and projected temperatures. This result is particularly concerning given that fertilisation in *Galeolaria* (and most other species) usually occurs under similar temperatures to early developmental

stages, and therefore the carryover effects of fertilisation environment may limit the adaptive potential of larval survival under projected warming. My findings in Chapter 4 may also question the accuracy of several past studies (including Chapter 3), where fertilisation was conducted under benign conditions before assessing additive genetic variation in early survival under environmental stress. However, the genetic variation lost when fertilisation occurred under projected warming in Chapter 4 may be due to the *change* in temperature between gametogenesis in parents and fertilisation in the external environment, rather than due only to the *stress* of fertilisation under projected warming. Similarity, an intriguing question unanswered by Chapters 3 and 4 is if parental and fertilisation environments have interactive effects on additive genetic variation in offspring survival. For instance, parents exposed to warming during gametogenesis may produce gametes with a greater resilience to such conditions (but see Guillaume et al. 2016), and consequently fertilisation under warmer conditions may affect additive genetic variation in offspring survival less profoundly than seen in Chapter 4. Yet, future tests of this three-way interaction (i.e., parental × fertilisation × offspring environment) in a quantitative genetic framework requires a huge logistical undertaking. Nevertheless, Chapters 3 and 4 highlight the importance of assessing the impacts of projected warming beyond a single life stage, as they show that experiencing warming earlier in the life cycle, or in preceding generations, can mediate both the susceptibility to warming in later life stages and the adaptive potential to respond to it.

A priority going forward should be to examine how the adaptive potential of marine populations will be influenced by additive genetic correlation across traits in different life stages. As with the across-environment genetic correlations discussed in the earlier chapters, the presence of genetic correlations between life stages may facilitate or constrain adaptation (Marshall and Morgan 2011, Collet and Fellous 2019). However, theory predicts that the complex life histories of many marine species makes them prone to genetic correlations among life stages that will constrain them from maximising lifetime fitness (Schluter et al. 1991, Marshall et al. 2016). For instance, a genetic correlation between larval and adult survival constrains adaptive potential in the ascidian, *Ciona intestinalis*, forcing evolutionary increases in survival at one stage to cause corresponding decreases in survival at the other (Aguirre et

al. 2014). To the best of my knowledge, no empirical study has examined whether stress tolerance is genetically correlated across life stages, and addressing this knowledge gap will be important step in assessing the adaptive potential of marine populations under future environmental stress.

My findings add to a growing body of evidence that nonadditive genetic effects will account for a greater proportion of fitness variation under the stressors imposed by future warming. Nonadditive genetic variation for larval survival was increased by exposure to projected warming during the preceding parental stages (Chapter 3), fertilisation (Chapter 4), and larval development (Chapter 2). Similarly, several others have also found nonadditive genetic variation in key traits increases under stress (Blows and Sokolowski 1995, Bubliy and Loeschcke 2002, Lymbery and Evans 2013, Rudin-Bitterli et al. 2018), suggesting the evolutionary role of nonadditive genetic effects under the stressors of global change is currently ripe for exploration. One potential avenue for such exportation is that theory predicts that nonadditive variation can be converted into additive variation following population bottlenecks, as a consequence of allele frequencies changing by genetic drift (Goodnight 1988). Additive variation resulting from this conversion could be mostly deleterious and subsequently removed by selection, but could also have a lasting impact if it allows the population to move to a new evolutionarily stable state (Barton and Turelli 2004). So far, these theoretical predictions have only once been tested empirically in a global change context, whereby bottlenecking a *Drosophila bunnanda* population increased the additive genetic variation in traits, but not their adaptive evolution under projected levels of environmental change (van Heerwaarden et al. 2008). Nonadditive genetic effects are also involved in longer-term evolutionary processes, such as population divergence and speciation (Carroll 2007, Hendry 2013), and thus increases in nonadditive genetic variation may facilitate these processes under future conditions. Although the role of nonadditive genetic effects in adaptation is complex and debated (Crow 2010, Hansen 2015, Barton 2017), my findings in Chapters 2 to 4 suggest, at the very least, that their influence on populations responding to projected warming warrants greater research attention.

Future work is needed to identify the underlying mechanisms of how parental and fertilisation environments impact offspring survival, and levels of genetic variation in offspring survival. For instance, transcriptomic methods are rapidly improving our ability to disentangle how populations respond to environmental stress via changes in gene expression (Franks and Hoffmann 2012, DeBiasse and Kelly 2016, Wong et al. 2018), and thus could provide valuable tools for examining why parental and fertilisation environments have such contrasting effects on offspring survival. Also, genomics approaches are increasingly able to identity the complex genetic basis of quantitative traits, and may eventually offer more cost effective ways to estimate quantitative genetic variance than traditional breeding designs or pedigrees (Robinson et al. 2013, Harrisson et al. 2014, Razgour et al. 2019). Moreover, advances in such 'omics approaches pave the way for more studies to track the evolutionary responses of natural population to environmental change in the field, and in doing so provide a more ecologically realistic view of adaptive evolution than is possible in lab-based studies (Charmantier et al. 2014, Razgour et al. 2019). Yet 'omics approaches are not without shortcomings (Harrisson et al. 2014, Evans 2015, Shaw 2018). Ultimately, we should strive to integrate the phenomenological approach of quantitative genetics with the mechanistic approaches of 'omics to best understand the potential of populations to adaptively evolve in response to future warming.

Selection targets early development and gametes, and is likely to be altered by warming

Selection will shape how natural populations adapt their trait phenotypes under projected warming. Yet, we currently have a scarce understanding of how selection acts on traits involved in the fertilisation and early development of marine populations, and even less about how selection on traits will be influenced by projected warming. In the second half of my thesis, I provide a rare disentanglement of how selection acts on size and development time at key early life stages (Chapter 5). Also, I add to a growing number of studies that show sperm morphology is an important target of selection, and provide the first example of how this selection can be altered by projected warming (Chapter 6). In doing so, I present new insights into how selection may shape marine populations to adapt key traits under future conditions.

Selection will often favour the evolution of specific trait combinations and will usually act alongside various types of functional, developmental, or genetic constraints on those traits (Arnold 1992). In Chapter 5, I found that selection directly acts on how individuals combine development time and subsequent size, and in a way that should weaken the positive correlation between them. Conversely, I detected no selection on combinations of initial size and development time, indicating that their positive correlation could reflect functional constraints set by physiology and allometry. Gillooly et al. (2002) argues that initial size and development time should be positively correlated as a functional consequence of larger individuals typically having lower mass-specific metabolic rates. Yet even if this is the case, my findings also indicate that initial size and development time are only weakly correlated, and thus have scope to evolve independently. Therefore, my findings imply that appreciating how selection will act alongside these functional constraints is needed to understand current patterns in size and development time, and for forecasting how these fundamental traits will evolve under future warming.

I originally planned to follow up Chapter 5 with a study that examined the temperature dependence of selection on the size and development time in *Pyura*. Size and development time often show enormous phenotypic plasticity in response to temperature (Kingsolver and Huey 2008), but the stability of selection across temperatures – or other environmental factors – is virtually unexplored. As such, selection may favour different combinations of initial size and development time in different environments, which may help explain why the phenotypic correlation between size and development time vary in strength and direction among populations (Pettersen et al. 2018). Furthermore, if such selection does depend on temperature, this could have key implications for how early developmental stages will evolve under future warming. Unfortunately, unforeseeable circumstances precluded this experiment, but testing the environmental dependence of selection on size and development time is still an important avenue for future research.

In the final study of my thesis, I found that selection on sperm morphology is shifted by the dual physiological and the physical effects of projected ocean warming. More specifically, narrower sperm

midpieces, relative to the population average, improved fertility under current-day conditions, but the effects of higher temperature and lower viscosity shifted this selection towards favouring shorter and/or wider midpieces under projected levels of warming. As midpieces house the mitochondria that produce energy (i.e., ATP) for sperm function, these shifts in selection may reflect the energetic challenges of functioning under stressful conditions. However, future work is needed to elucidate what energetic benefits a shorter or a wider midpiece would provide sperm, as past studies that have linked the morphology of sperm midpiece to sperm performance have found few consistent patterns (Mendonca et al. 2018), and have not examined how midpiece morphology influences sperm performance at a multiple temperatures. An additional future step could also be to examine the genetic basis of sperm morphology in *Galeolaria*, potentially via similar breeding designs as in Chapters 2 to 4, to assess whether *Galeolaria* will evolve in response to selection on their sperm traits. While I only measured selection on sperm traits here, egg traits of external fertilisers are also expected to be under strong selection (Levitan 2006), and how warming may alter selection on egg traits undoubtedly requires future exploration. Addressing these knowledge gaps should be a goal going forward, as my findings indicate that gamete morphology may be a key factor for marine populations sustaining their reproductive output under future warming.

My findings in Chapter 6 may also have implications beyond gametes stages, by highlighting the neglected physical consequences of lower water viscosity under projected warming. In marine systems, the vast majority of research has focus on the physiological consequences of ocean warming, but many marine populations may also be vulnerable to the physical consequences of warming. For instance, the tiny size of many marine species during key life stages (e.g., gametes, embryos, and larvae), or even their entire lifecycles (e.g., phytoplankton and prokaryotes; Podolsky and Emlet 1993, Humphries 2013), mean their motility will near-inevitably be affected by lower water viscosity under projected ocean warming. In turn, the physical impacts of global change in marine (and other aquatic) ecosystems may have widespread implications that are yet to be discovered.

On a final note, the spectacular diversity of life in the sea provided the motivation for my thesis, but also served as a reminder that the evolutionary processes I sought to predict often act in unintuitive and complex ways. Continued work is vital to understand this complexity, but this thesis provides novel empirical studies and new ideas that hopefully pushes forward our understanding of how marine populations will adaptively evolve in response to the challenges posed by global change.

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