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Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer?

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Summary

1. For most organisms, early life-history stages are the most sensitive to environmental stress and so transgenerational phenotypic plasticity, whereby the parental environment and offspring environment interact to alter the phenotype of the offspring, is viewed as key to promoting persistence in the face of environmental change. While there has been long-standing interest in the role of transgenerational plasticity via the maternal line (traditionally the field of maternal effects), increasingly it appears that paternal effects can also play a role.

2. Despite the emerging role of paternal effects in studies of global change, key knowledge gaps remain: first, whether paternal effects act to increase or decrease offspring performance remains largely unexplored; second, the relative roles of maternal and paternal effects are rarely disentangled; and third, the role of environmental variation, a key determinant of the benefits of transgenerational plasticity, has not been explored with regard to paternal effects.

3. Here, we explore all three issues using the marine tubeworm *Galeolaria caespitosa*, an important habitat-forming species in southern Australia.

4. We found that both paternal and maternal experiences affected key stages of offspring performance (fertilization and larval development) and, surprisingly, paternal effects were often stronger than maternal effects. Furthermore, we found that paternal effects often reduced offspring performance, especially when environments varied compared with when environments were stable.

5. Our results suggest that, while transgenerational plasticity may play an important role in modifying the impacts of global change, these effects are not uniformly positive. Importantly, paternal effects can be as strong, or stronger, than maternal effects and environmental variability strongly alters the impacts of paternal effects.

Key-words: environmental variation, fertilization success, marine invertebrate, maternal effects, nongenetic inheritance, offspring fitness, parental effects, phenotypic plasticity

Introduction

Global change imposes new pressures on natural populations. The extent to which populations persist under global change depends on the ability of the population to respond via migration, adaptive evolution and phenotypic plasticity (Hoffmann & Sgro 2011). Although all three mechanisms are important, their relative importance varies both over time and among species (Bell & Collins 2008). Migration to favourable habitats is only a viable outcome if the organism has sufficient dispersal potential and if suitable alternative habitats exist. Evolutionary adaptation to new selection regimes will determine the longer term capacity of organisms to persist in the face of climate change (sometimes termed 'evolutionary rescue'; Bell & Collins 2008; Pandolfi *et al.* 2011), although the extent to which evolution can rescue species coping with climate change depends on both the availability of heritable variation and the demographic costs of adaptation (Bell & Gonzalez 2009). If populations suffer too much mortality during adaptation, then even as evolution occurs, the demographic costs of adaptation extirpate the population before tolerance to the stressor evolves (Bell & Gonzalez 2009). Under these circumstances, phenotypic plasticity plays a key role (Chevin & Lande 2010).

Phenotypic plasticity involves the differential expression of phenotypes according to local conditions. Because phenotypic plasticity provides scope for the same genotype to express beneficial phenotypes in response to change, phenotypic plasticity is an important means of coping with relatively rapid environmental fluctuations (Chevin & Lande 2010), where organisms can effectively 'buy more time' in

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which to evolutionarily adapt to global change stressors (Munday *et al.* 2013). By expressing resistant phenotypes via phenotypic plasticity, populations may experience fewer demographic costs while they continue to evolve in response to global change. Phenotypic plasticity is thought to be particularly important with regard to climate change when it occurs across generations – a process known as transgenerational phenotypic plasticity (transgenerational plasticity for short).

Transgenerational plasticity occurs when the environment or phenotype of the parent affects the phenotype of the offspring, often in adaptive ways (Agrawal 2001). Because new offspring are particularly vulnerable to environmental stressors, it is predicted that parents from a range of taxa will alter the phenotype of their offspring in response to their local environmental conditions (Munday *et al.* 2013). Such responses can be adaptive when the parental environment is a good predictor of the offspring environment (Burgess & Marshall 2014). For example, beetle mothers produce better provisioned offspring when they lay their eggs in nutritionally poor conditions so as to buffer their offspring against starvation (Fox, Thakar & Mousseau 1997). Similarly, when bryozoans are exposed to toxicants or competitors, mothers produce toxicant-resistant offspring or better competitors respectively (Allen, Buckley & Marshall 2008; Marshall 2008). Recent studies show that parents that experience stressors associated with global change alter the phenotype of their offspring so as to increase performance (Munday *et al.* 2013; Sunday *et al.* 2014). For example, in fish, parental exposure to higher temperatures enhances the thermal tolerance of offspring (Donelson *et al.* 2012; Salinas & Munch 2012). Similarly, parents exposed to decreased pH levels (increases in atmospheric CO_2 reduce the pH of seawater) produce offspring that are themselves better able to cope with more acidic seawater in both invertebrates and fish (Miller *et al.* 2012; Parker *et al.* 2012; Thor & Dupont 2015). These studies show that transgenerational plasticity has the potential to buffer organisms from the impacts of climate change, and release early life-history stages from the crucial bottlenecks that would otherwise hamper population persistence (Munday *et al.* 2013). Although there has been a growing recognition of the role of both maternal and paternal effects over the past few years, the majority of transgenerational plasticity studies have not disentangled the two parental contributions to offspring phenotype (but see Jensen, Allen & Marshall 2014; Shama & Wegner 2014; Lane *et al.* 2015). Likewise, while studies are beginning to focus more on the role of predictability in the parental environment on offspring phenotype (Burgess & Marshall 2011, 2014; Marshall & Burgess 2015; Shama 2015), more remains to be investigated on the nature and strength of environmental predictability.

While the number of studies investigating parental effects is growing, in the past studies of transgenerational plasticity in response to global change stressors have tended to focus on maternal transgenerational plasticity (Munday *et al.* 2013), also known as maternal effects (Marshall & Uller 2007), and the influences of maternal effects are more nuanced than is sometimes initially recognized. While long disregarded as a nuisance in quantitative genetics studies, we now recognize maternal effects as major determinants of offspring phenotype (Wade 1998). Crucially, maternal effects are not always adaptive for offspring, rather a maternal effect may increase or decrease offspring fitness depending on a complex set of interacting factors (Wolf & Wade 2001; Marshall & Uller 2007). For example, mothers may manipulate offspring phenotypes to increase their own, rather than their offspring's fitness, sometimes to the offspring's detriment (Cunningham & Russell 2000; Marshall & Uller 2007). Alternatively, maternal effects may not buffer offspring from stressful conditions at all, rather they may act as conduit by which stressors reduce performance in both the maternal and offspring generation (Bernardo 1996; Rossiter 1996; McCormick 2006). It is perhaps unsurprising, therefore, that a recent meta-analysis found that the incidence of adaptive transgenerational plasticity, whereby parents (most commonly mothers) increase offspring performance in response to change, is far less widespread than was initially imagined (Uller, Nakagawa & English 2013). As such, while transgenerational plasticity undoubtedly plays a key role in modifying the impacts of stressors associated with global change across generations, it may not be a panacea. Instead, transgenerational plasticity may sometimes exacerbate the impacts of climate change, transmitting the negative effects of stressors across generations. Although the number of studies investigating transgenerational plasticity has increased exponentially over the last few years, the role of transgenerational plasticity in mitigating global change still remains unclear at this stage. A key factor in whether transgenerational plasticity will influence offspring performance in adaptive ways is the likelihood with which parents can anticipate the environment of their offspring – when environments are unpredictable, adaptive plasticity is unlikely (Burgess & Marshall 2014). While environmental predictability effects are implicated in several general studies of transgenerational plasticity, very few studies have addressed this issue in the context of global change (but see Donelson & Munday 2015; Shama 2015). This dearth of studies on environmental predictability and transgenerational plasticity is surprising, given future global change is likely to alter not only the mean but also the variability of future conditions.

In studies of transgenerational plasticity in response to global change stressors, both parents are often exposed to near-future conditions, yet the relative roles of both mothers and fathers in altering the phenotype of offspring remain largely unexplored (but see Ducatez *et al.* 2012; Shama *et al.* 2014; Lane *et al.* 2015). Under the traditional view of inheritance (Falconer & Mackay 1996), this emphasis on the maternal line makes sense; mothers are assumed to influence the phenotypes of their offspring via nuclear genes, extra-nuclear genetic elements, hormones

and via changes in provisioning (Lynch & Walsh 1998). Fathers, on the other hand, have long been thought to only influence offspring phenotypes via their genes alone, unless they also provide paternal care (Day & Bonduriansky 2011). Consequently, studies that did not distinguish between maternal and paternal effects in response to global change stressors were an appropriate first step in determining the role of transgenerational plasticity in responses to global change. However, new evidence suggests that the contribution of fathers to their offspring's phenotype is not restricted to genes alone, rather transgenerational plasticity via the paternal line seems increasingly likely (Crean & Bonduriansky 2014) such that studies should now seek to disentangle the relative roles of maternal and paternal effects.

A growing number of studies have demonstrated effects of paternal phenotype and environment, broadly termed paternal effects, on offspring phenotype (Crean & Bonduriansky 2014; Jensen, Allen & Marshall 2014). For example, the paternal food environment and body size have been shown to affect offspring performance in insects (Bonduriansky & Head 2007; Crean, Kopps & Bonduriansky 2014). Paternal effects even show evidence of being adaptive for offspring: a recent study showed that fathers exposed to more competitive environments produce offspring that are better competitors themselves (Crean, Dwyer & Marshall 2013). Whilst they are increasingly being revealed in a range of organisms, few studies have yet explicitly explored the specific role of paternal effects in modifying the impact of global change (but see Lane *et al*. 2015).

The study of paternal effects has been hampered by the difficulty in producing unequivocal demonstrations, as maternal effects can often confound experimental studies. Mothers sometimes alter the phenotype of their offspring in response to the phenotype of their mating partner such that some apparent effects of paternal phenotype on offspring phenotype may actually be driven by maternal responses to differences in paternal phenotype (Bonduriansky & Day 2009; Ducatez *et al*. 2012). Theory predicts that mothers and fathers should be under very different selection pressures with regard to maximizing offspring fitness (Kamel, Grosberg & Marshall 2010), and so the effects of maternal and paternal effects could be quite different. Disentangling the role of mothers and fathers is therefore necessary.

One way to disentangle maternal and paternal effects while avoiding the confounding issues discussed above is to use species with external fertilization (e.g. Jensen, Allen & Marshall 2014), which precludes mothers from altering the phenotype of offspring after fertilization. Here, we use the marine broadcast spawning polychaete *Galeolaria caespitosa* to disentangle the effects of male and female environmental conditions on subsequent offspring performance. We performed three full-factorial experiments where we manipulated the thermal environment of both mothers and fathers prior to fertilizations, after which we measured offspring traits related to fitness when offspring environment matched or mismatched the parental environments. Specifically, we asked: (i) How do parental thermal environments influence gamete morphology and fertilization success? (ii) How do parental thermal environments influence larval survival? (iii) How does variability in the father's thermal environment influence larval survival?

Materials and methods

STUDY SPECIES AND SITE

Galeolaria caespitosa (L.) is a marine tubeworm that is endemic to the southern and eastern coasts of Australia (Halt *et al*. 2009). Adults form mixed-sex clusters on artificial structures along intertidal zones and spawn year-round by releasing gametes into the water column (Andrews & Anderson 1962). Gonad cycles indicate that spawning occurs every 3–4 weeks in nature (unpublished data), and while data on adult longevity are lacking, casual observations suggest that individuals can live for multiple years. We regularly collected *G. caespitosa* clusters from March to September 2013 and March to May 2014 at Middle Brighton Pier in Port Phillip Bay, Melbourne, Australia (37°54′S, 144°59′E). Temperature loggers attached to pier pilings adjacent to our study population indicate that adults experience large temperature fluctuations throughout the year, as well as on smaller time-scales. For example, temperatures can differ by as much as 6 °C during a single week, and average weekly temperature can differ by up to 2·1 °C within a month. As larvae in this species are free-swimming and feed planktonically for around 11 days, it is likely that larvae disperse into Port Phillip Bay where temperatures remain relatively stable and vary by 2–3 °C at most in a fortnight. Importantly, the mean temperatures in the adult habitat and the larval habitat are highly correlated (correlation between the mean weekly temperatures from the middle of Port Phillip Bay and data loggers at Middle Brighton Pier in 2013: $R = 0{\cdot}94$; Guillaume unpublished data), indicating that the parental environment is a good predictor of the offspring environment such that adaptive transgenerational plasticity should be expected (Burgess & Marshall 2014). Furthermore, we formally estimated autocorrelation in seawater temperature at our study site (based on data loggers for 3 months at our field site) according to the methods outlined in Burgess & Marshall (2011). Our approach differed slightly in that the assumption of stationarity was violated and so we first detrended our data (see Legendre & Legendre 1998; Burgess & Marshall 2011 for details).

GENERAL METHODS

We transported clusters of worms without water in insulated aquaria to a controlled temperature room (CT room; 16·5 ± 1·0 °C) at the School of Biological Sciences, Monash University, Clayton. We placed clusters containing ~200 individuals in individually aerated plastic aquaria containing unfiltered seawater at 18·0 ± 1·0 °C. We had a total of four parental temperature acclimation combinations: clusters were kept at one of two acclimation temperatures (cooler = 15·5 °C and warmer = 21·5 °C) and the sexes were held separately. Note that because clusters are mixed sex, females or males were selected after acclimation during gamete collection depending on which sex that cluster was allocated to. Each aquarium was an independent unit of replication ($n = 1$ per treatment combination per run), where fresh clusters were collected for each run. Based on logged temperature data at our site, the selected shifts in temperature are not the most extreme experienced by the study species but represent more

commonly experienced shifts in the temperature regime. We used aquarium heaters to ramp warmer treatment aquaria to the target temperature over a 24- to 48-h period. We also equipped the cooler treatment aquaria with heaters to control for any potential artefacts introduced by heaters themselves, but left these heaters inactive so that holding aquaria could equilibrate with room temperature (~15·5 °C).

We allowed clusters of *G. caespitosa* to acclimate under the conditions described above for ~14 or 28 days prior to use in experiments. A 14-day time frame was chosen, as seawater temperature at our field site on a given day was a good predictor of seawater temperature ~13 days later (see Results). During the acclimation period, we changed water two to three times a week, then fed each cluster ~0·5 mL of commercially prepared phytoplankton (Seachem Reef Phytoplankton). We monitored salinity and water temperature to maintain water at 37 ppt and at the appropriate treatment temperature throughout the acclimation period.

To obtain gametes, we used standard methods (see Marshall & Evans 2005 for details). Briefly, we removed adults from their calcareous tubes using fine forceps and placed them into individual 40 mm Petri dishes with filtered seawater (0·22 μm screen size), where reproductively mature adults began spawning immediately. We conducted fertilizations using gametes pooled from the required number of mature adults in each treatment group (see 'Experimental Design and Analysis') to reduce variability in fertilization success due to male–female compatibility and egg fertilizability (Kupriyanova & Havenhand 2002; Marshall & Evans 2005). To minimize differences in sperm or egg contribution from each adult, we took only 0·1 mL of gametes from each individual. We used gametes up to an hour after spawning for fertilization experiments, as egg and sperm viability does not change significantly within this time (Kupriyanova & Havenhand 2002). Sperm concentrations were standardized prior to fertilizations (see 'Experimental Design and Analysis'), as sperm concentration has been shown to alter fertilization success (Kupriyanova 2006) and the effects of stress (Marshall 2006). To create different temperature environments for assays of fertilization success and larval survival, we placed centrifuge tubes in MD-mini thermoblocks (Major Science) set to the required assay temperatures for the desired time. Fertilization success and larval survival were then determined as outlined below. Note that gamete extraction and the mixing of sperm and eggs occurred at room temperature (17 ± 1 °C), but were then immediately incubated at the appropriate fertilization assay temperature.

EXPERIMENTAL DESIGN AND ANALYSIS

Experiment 1. Temperature-mediated parental effects on gamete morphology and fertilization success

In our first experiment, we investigated the effect of a single parental environment on gamete morphology and fertilization success. Adults were acclimated to their allocated temperature treatment for 14 days, as outlined above in 'General methods'. We performed fertilization assays for all combinations of male and female adults acclimated to warmer or cooler treatments, where eggs were pooled from four females and sperm were pooled from four males for each treatment group. Gamete morphology was measured for pooled gametes, and fertilization success was then assayed at either 16·5 °C or 22·0 °C.

We measured gamete size within 15 min of spawning by taking digital images with a Moticam digital camera (5.0 or 10.0 MP) attached to an Olympus CX41 compound microscope, using the computer program MOTIC IMAGES PLUS (version 2.0). Gamete size was later analysed in IMAGEJ 1.46r software (Rasband 1997–2012), where we measured egg diameter in a straight line across a round egg and sperm lengths from head to tail as per Johnson, Monro & Marshall (2013). In each run, we measured 30 eggs and 15 sperm of pooled stock for each male and female temperature treatment.

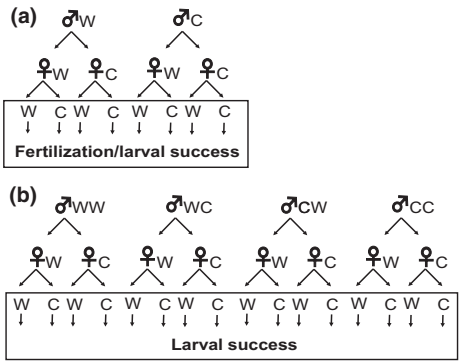

Fig. 1. Full-factorial experimental designs for testing transgenerational phenotypic plasticity in *Galeolaria caespitosa*. Adults were acclimated to either cooler (C, 15·5 °C) or warmer (W, 21·5 °C) temperatures for either (a) 14 days (Experiments 1 and 2) or (b) 28 days (Experiment 3, where half the males were swapped into the alternative temperature after 14 days). Assays of fertilization and larval success (surrounded by box) occurred at 16·5 °C (C) or 22·0 °C (W) and were performed for all combinations of male–female temperatures.

To assay fertilization success, we performed a series of 10-fold sperm solution dilutions for each male × female × fertilization temperature combination, such that eggs were exposed to sperm diluted by factors from 10^0 to 10^5 times the sperm stock concentration (a total of six sperm concentrations for each combination, resulting in 48 fertilization assays per run; Fig. 1a). Sperm concentration was standardized prior to fertilizations so that sperm stock at the beginning of each serial dilution was at $6{\cdot}5 \times 10^7$ sperm mL^{-1}, with the exception of one replicate where the stock concentration was not high enough and $5{\cdot}3 \times 10^7$ sperm mL^{-1} was used instead. We added 0·1 mL of pooled eggs to 0·9 mL of sperm diluted to the desired concentration, leaving a subsample of pooled eggs that were not exposed to sperm and assigned them as a control to account for any potential contamination of eggs prior to fertilizations. If the control showed >3% fertilization, the run was discarded (Tait, Atapattu & Browne 1984). Fertilization success was calculated by counting the proportion of successfully fertilized eggs in each treatment (i.e. cleavage was observed without evidence of polyspermy; Styan 1998). We repeated this experiment nine times.

Experiment 2. Temperature-mediated parental effects on offspring survival

Our second experiment investigated the effect of single parental environment on offspring performance. Adults were acclimated to their allocated temperature treatment for 14 days, as outlined above in 'General methods'. We performed fertilizations for all combinations of male and female adults acclimated to warmer or cooler treatments, with incubation temperatures at either 16·5 °C or 22·0 °C and two replicates for each combination (total of 16 fertilizations per run; Fig. 1a). Eggs were pooled from four females in each treatment group, and sperm were pooled from four males in each treatment group.

Prior to fertilizations, we diluted pooled sperm by a factor of 10^4 to maximize fertilization success while minimizing polyspermy, as indicated by pilot studies (data not shown). For

each fertilization, we added 0·1 mL diluted sperm to 0·1 mL pooled eggs three times, 10 min apart. After exposure to sperm for 2–3 h (depending on assay temperatures), we rinsed the eggs in sterilized and filtered seawater before placing ~25 successfully fertilized eggs in a clean centrifuge tube. We then incubated the samples for 48 h at the same temperature that they experienced during fertilization. To assay larval survival, we used standard methods developed for this species (Chirgwin *et al.* 2015) and describe them here only briefly. We poured out the contents of the centrifuge tube into a microscope counting tray and used two drops of Lugols solution (5% iodine, 10% potassium iodide) to stain and count larvae. We calculated offspring success as the proportion of fertilized eggs that developed into normal larvae. We repeated this experiment 13 times.

Experiment 3. Variable temperatures and paternal effects on offspring survival

In our third experiment, we investigated the effect of variability in the paternal environments on offspring performance. For 14 days, males and females were allocated to one of two temperatures as discussed above. After the first 14 days, we swapped half of the clusters allocated to the male treatment into the alternative treatment group for a further 14 days. To control for effects of changing aquaria, we moved all clusters into new aquaria, regardless of whether they were allocated to a different temperature. Males will be referred to as the temperature that they received either 'early' or 'late' in the acclimation period. We performed fertilizations for all combinations of male and female adults acclimated to warmer or cooler treatments, with incubation temperatures at either 16·5 °C or 22·0 °C and two replicates for each combination (total of 32 fertilizations per run; Fig. 1b). Eggs were pooled from five females in each treatment group, and sperm were pooled from four males in each treatment group. Fertilizations and larval survival were performed as outlined above in Experiment 2. We performed five experimental runs.

STATISTICAL ANALYSIS

We used a linear mixed effects REML framework for analysing data. The specific analytical model for each experiment is outlined below. In general, however, we first tested full models (i.e. with all possible interactions between fixed and random effects) and then reduced nonsignificant interactions with random effects as per Quinn & Keough (2002). Analyses were performed in SYSTAT 13.0 (Systat Software, San Jose, CA, USA).

Experiment 1. In the first experiment, we fitted a model to fertilization success as the response variable, with the paternal, maternal and fertilization environments as fixed categorical effects, with sperm concentration as covariate, and with run as a random effect.

Experiment 2. In the second experiment, we fitted a model to larval survival as the response variable, with the paternal, maternal and offspring environments as fixed categorical effects, and with run as a random effect.

Experiment 3. In the third experiment, we fitted a model to larval survival as the response variable, with the early paternal, late paternal, maternal and offspring environments as fixed categorical effects, and with run as a random effect.

Results

TEMPORAL AUTOCORRELATION IN SEAWATER TEMPERATURE AT THE STUDY SITE

Seawater temperature on any one day was a good predictor of temperature for almost two weeks into the future at our field site. Formal autocorrelation analysis revealed significant autocorrelation across a lag of 12·9 days, beyond which autocorrelation was substantial but not significantly different from zero.

Experiment 1. Temperature-mediated parental effects on gamete morphology and fertilization success

Neither egg size nor sperm size was affected by parental temperature. Egg diameters varied across experimental runs, but did not differ between maternal temperatures ($F_{1,9} < 0{\cdot}0001$, $P = 0{\cdot}986$). Similarly, sperm lengths varied across experimental runs, but showed no systematic variation between paternal temperatures ($F_{1,9} = 0{\cdot}02$, $P = 0{\cdot}89$).

Fertilization success depended on a combination of the thermal histories of both mothers and fathers (Table 1). Maternal experience had no effect on fertilization success when fathers had experienced a warmer environment, but when fathers experienced a cooler environment, fertilization success was higher when mothers experienced a warmer environment (Fig. 2). These effects were consistent across sperm concentrations. Fathers that experienced

Table 1. Effect of parental thermal experience and temperature on fertilization success (Experiment 1).

Effect	Numerator d.f.	Denominator d.f.	*F*	*P*
Fertilization env.	1	8	0·01	0·921
Paternal env.	1	8	5·68	**0·044**
Maternal env.	1	8	0·668	0·438
Sperm conc.	1	40	71·25	**< 0·001**
Paternal env. × Maternal env.	1	331	4·24	**0·040**
Fertilization env. × Paternal env.	1	8	0·13	0·733
Fertilization env. × Maternal env.	1	16	0·03	0·862
Fertilization env. × Paternal env. × Maternal env.	1	331	2·143	0·144

Note that model is reduced after testing for nonsignifcant interactions between treatments and the covariate (sperm concentration) as well as random factors and their interactions that did not explain significantly more variance (see Quinn & Keough 2002 for details). Significant *P*-values shown in bold.

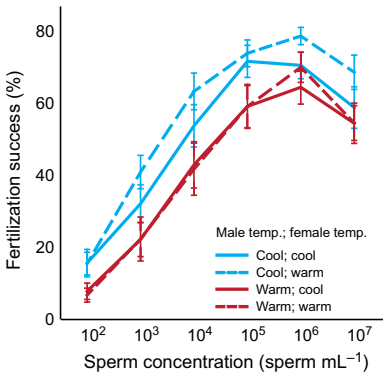

Fig. 2. Fertilization curves showing the combined effects of male and female acclimation temperatures on fertilization success in *Galeolaria caespitosa*. Adults were acclimated to either cooler (15·5 °C) or warmer (21·5 °C) water temperatures for 14 days before use in fertilization assays. Fertilization success is pooled across assay temperatures (see text and Fig. 1a). Nine experimental runs were performed. Error bars are ±SE.

warmer temperatures achieved lower fertilization success across all sperm concentrations and maternal experiences (Fig. 2). The temperature at which fertilization occurred had no effect on fertilization success.

Experiment 2. Temperature-mediated parental effects on offspring survival

The survival of offspring depended on paternal experience: offspring from fathers that experienced warmer temperatures had poorer survival than offspring from fathers that experienced cooler temperatures (Table 2; Fig. 3a). The effect of maternal experience on offspring survival depended on the offspring's environment, where offspring performance seemed to be more driven by offspring produced by warm mothers rather than cool mothers. Offspring survival was highest when there was a mismatch between the temperatures mothers experienced and the temperatures offspring experienced (Fig. 3b). There was no evidence for a matching effect between parental experience and offspring experience in any combination.

Table 2. Effect of parental and offspring thermal environments on offspring survival (Experiment 2).

Effect	Numerator d.f.	Denominator d.f.	*F*	*P*
Paternal env.	1	162	4·50	**0·035**
Maternal env.	1	12	0·011	0·92
Offspring env.	1	12	2·93	0·113
Paternal env. × Maternal env.	1	162	0·15	0·698
Offspring env. × Maternal env.	1	162	4·64	**0·033**
Offspring env. × Paternal env.	1	162	1·3	0·256
Offspring env. × Paternal env. × Maternal env.	1	162	1·14	0·287

Note that model is reduced after testing random factors and their interactions that did not explain significantly more variance (see Quinn & Keough 2002 for details). Significant *P*-values shown in bold.

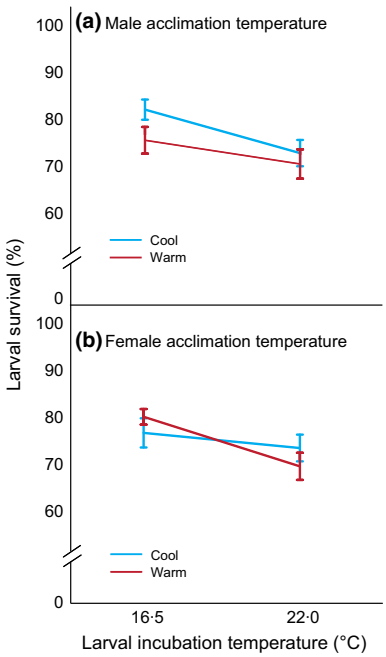

Fig. 3. The relationship between larval incubation temperature and (a) male acclimation temperature or (b) female acclimation temperature on larval survival in *Galeolaria caespitosa*. Adults were acclimated to either cooler (15·5 °C) or warmer (21·5 °C) water temperatures for 14 days before use in fertilization assays. Larval survival is pooled across (a) female acclimation temperatures or (b) male acclimation temperatures, as well as offspring incubation temperatures (see text and Fig. 1a). Thirteen experimental runs were performed. Error bars are ±SE.

There were significant interactions between our main effects of interest and experimental run. Maternal environment interacted with run (Maternal env. × Run: $\chi^2 = 20{\cdot}67$, $P < 0{\cdot}001$), with 6 out of 13 runs in which mothers experiencing cooler temperatures produced offspring with lower survival (see Fig. S1). Similarly, there was an interaction between run and offspring environment (Offspring env. × Run: $\chi^2 = 27{\cdot}428$, $P < 0{\cdot}001$), with higher offspring survival in cooler temperatures in all but three runs.

Experiment 3. Variable temperatures and paternal effects on offspring survival

Paternal acclimation temperature affected offspring survival such that fathers that experienced the same temperatures across the 4-week period produced offspring with higher survival than fathers that experienced variable temperatures (Table 3; Fig. 4). Post-hoc tests detected no difference in the survival of offspring between those whose fathers had experienced either con-

Table 3. Effect of parental and offspring thermal environments on offspring survival when paternal environment varies (Experiment 3).

Effect	Numerator d.f.	Denominator d.f.	*F*	*P*
Paternal env. 1	1	8	0·05	0·820
Paternal env. 2	1	12	0·02	0·880
Maternal env.	1	12	1·25	0·284
Offspring env.	1	118	1·66	0·200
Paternal env. 1 × Paternal env. 2	1	118	4·36	**0·039**
Maternal env. × Paternal env. 1	1	12	0·11	0·750
Offspring env. × Paternal env. 1	1	118	0·04	0·841
Maternal env. × Paternal env. 2	1	118	2·22	0·139
Offspring env. × Paternal env. 2	1	118	0·99	0·323
Offspring env. × Maternal env.	1	118	1·95	0·165
Maternal env. × Paternal env. 1 × Paternal env. 2	1	118	0·83	0·365
Offspring env. × Paternal env. 1 × Paternal env. 2	1	118	0·07	0·795
Offspring env. × Paternal env. 1 × Paternal env. 2	1	118	0·25	0·618
Offspring env. × Paternal env. 1 × Paternal env. 1	1	118	0·14	0·706
Offspring env. × Maternal env. × Paternal env. 1 × Paternal env. 2	1	118	1·51	0·222

Here, Paternal env. 1 refers to male early acclimation treatment and Paternal env. 2 refers to male late acclimation treatment. Female acclimation temperatures were kept constant over this time. Note that model is reduced after testing random factors and their interactions that did not explain significantly more variance (see Quinn & Keough 2002 for details). Significant *P*-values shown in bold.

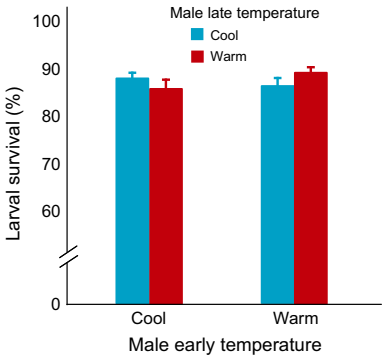

Fig. 4. The effect of variability in paternal acclimation temperature on larval survival in *Galeolaria caespitosa*. Adults were acclimated to either cooler (15·5 °C) or warmer (21·5 °C) water temperatures for 28 days before use in fertilization assays. After the first 14 days of acclimation, half of the males were swapped into the alternative treatment group. Acclimation then continued for a further 14 days. 'Early' refers to the temperature that males experienced before the swap, and 'late' refers to the temperature that males experienced after the swap. Larval survival is pooled across female acclimation and offspring incubation temperatures (see text and Fig. 1b). Five experimental runs were performed. Error bars are ±SE.

stant warmer or constant cooler conditions ($F_{1,70}$ = 0·001, P = 0·991). Similarly, there was no difference in survival of offspring from fathers that had experienced warmer conditions, then cooler, or cooler then warmer ($F_{1,72}$ = 0·057, P = 0·812).

Discussion

The parental thermal environment affected subsequent offspring performance both during and after fertilization. These parental effects were not beneficial for offspring, rather they affected offspring performance in complex ways. Surprisingly, paternal effects were equal to or greater than maternal effects on subsequent offspring performance. The parental experience of increased temperature had a number of negative effects throughout the life history that are likely to reduce the recruitment and productivity of the focal species. We found that the impacts of transgenerational plasticity depended on the duration of exposure, where the negative paternal effects that we observed disappeared when fathers were exposed to higher temperatures for longer. Overall, we found that parental effects acted as a conduit rather than a buffer to short-term environmental stress in the parental generation, and in some instances can act to exacerbate rather than ameliorate the likely impacts of global change.

The fertilization success of gametes depended on the experience of both mothers and fathers, but not in adaptive ways. Several species (including our study species) with external fertilization exhibit transgenerational plasticity to maximize fertilization success in the face of stress (Roller & Stickle 1994; Crean & Marshall 2008a; Jensen, Allen & Marshall 2014), but as far as we are aware, no other transgenerational plasticity study has examined temperature. Our study species in particular shows adaptive plasticity in response to other stressors (parental exposure to hyposalinity reduces the impact of hyposalinity on fertilization; Tait, Atapattu & Browne 1984), but it seems the effects of temperature are less straightforward. The reason why this species shows adaptive transgenerational plasticity in response to salinity but not temperature may be because the temperature at which fertilization occurs is simply too variable to predict. While we have no data on salinity at our site, in similar bays salinity shows less short-term variation relative to temperature (Jensen, Allen & Marshall 2014). Theory predicts that parents should only express transgenerational plasticity if the offspring environment can be anticipated by parents (Burgess & Marshall 2014) – temperature data from our site suggest

that despite mean weekly temperatures near our parental colonies being a good predictor of mean temperature, temperature can still show dramatic differences from hour to hour. Given that fertilization success occurs over very short time-scales in this species and others, it may be that temperature in these very short temporal windows is simply too difficult to anticipate such that adaptive plasticity to maximize fertilization success in variable temperature cannot evolve.

We found a surprising interaction between both the maternal and paternal environment: when fathers experienced a cooler environment, fertilization success was maximized when mothers experienced a warmer environment. The driver of this interaction remains unclear. The effect was consistent across all sperm concentrations (there was no sperm concentration × maternal × paternal environment interaction), suggesting that the parental environment did not affect processes related to polyspermy or sperm limitation (otherwise differential effects would have been expected at different sperm concentrations; Marshall 2006). We saw no overt changes in gamete morphology in our experiments; warmer and cooler mothers produced eggs that were similar in size, and sperm did not differ in either their head or tail length, such that morphological differences did not drive the patterns we observed. More detailed explorations of the genomic and proteomic signatures on gametes from parents of different temperatures are now underway.

Over and above the interaction between maternal and paternal environments, there was a clear effect of paternal thermal experience on fertilization success. Across all of the sperm concentrations in our experiment, sperm from fathers that had experienced a warmer temperature achieved around 30% lower fertilization success than sperm from fathers that experienced a cooler temperature. These fertilization dynamics suggest that the effect of temperature on fathers does not produce sperm that cannot contact eggs (such an effect would manifest as a sperm concentration-dependent effect; Marshall 2006); instead it seems that a consistent proportion of sperm are rendered incapable of initiating a fertilization reaction in eggs. There is some evidence for plasticity in sperm phenotypes to affect both fertilization capacity and offspring phenotype (Marshall 2015). In a number of systems, plasticity in sperm phenotype affects sperm fertilization capacity (Crean & Marshall 2008b), offspring phenotype (Crean, Dwyer & Marshall 2013) and methylation patterns in sperm DNA (Jiang *et al.* 2013). Together these studies suggest that changes in sperm phenotype affect both heritable and inheritable components of offspring phenotype. Regardless of the mechanism driving the effects we observed, given the fecundity of our species (a single female can produce 1000s of eggs), the large effect on fertilization success that we observed is likely to have significant consequences for recruitment in the next generation.

Paternal exposure to increased temperature also affected subsequent offspring performance. Fathers that experienced warmer temperatures sired offspring with lower survival than fathers that experienced cooler temperatures. It may be that whatever effect of increased temperature that reduces the fertilization potential of sperm also reduces the performance of offspring. That sperm from fathers exposed to warmer temperatures have both reduced fertilization and reduced offspring performance suggests that DNA damage has occurred. The causes and consequences of damage to sperm DNA are poorly understood, but temperature stress could induce oxidative stress or aberrant apoptosis (Lewis & Aitken 2005). In mammals at least, sperm repair mechanisms are down-regulated during late-stage spermatogenesis; it will be interesting to determine whether the effects we observed here are due to damage during the production of sperm or occur while being stored. Longer exposure times, particularly if the exposure occurred during the father's development, could potentially alleviate some of the physiological damage caused by acute temperature treatments. The cellular and physiological responses to chronic high temperatures may be very different from the responses activated during acute high-temperature exposure. Regardless of the mechanism, populations are receiving a 'double hit' from increases in paternal temperature – fertilization success drops by ~30% and larval survival drops by a further ~10%. In this regard, rather than buffer offspring from environmental stress, it seems that transgenerational plasticity is acting as conduit, transmitting stress from the parental generation into reduced performance in the offspring generation. Interestingly, the temperatures we used in our experiment were not extreme relative to those experienced regularly in the field at that site during the summer. Clearly even fairly mild thermal stresses can be transmitted to the offspring generation via the paternal line.

The temperature that mothers experienced also affected offspring survival, although these maternal effects were less consistent than the paternal effects and were in the opposite direction. It seemed that offspring produced by warm mothers drove this pattern more than offspring produced by cool mothers. It is possible that larval thermal stress may be more detrimental when mothers have experienced a thermal stress themselves, suggesting that this is a transmissive maternal effect rather than an anticipatory one (Marshall & Uller 2007). In six experimental runs, mothers that experienced cooler temperatures produced offspring that had lower performance than mothers that experienced warmer temperatures. We observed no overt changes in gamete morphology, but it is possible that mothers changed the biochemical composition of their eggs in response to temperature. For example, birds often alter the hormonal and epigenetic state of their eggs without altering offspring size, with important consequences for offspring phenotype (Groothuis & Schwabl 2008).

Despite evidence that offspring temperature affected survival, we found no evidence for adaptive transgenerational plasticity on the maternal line – offspring from mothers that experienced higher temperatures were no better able

to cope with higher temperature than offspring from cooler temperatures. Instead, we found evidence for maladaptive maternal effects – offspring performance was actually highest when mothers experienced a different environment from that of their offspring. This failure to detect adaptive maternal effects in response to temperature differs from previous studies (Bownds, Wilson & Marshall 2010; Donelson *et al*. 2012; Salinas & Munch 2012), including organisms that live in similar habitats (Burgess & Marshall 2011). Clearly adaptive maternal effects in response to temperature cannot be assumed to be a ubiquitous response and may not buffer many species from the impacts of future global change. Nevertheless, our results have unsettling implications for the impacts of global change: offspring survival was lowest when both maternal and offspring temperatures were higher, suggesting that higher temperatures will reduce larval survival via both direct effects on offspring and via indirect maternal effects.

The effect of temperature on larval survival depended on how long parents, specifically fathers, were exposed to higher temperatures. We found that the negative effects on larval survival disappeared after the acclimation period doubled from a 2-week acclimation period to 4 weeks. This highlights that the short-term nature of the temperature treatment (14 and 28 days) could produce a different result than if the adults were acclimated for their whole life. Donelson & Munday (2015) found that biased offspring sex ratios in reef fish caused by higher temperatures was compensated when parents developed in the warmer conditions for their whole lives, but not when they were exposed to warmer temperatures only during the reproductive period. In this example, developmental plasticity in one generation may be necessary to induce adaptive transgenerational plasticity in the next. Future global change is likely to result in higher frequency and intensity of sudden temperature spikes (Thompson *et al*. 2013) and our results suggest that these may be more problematic than temperature increases overall. In our system at least, a range of temperatures can be tolerated, but rapids shifts in temperature invoke transitory decreases in offspring performance. Experiments that disentangle duration of exposure and variance are necessary to determine how different temperature regimes will alter estimates of transgenerational plasticity.

Our results suggest that variability, rather than mean temperature per se, drives the paternal effects we observed. Given that our experiments involved much more constant temperatures (even the variable treatment shows less variation than the typical field environment), it seems that natural variation in temperature is likely to have significant, yet largely unrecognized, downstream effects on offspring performance. One of the predicted impacts of global change is not only higher temperatures on average but also more variable and extreme temperatures. Our results suggest that increased temperature variation will have negative effects on larval performance via paternal effects – whether such effects occur in other systems should now be explored.

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.580bh (Guillaume, Monro & Marshall 2015).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Figure S1. The effect of female acclimation temperature on larval survival 48 h after fertilization in each of the 13 experimental runs in Experiment 2.