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Adaptive maternal and paternal effects: gamete plasticity in response to parental stress

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Summary

1. Transgenerational phenotypic plasticity is increasingly recognized as an important buffer of environmental change – many studies show that mothers alter the phenotype of their offspring so as to maximize their performance in their local environment. Fewer studies have examined the capacity of parents to alter the phenotype of their gametes to cope with environmental change. In organisms that shed their gametes externally, gametes are extremely vulnerable to local stresses and transgenerational plasticity in the phenotypes of gametes seems likely in this group.

2. In a marine tubeworm, *Hydroides diramphus,* we manipulated the salinity environment that mothers and fathers experienced before reproduction and then examined the phenotype of their gametes, as well as the performance of those gametes and the resultant larvae in different salinities.

3. We found strong evidence for gamete plasticity – both mothers and fathers adaptively adjust the phenotype of their gametes to maximize the performance of those gametes in the salinity regime experienced by their parents. Parents were quite flexible in the phenotype of gametes that they produced: they could switch the salinity tolerance of their gametes back and forth depending on their most recent experience.

4. Gamete plasticity was not without risks, however. We observed strong trade-offs in performance when gametes experienced an environment that did not match that of their parents. These effects of the parental environment persist for the duration of the larval phase such that larvae may not be able to disperse to environments that do not match their parents. Gamete plasticity may therefore represent an important source of phenotype–environment mismatches.

5. Gamete plasticity may represent an important mechanism for coping with environmental change and an important source of maternal and paternal effects in species with external fertilization. Studies that seek to predict the impacts of stresses that persist across generations (e.g. ocean acidification) should include parental exposures to the stress of interest.

Key-words: epigenetics, non-genetic parental effects, transgenerational phenotypic plasticity

Introduction

In most organisms, early life-history stages tend to have much lower tolerances to stress than that of later, more-robust adult stages (Moran 1994; Byrne 2012). In many instances, this early vulnerability to stress is ameliorated somewhat by anticipatory maternal effects (Marshall & Uller 2007): mothers adaptively manipulate the phenotype of their offspring in response to predictable environmental stresses (Agrawal, Laforsch & Tollrian 1999). This form of adaptive, transgenerational plasticity has important ecological and evolutionary consequences. Ecologically, transgenerational phenotypic plasticity (TPP) can be an important source of variation in the phenotype and performance of individuals within a population (Uller 2008). Evolutionarily, TPP may provide scope and time for populations to adapt to changing conditions (Reed, Schindler & Waples 2011; Miller *et al.* 2012; Parker *et al.* 2012). In

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some instances, the causes and consequences of TPP are increasingly well understood, but most investigations have been restricted to specific phases of the life history.

Most studies of adaptive transgenerational plasticity have focused on the phenotype of the post-zygotic stage, usually, the larval or juvenile phase (Galloway 2001; McCormick 2006; Uller 2008; Parker *et al.* 2012). For internal fertilizers, this focus on later life-history stages makes sense. The gametes of internal fertilizers are released into a relatively stable environment that is controlled by maternal homeostasis, so they are unlikely to experience substantial environmental variation, precluding the need for TPP. In contrast, most fish, anurans and marine invertebrates have external fertilization, and their gametes will face all the challenges and stresses associated with variable environments, but do so at much smaller sizes, and often with only the simplest mechanisms for coping with stress relative to the offspring of internal fertilizers (Levitan 1995). Indeed, the gametic phase is often the most vulnerable stage of an organism's life (Marshall 2006), and successful fertilization is so rare that some populations may be limited by variation in fertilization rates (Levitan & Petersen 1995). Because gametes are so sensitive and the consequences of environmental variation so profound, we expect selection to favour the evolution of TPP in gamete phenotype (which we will call gamete plasticity) as a way of ensuring that fertilization is successful in heterogeneous environments.

Gamete plasticity has the potential to be an important source of both maternal and paternal effects. Maternal effects have long been recognized as an important non-genetic source of phenotypic variation in a range of systems (Mousseau & Fox 1998). More recently, there has been a dramatic increase in the number of studies demonstrating that non-genetic paternal effects on the offspring phenotype also exist (Bonduriansky & Head 2007; Uller 2008; Bonduriansky & Day 2009). Both maternal and paternal effects occur when changes in the environment or phenotype of the parent affect the phenotype of the offspring – gamete plasticity may be an important, although largely unexplored, conduit by which such effects are transmitted (Crean, Dwyer & Marshall in press).

While there are good reasons to expect gamete plasticity to be common, there are equally good reasons to expect it to be rare. First, parents require some degree of environmental predictability, if they cannot anticipate the environment their gametes will experience, they cannot adjust their gametes in an adaptive way (Crean & Marshall 2009). Secondly, phenotypic changes in one life-history stage almost always have consequences for subsequent life-history stages (Schluter, Price & Rowe 1991). For example, tadpoles that express plasticity in response to predators have narrower bodies and longer legs as adults (Relyea 2001, 2002). These strong phenotypic links among life-history stages can act to constrain the evolution of plasticity in any one stage, if optimizing performance in one stage comes at the cost of performance in another stage, and there may be no net selection for change (Schluter, Price & Rowe 1991; Marshall & Morgan 2011). These links may form earlier than was once thought, crossing the fertilization boundary: recent studies show that fertilization does not represent a 'new beginning' for zygotes; rather, changes in the phenotype of both eggs and sperm can have consequences for the subsequent embryo (Bonduriansky & Day 2009; Crean, Dwyer & Marshall 2012; Ritchie & Marshall 2013). The benefits associated with gamete plasticity to enhance fertilization may therefore be offset by the costs of such changes for the remainder of the zygote's life. As such, it is difficult to predict the prevalence of gamete plasticity in nature.

There are some indications that parents alter their gametes to increase the likelihood of successful fertilization. A number of studies, particularly in internal fertilizers, have demonstrated that fathers alter the phenotype of their sperm according to the local threat of sperm competition: males that are likely to compete with many males for a mate produce more competitive ejaculates (Birkhead & Pizzari 2002; Gage & Morrow 2003). Similarly, one study has shown that mothers increase the target size of their eggs to make them more easily fertilized when the local density of males is low and the probability of sperm limitation is high (Crean & Marshall 2008). Finally, some studies have shown that the negative effects of low salinity on fertilization rates are diminished when adults are first kept in lower salinity conditions (Tait, Atapattu & Browne 1984; Hintz & Lawrence 1994; Roller & Stickle 1994), suggesting that some gamete plasticity has occurred in one or both gamete types but the consequences of this plasticity for later life-history stages remain unclear. These consequences are particularly important to explore given the increased recognition of the role of both maternal and paternal effects.

Here, we manipulated the environmental conditions that both mothers and fathers experienced by exposing adults of a broadcast spawning marine invertebrate (*Hydroides diramphus*, Fig. 1) to different levels of salinity, a naturally varying stressor in field populations (Tait, Atapattu & Browne 1984). We then determined whether the parental environment had any consequences for the performance of gametes during fertilization across high and low levels of salinity. Next, we determined whether gamete plasticity occurred in both eggs and sperm, and what aspects of fertilization and development were affected by our manipulations. Finally, we determined whether the gamete plasticity that we observed had lasting consequences for the phenotype offspring beyond fertilization and early development.

Materials and methods

STUDY SPECIES AND HUSBANDRY

Hydroides diramphus is a dioecious marine polychaete that occurs in sparse to highly dense aggregations or 'colonies' on rocks or pontoons at shallow depths and reproduces by broadcast

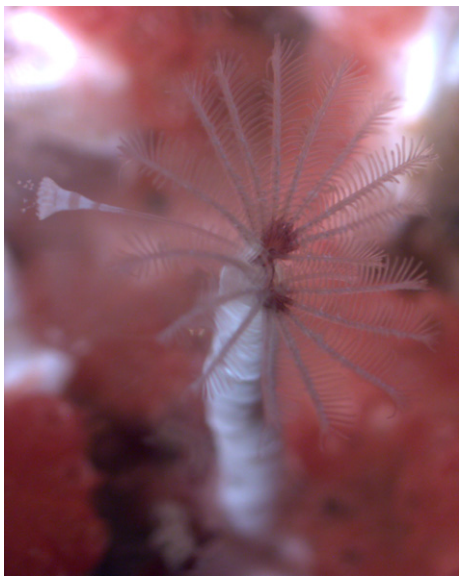

Fig. 1. Adult *Hydroides diramphus* with its feeding tentacles extended. Photograph by Richard Allen.

spawning (Basttida-Zavala & Ten Hove 2002). *Hydroides diramphus* is native to the lagoonal habitats in the Caribbean; however, it has also been recorded from harbours and ships hulls worldwide (personal communication, Robin Wilson, Museum of Victoria). At our field site (Scarborough Marina, Redcliffe, Australia: 27°11′ 153°06′), salinity is strongly affected by rainfall events and associated runoff and can drop from 35‰ to 25‰ in a few hours and can stay this low for weeks at a time (Ecosystem Health Monitoring Program, Healthy Waterways Ltd, Brisbane, Queensland, Australia). While drops in salinity can occur at any time, more sustained periods of low salinity tended to occur in the Austral summer during periods of frequent and intense rainfall.

Hydroides diramphus colonies were collected from the pontoons at Scarborough Marina. Colonies were then placed into an insulated box filled with freshly collected seawater and transferred to the University of Queensland, St. Lucia. Colonies were then transferred to aerated tubs filled with 6 L of seawater and were maintained at 35‰ and 26‰ (field-collected water diluted with distilled water). Colonies were maintained in a constant temperature room (22 °C ± 1), on a 12 h:12-h photoperiod, and were fed on a diet of *Isochrysis galbana*. Water changes and feeding occurred every 3 days.

IN VITRO FERTILIZATION METHODS

To obtain gametes, adult *H. diramphus* were carefully removed from their calcareous tubes (Allen & Marshall 2010) and placed into a 5-mL Petri dish containing 2 mL of filtered seawater (FSW, 0·22 μm) of the parental environment (unless manipulations of the fertilization environment were required). For all experiments, motile sperm from three males and mature eggs from three females were each pooled before being used in our assays. We pooled sperm and eggs because we wished to minimize the impacts of male × female interactions at fertilization and beyond affecting subsequent success (Marshall 2006). This approach precludes the estimation of individual effects but maximizes statistical power.

A drop of the pooled egg solution was placed on a Neubauer Improved haemocytometer and adjusted to an egg concentration of 500 eggs.mL^{-1}. A drop of pooled sperm was then placed on the haemocytometer to determine the sperm concentration. Sperm concentration was adjusted to $4{\cdot}8 \times 10^6$ sperm.mL^{-1} ($\pm 10^3$ sperm.mL^{-1}), and subsequent dilutions were made for other experiments requiring a lower sperm concentrations. Theory predicts that tests of stressors at sperm concentrations that result in 100% fertilization success in the controls underestimates the impacts of the stressor of interest (Marshall 2006), so we deliberately used sperm concentrations that were lower than those that resulted in 100% fertilization success. Any unused sperm was discarded within 1 h of spawning unless otherwise stipulated.

In vitro fertilization assays were carried out by adding 2 mL of egg solution to 0·2 mL of sperm solution. Eggs were washed through a 25 μm nitrex mesh with FSW of the appropriate salinity 15 min after mixing with sperm. Ninety minutes after mixing eggs and sperm, 0·20 μL of eggs (*c.* 100 eggs) was placed on a single cavity well slide and photographed under an Olympus CX41 compound microscope with the program pixeLINK capture OEM. The number of normally cleaved versus non-cleaved eggs was counted from the digital image.

EXPERIMENT 1: THE EFFECT OF THE PARENTAL ENVIRONMENT ON FERTILIZATION SUCCESS UNDER DIFFERENT SALINITIES

Parental and gamete salinity environments were manipulated orthogonally to determine whether the changes in the parental environment affected fertilization success. Fertilization assays were carried out at two salinities and three sperm concentrations (Fig. S1, Supporting information). *Hydroides diramphus* colonies were maintained in the laboratory at salinities of either 35‰ or 26‰, with six replicate tubs per salinity ($n = 6$). After 14 days at those salinities, fertilization assays were carried out where the gametes from each parental environment were exposed to two salinities, 35‰ and 26‰ (Fig. S1). We chose 14 days arbitrarily – gametogenesis is likely to exceed this time substantially but we felt that 14 days of reduced salinity best reflected the conditions that these organisms experience in the field following intense rainfall (Ecosystem Health Monitoring Program, Healthy Waterways Ltd). The gametes from males and females were extracted, and *in vitro* fertilization assays were carried out. Fertilization success was assessed over three sperm concentrations: (i) high ($4{\cdot}8 \times 10^7$ sperm.mL^{-1}); (ii) medium ($4{\cdot}8 \times 10^6$ sperm.mL^{-1}); and (iii) low ($4{\cdot}8 \times 10^4$ sperm.mL^{-1}). Three subreplicate fertilization assays were carried out per tub (Fig. S1).

EXPERIMENT 2: THE EFFECT OF SALINITY ON GAMETE PERFORMANCE: PHENOTYPIC PLASTICITY VS. SELECTION

We found that the parental environment strongly affected fertilization success under different salinities (see Results, Experiment 1). Our next step was to determine whether this effect was reversible, thereby indicating plasticity rather than selection drove our results. We therefore repeated our experiment but after 2 weeks of exposure to one salinity, half of the adults were exposed to the other salinity before conducting fertilization assays as before. If selection drove the effects we observed, then we would expect an effect of the second salinity environment on fertilization success.

Adult colonies were collected and again were maintained at either 26‰ or 35‰ for 2 weeks. After 2 weeks, the colonies from each tub were equally split into two tubs, one of each salinity treatment (see Fig. S2, Supporting information), which resulted in the following four adult treatments combinations per replicate 35–35‰, 35–26‰, 26–26‰ and 26–35‰, where the former value refers to the salinity, the adults were initially exposed to for

2 weeks, and the later value was the salinity experienced in weeks 3 and 4. Colonies that remained in the same salinity treatment acted as controls to ensure there was no effect of being moved from tub to tub or of being maintained in the laboratory over 4 weeks. Again, all treatments were fully crossed, and we used three replicate tubs per treatment combination.

After 4 weeks, fertilization assays were carried out under two different salinities as described in Experiment 1. From this experiment onwards, however, only a single sperm concentration of $4{\cdot}8 \times 10^6$ sperm.mL^{-1} (one which resulted in 60–80% fertilization success) was used because Experiment 1 showed that our findings were independent of the sperm concentration that was used (see Results).

EXPERIMENT 3: TIMING OF THE INDUCTION OF TRANSGENERATIONAL PLASTICITY

We found that parents exposed to different salinities for 2 weeks induced gamete plasticity (see Results), and so we were interested in whether parents exposed to different salinities for shorter periods also induced gamete plasticity. We therefore repeated Experiment 1, but exposed adults for shorter periods ranging from 0, 3, 5 to 7 days.

EXPERIMENT 4: THE EFFECT OF THE PARENTAL ENVIRONMENT ON FERTILIZATION AND DEVELOPMENT

We were interested in whether the parental environment affected fertilization or post-fertilization development in the same manner and magnitude. We separated the effects of salinity in our fertilization assays into two components and manipulated the fertilization environment and egg development environments separately (Pechenik, Pearse & Qian 2007; Allen & Pechenik 2010).

Colonies of *H. diramphus* were collected and maintained in the laboratory under two experimental salinities – 35 ‰ and 26 ‰ – with four tubs per salinity. After 2 weeks, a series of *in vitro* fertilization assays were carried out as described above, but the gamete environment was separated into the fertilization environment and development environment (Pechenik, Pearse & Qian 2007; Allen & Pechenik 2010). As per earlier studies on this topic (Allen & Pechenik 2010), the fertilization environment was defined as the environment in which sperm and eggs fuse (i.e. the first 15 min after mixing the gametes). The development stage was defined as the environment developing eggs were exposed to after fertilization and until fertilization success was scored (i.e. after eggs were rinsed free of sperm). Fertilization success was scored at 90 min egg–sperm mixing. Every treatment combination was included yielding a total of eight treatment combinations with five replicates per combination.

EXPERIMENT 5: MATERNAL AND PATERNAL EFFECTS ON GAMETE PERFORMANCE

In this experiment, we examined whether the maternal or paternal environments were responsible for changes in fertilization success by crossing the eggs and sperm from parents from a both salinity conditions in every combination and carrying out *in vitro* fertilization assays in both salinities.

Colonies were maintained in the laboratory for 2 weeks at either 35‰ or 26‰ with four tubs per salinity. Fertilization assays were carried out as described in Experiment 1, but we also crossed males and females from the different salinity treatments. Three subreplicate fertilization assays were carried out for each tub. In experiments where males and females were crossed from the same salinity environment, each male and female was taken from separate tub.

EXPERIMENT 6: THE EFFECT OF PARENTAL ENVIRONMENT ON GAMETE MORPHOLOGY AND SPERM LONGEVITY

We found that the performance of both male and female gametes was altered in response to changes in parental experiences of salinity (see Results), so we then examined whether the morphology of gametes also changed with parental experience.

We collected the sperm and eggs of five males and five females from each of the five tubs maintained at each salinity for 2 weeks. We fixed the gametes in 10% formaldehyde in seawater and later measured a sample of *c.* 50 eggs and 50 sperm from each replicate. We estimated the diameter of eggs and the head area and tail length of sperm with the digital analysis software Image J (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012).

We also measured the longevity of sperm from males from the different environments by estimating the capacity of sperm to fertilize freshly collected eggs under different salinity environments every hour for up to 3 h after collection.

EXPERIMENT 7: THE PERSISTENCE OF GAMETE PLASTICITY EFFECTS OVER THE LARVAL PERIOD

We were interested in whether the effects of parental experience of salinity on gamete performance persisted beyond development to affect larval performance. We therefore exposed parents as described above for Experiment 1 (four tubs per salinity), created zygotes in the salinity that their parents had experienced and placed 30 fertilized eggs in each of twenty 5 mL vials. Then at 1, 48 and 96 h after fertilization, we transferred the developing larvae in five vials from their original salinity (and that of their parents) to the other salinity. At each time of change, all other larvae were also transferred to new vials but with the same salinity to control for movement effects. Two days after larvae were moved from their original salinity to their new salinity, larval survival was estimated (Fig. S3, Supporting information).

DATA ANALYSIS

Our main treatment of interest (parental exposure to salinity) was applied at the scale of tub, so our unit of replication for this treatment was tub. Our gamete treatments were applied at the scale of individual batches of eggs and sperm, and so we also replicated at this scale. The appropriate analysis for our design therefore was a standard, partly nested ANOVA where adult treatment effects are tested at the scale of tubs and gamete treatment effects are tested at a lower scale (Quinn & Keough 2002). All of our analyses treated tub (Adult Salinity) as a random factor, Adult salinity as a fixed factor and the various gamete treatments as fixed factors. We used standard model reduction procedures to remove non-significant terms that were of no biological interest (Quinn & Keough 2002). To analyse the effect of paternal experience on sperm morphology, we used nested MANOVA where Adult salinity was a fixed factor, tub (Adult Salinity) was a random factor, and sperm head area and tail length were response variables.

Results

EXPERIMENT 1: THE EFFECT OF THE PARENTAL ENVIRONMENT ON FERTILIZATION SUCCESS UNDER DIFFERENT SALINITIES

The salinity that parents experienced significantly affected fertilization success under different salinities (Table 1).

Table 1. A partly nested ANOVA (reduced model) showing the effect of the parental environment (Parent env.) and gamete environment (Gamete env.) on fertilization success over three sperm concentrations (Sperm conc.). All significant *P* values shown in bold

Source	d.f.	MS	*F*	*P*
Sperm conc.	2	5785	14·82	**< 0·001**
Parental env.	1	191	0·53	0·485
Gamete env.	2	13 766	58·30	**< 0·001**
Parental env. × Sperm conc.	2	88	0·22	0·797
Gamete env. × Sperm conc.	4	1511	3·87	**0·005**
Parental env. × Gamete env.	2	6119	15·68	**<0·001**
Parental env. × Gamete env. × Sperm conc.	4	170	0·43	0·781
Tub (Parental env.)	8	358	1·28	0·258
Error	182	390		

Table 2. Partly nested ANOVA showing the effect of parental experience in 4 weeks (Environment 1) and second 2 weeks (Environment 2) prior to reproduction on Gamete env. under different salinities. All significant *P* values shown in bold

Source	d.f.	MS	*F*	*P*
Parental env. 1	1	377	1·02	0·319
Parental env. 2	1	355	0·96	0·333
Gamete env.	1	434	1·17	0·285
Parental env. 1 × Gamete env.	1	408	1·10	0·299
Parental env. 2 × Gamete env.	1	13 836	37·42	**< 0·001**
Parental env. 2 × Parental env. 1	1	33	0·09	0·764
Gamete env. × Parental env. 1 × Parental env. 2	1	49	0·13	0·718
Tub (Parental env. 2 × Parental env. 1)	10	438	1·18	0·330
Error	39	369		

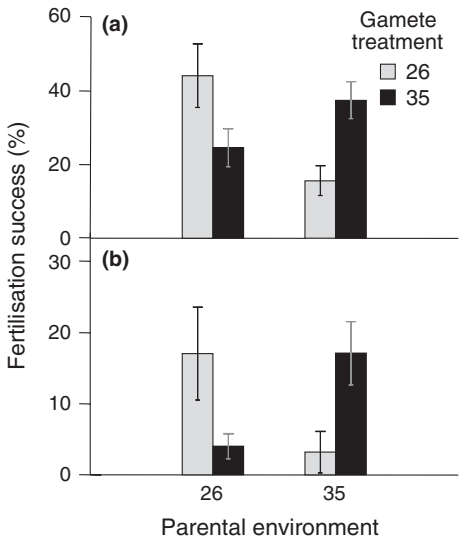

Fig. 2. Mean (±SE) fertilization success of *Hydroides diramphus* gametes under different salinity conditions and different parental experiences. Panel (a) shows fertilization success at a high sperm concentration and panel (b) shows fertilization success at a low sperm concentration. Black bars indicate success in normal salinity conditions (35 ppt), and grey bars indicate success under lower salinity conditions (26 ppt).

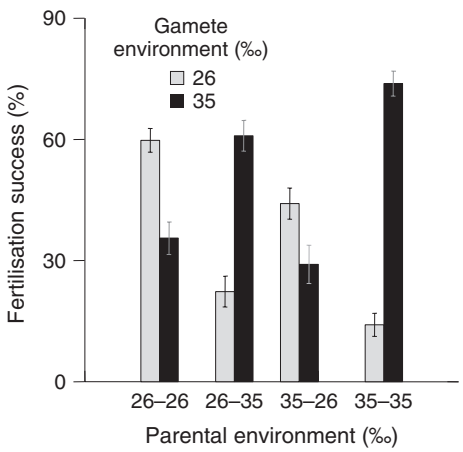

Fig. 3. Mean (±SE) fertilization success of gametes under different salinity conditions at fertilization (black bars show normal salinity at fertilization, grey bars show lower salinity at fertilization) of *Hydroides diramphus* gametes. The *x* axis indicates the salinity experience of parents, with the first value in each pair indicating the first salinity environment that parents experienced, while the second value indicates the second salinity environment that parents experienced.

Fertilization success was approximately twice as high when the gametes experienced the same salinity as their parents (Fig. 2). While sperm concentration significantly affected fertilization success, there was no significant interaction between sperm concentration, parental salinity and gamete salinity (Table 1).

EXPERIMENT 2: THE EFFECT OF SALINITY ON GAMETE PERFORMANCE: PHENOTYPIC PLASTICITY VS. SELECTION

Our findings in Experiment 1 appeared to be driven by plasticity rather than selection effects: the effects of adult environment were reversible. Fertilization success was greatest when the gamete environment matched the parental environment in the 2 weeks immediately preceding fertilization (Table 2, Fig. 3). Only the most recent experience of the parents determined fertilization success in the two gamete environments, and the environment that parents experienced in the first 2 weeks had no effect on fertilization (Table 2).

EXPERIMENT 3: TIMING OF THE INDUCTION OF TRANSGENERATIONAL PLASTICITY

Parents did not modify the resistance of their gametes immediately; gametes showed no change in resistance to hyposalinity when their parents had experienced hyposalinity for 3, 5 or 7 days ($F_{3,47} = 0{\cdot}773$, $P = 0{\cdot}515$). There were indications, however, that gametes from parents

exposed to low salinity for 7 days did have slightly higher fertilization success than gametes from parents exposed to normal salinity (fertilization success of 45·33% and 35·33%, respectively).

EXPERIMENT 4: THE EFFECT OF THE PARENTAL ENVIRONMENT ON FERTILIZATION AND DEVELOPMENT

Both the fertilization salinity and the salinity in which post-fertilization development took place affected fertilization success. The highest success was recorded when both the fertilization salinity and the developmental salinity matched the parental environment (Table 3, Fig. 4). While both the fertilization and developmental salinity affected success, the effects of the developmental environment appeared to have a slightly stronger effect than the fertilization environment. The reduction in fertilization success overall was greatest when the developmental environment did not match the parental environment. The reduction in fertilization success was much less when only the fertilization environment did not match the parental environment (Fig. 4).

EXPERIMENT 5: MATERNAL AND PATERNAL EFFECTS ON GAMETE PERFORMANCE

Both maternal and paternal experience of salinity affected fertilization success in different salinities (Fig. 5). Fertilization success was highest when both the maternal and paternal environment matched the fertilization environment and lowest when neither parental environment matched the fertilization environment (paternal environment × fertilization environment interaction: $F_{4,18} = 15{\cdot}21$, $P < 0{\cdot}001$; maternal environment × fertilization interaction $F_{4,18} = 11{\cdot}83$, $P < 0{\cdot}001$). The effects of maternal and paternal experience were additive: the non-significant interaction between paternal, maternal and fertilization environment indicates that the effect was simply the sum of the effects of both parents ($F_{8,18} = 2{\cdot}47$, $P = 0{\cdot}053$).

Table 3. Partly nested ANOVA showing the effect of parental experience, the fertilization environment and the developmental environment on subsequent fertilization success. All significant *P* values shown in bold

Source	d.f.	MS	*F*	*P*
Development env.	1	16	0·08	0·777
Fertilization env.	1	639	3·16	0·078
Parental env.	1	10 955	54·28	**< 0·001**
Development env. × Fertilization env.	1	2615	12·96	**< 0·001**
Development env. × Parental env.	1	27 842	137·9	**< 0·001**
Fertilization env. × Parental env.	1	9611	47·62	**< 0·001**
Dev. env. × Fert. env. × Parental env.	1	808	4·00	**0·048**
Tub (Parental env.)	12	608	3·01	**0·001**
Error	118	201		

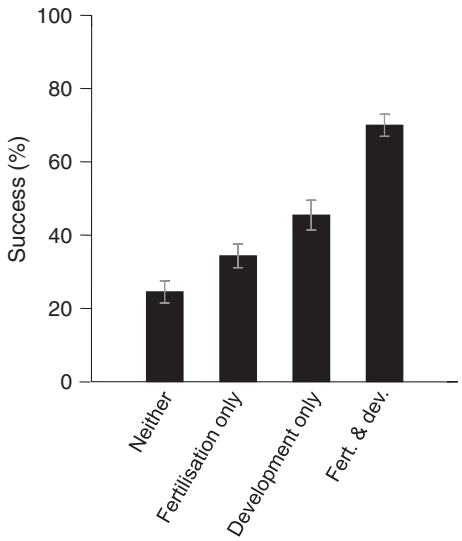

Fig. 4. Mean (±SE) fertilization success of *Hydroides diramphus* gametes under different salinity conditions. Bars show fertilization success only for those gametes whose parents experienced low salinity (similar results were found for gametes whose parents experienced normal salinity conditions). The *x* axis indicates whether the salinity environment that gametes and developing eggs experienced matched the environment of their parents. Moving from left to right, the first bar indicates fertilization success when neither environment matched that of the parents' environment, the second bar indicates fertilization success when only the fertilization environment matched the parents' environment, the third bar indicates fertilization success when only the developmental environment matched the parents' environment and the fourth bar indicates fertilization when both the fertilization and developmental environment matched the parents' environment.

EXPERIMENT 6: THE EFFECT OF PARENTAL ENVIRONMENT ON GAMETE MORPHOLOGY AND SPERM LONGEVITY

Parental salinity experience affected sperm morphology, but not egg morphology. Sperm from males exposed to hyposalinity produced sperm that had *c*. 50% smaller heads than sperm from males exposed to normal salinity (Pillai Trace: 0·79, $F_{2,5} = 9{\cdot}41$, $P = 0{\cdot}02$; Univariate *F* test: $F_{1,6} = 17{\cdot}63$, $P = 0{\cdot}006$). Both sperm tail length and egg diameter were unaffected by parental experience (Sperm: $F_{1,6} = 1{\cdot}32$, $P = 0{\cdot}294$; Eggs: $F_{1,4} = 0{\cdot}007$, $P = 0{\cdot}938$). The effects of parental and gamete environment on sperm longevity were similar to the effects on fertilization – sperm lived longer when the gamete environment matched the adult environment (Repeated Measures ANOVA: Time × Parental Env. × Gamete Env: $F_{3,\ 138} = 23{\cdot}95$, $P < 0{\cdot}0001$). Specifically, when sperm were exposed to the same environment as their fathers, they were still capable of fertilizing *c*. 25% of eggs 3 h after spawning, whereas sperm exposed to environments that did not match their father fertilized <3% of eggs after the same period of time.

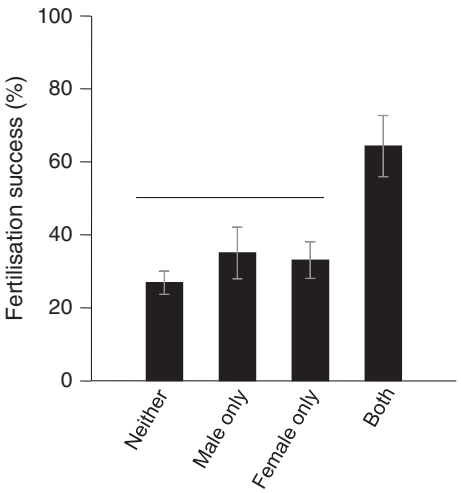

Fig. 5. Mean (±SE) fertilization success of *Hydroides diramphus* gametes under different salinity conditions. Bars show fertilization success only for those gametes whose parents experienced low salinity (similar results were found for gametes whose parents experienced normal salinity conditions). The *x* axis indicates whether salinity environment that gametes experienced matched the environment their parents. Moving left to right, the first bar indicates neither parents' environment matched the gametes' environment, the second bar indicates that only the father's environment matched the gametes' environment, the third bar indicates only the mother's environment matched the gametes' environment and the fourth bar indicates that both parents' environment matched the gametes' environment.

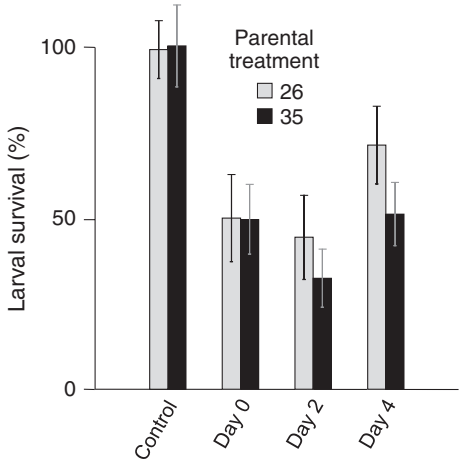

Fig. 6. Mean (±SE) survival of larvae under different salinity conditions. Grey bars indicate the survival of larvae whose parents experienced low (26 ppt) salinity conditions and black bars indicate the survival of larvae whose parents experienced normal (35 ppt) salinity conditions. The *x* axis indicates when larvae were switched from the environment that matched their parents to the salinity that did not match their parents. Moving left to right, the first bar indicates a control where larvae were kept in conditions that matched their parents throughout larval life, the second bar indicates survival of larvae after being switched immediately after fertilization success was assessed, the third and fourth bars indicate larval survival after being switched 2 and 4 days after fertilization, respectively.

EXPERIMENT 7: THE PERSISTENCE OF GAMETE PLASTICITY EFFECTS OVER THE LARVAL PERIOD

Parental experience had a strong effect on larval survival in different salinities. Larvae that were reared in the same environment as their parents had the highest survival after 6 days in culture (Fig. 6). When larvae were transferred from the salinity their parents experienced to a new salinity, their survival was decreased (Parental Env. × Larval Env. $F_{3,18} = 8{\cdot}67$, $P = 0{\cdot}001$), and this effect was largely consistent regardless of the salinity that parents experienced (Parental Env. × Timing of Transfer: $F_{3,6} = 1{\cdot}97$, $P = 0{\cdot}219$). The timing of when larvae were transferred from their natal environment to a new salinity strongly affected survival with larvae that were transferred the day that they were fertilized having the lowest survival and larvae transferred later having higher survival (Fig. 6).

Discussion

We found strong evidence for gamete plasticity in response to parental exposure to stress. The gametes, embryos and larvae from parents that had been exposed to a lower salinity were better able to cope with that stress themselves. Importantly, there were equivalent consequences of a mismatch between the parental and offspring environment: offspring from hyposalinity-exposed parents performed poorly in normal salinity conditions and *vice versa*. Gamete plasticity was not sex specific: both mothers and fathers altered the properties of their gametes in response to osmotic stress and both gamete types as well as the post-fertilization development of zygotes were sensitive to exposure to a salinity environment that did not match the parents. This plasticity was reversible during the adult stage; adults were capable of coping with variable salinities, switched between producing hyposalinity-tolerant gametes and hyposalinity-intolerant gametes if exposed to each environment for sufficient time. In contrast, the offspring themselves appeared to be much less flexible in their ability to cope with environmental change. Offspring had much poorer survival when they were transferred to a salinity that did not match the most recent experience of their parents. Our results show that parents of both sexes can exercise a remarkable degree of control over the phenotypes of their gametes of offspring, but our study also reveals a number of important limits and consequences of gamete plasticity.

Many studies have shown the strength and prevalence of maternal effects (Uller 2008) and fewer have demonstrated the paternal effects we observed in this study. We found good evidence for paternal effects on the phenotype of sperm being as important for successful fertilization as maternal effects were for the phenotype of eggs. It is easy to imagine how changes in the ion and transport and permeability of membranes of eggs could affect the osmoregulation of eggs (Charmantier 1998), but less is known about osmoregulation in sperm generally (Chen *et al.* 2011) and marine invertebrate sperm specifically. We saw no change

in the morphology of eggs when mothers were exposed to lower salinity, but we found that sperm from low-salinity fathers were much smaller than sperm from fathers that experienced normal salinity. Whether this change was adaptive (i.e. associated with the increased resistance to lower salinity) or was simply a coincidental side effect of paternal stress is unclear. We are not aware of any studies that have documented similar decreases in sperm size in response to salinity changes or any environmental stress, and further study in other systems is clearly warranted. Regardless of the mechanisms that drove our results that gamete plasticity occurs in response to stress at all has important implications for the way we study and predict the impact of stress.

Because fertilization is often the most sensitive stage in marine life cycles, it is often a focus of ecotoxicology (Marshall 2006; Byrne 2012). Many of these studies predict that stresses such as pollution or climate change will dramatically lower fertilization success, reducing population growth rates (Hollows, Johnston & Marshall 2007; Havenhand *et al*. 2008; Parker, Ross & O'Connor 2009; Reuter *et al*. 2011; but see Byrne *et al*. 2009; Havenhand & Schlegel 2009). Such studies typically focus on fertilization alone and preclude the exposure of the parental generation to the stress itself. Our results here, and that of others (Tait, Atapattu & Browne 1984; Parker *et al*. 2012), suggest that parental exposure to the stress of interest can strongly affect the impact of that stress on fertilization, and as such, risk of overestimating the direct impacts of the stress on fertilization success. However, theory predicts that such plasticity should only be expected under some specific conditions (Marshall & Uller 2007; Burgess & Marshall 2011): first, the focal species has been exposed to the stressor of interest for a sufficient period of its evolutionary history; secondly, that the stressor varies over time such that plasticity is favoured; and thirdly, that the stressor's incidence is predictable from the experience of the parents. Furthermore, gamete plasticity is unlikely to completely neutralize the effects of climate change or pollutants. Studies on other forms of transgenerational plasticity have shown that manipulating the phenotype of offspring is not without costs: increasing the resistance of offspring to one stress can result in decreases in their resistance to other, equally important stresses (Marshall 2008; Moran, Muniz Dias & Marshall 2010). As such, even if gamete plasticity in response to stress is widespread, pollutants and climate change are still likely to have negative effects, although they may be more complex than simple reductions in fertilization success.

Gamete plasticity may be an important means of coping with environmental fluctuations, but the strategy of altering gametes to perform best in local conditions is not without risks. We found that stress-resistant offspring performed more poorly in the absence of the stress. Such context-dependent costs and benefits of induced phenotypes are increasingly common in studies of phenotypic plasticity both within and between generations (Kraaijeveld & Godfray 1997; Relyea 2003; Marshall 2008). Thus, if parents do not predict the environment that their offspring will encounter accurately, then they risk producing offspring that have an inappropriate phenotype for that environment (sometimes termed as 'phenotype–environment mismatch' or PEM; (DeWitt, Sih & Wilson 1998)) such that manipulating the phenotype of the offspring generates significant costs. Thus, parents should only manipulate the phenotype of their offspring if the risks of producing offspring that will suffer a PEM are small (Crean & Marshall 2009; Burgess & Marshall 2011). In our system, brief rain showers produce ephemeral hyposaline conditions while heavy, sustained rain results in reductions in salinity that last longer and extend over a wider area. We found that parents did not express gamete plasticity when exposed to hyposalinity for short periods. This phenotypic lag in the production of gametes may be due to physiological constraints: parents may simply be unable to alter the properties of their gametes instantaneously. Alternatively, parents may refrain from altering the phenotype of their gametes immediately as a strategy to reduce the risk of their offspring suffering a PEM. Persistent low salinities tend to occur during the wet season at our study site, and parents may use the cue hyposaline conditions occurring for >1 week as an indicator that their offspring are also likely to experience hyposaline conditions.

CONSEQUENCES OF TRANSGENERATIONAL PLASTICITY FOR POPULATION CONNECTIVITY

The manipulation of the offspring phenotype was remarkably persistent: larvae suffered a significant reduction in performance if they were moved out of their parent's environment for up to 6 days after fertilization. In other words, the risk of PEM persists for the entire larval phase of this species. Later adult life-history stages can tolerate switches between lower salinity and normal environments, but it is the larval stage that can strongly affect the final distribution and abundance of adults in sessile organisms (Hunt & Sheibling 1997), and it is this stage that shows limited flexibility in tolerating low salinity. As such, dispersing larvae are likely to suffer major reductions in fitness if they move out of the environment that matches their parents. Such an effect will have outcomes that are analogous to natal imprinting effects. Natal imprinting refers to the process, whereby dispersers show a preference for habitat conditions that match those of their natal environment (Stamps 2006; Remy *et al*. 2011). In our study, offspring do not prefer the natal environment necessarily, but rather, they are more likely to survive if they encounter their natal environment, resulting in differential effective dispersal (sensu Pineda, Hare & Sponaugle 2007) to natal environments. Previously, it has been shown that local adaptation can generate PEMs that reduce connectivity among populations (Marshall *et al*. 2010), and here, it seems that parental effects can also affect population connectivity via PEMs, but potentially much more rapidly than those involving local adaptation.

Overall, we suspect that the effects we observed here are widespread among marine invertebrates with external fertilization – there are strong indications that such effects occur in both other species of annelid and in echinoderms and molluscs (Tait, Atapattu & Browne 1984; Hintz & Lawrence 1994; Roller & Stickle 1994; Parker *et al*. 2012), we eagerly await further studies exploring the prevalence of gamete plasticity in this group.

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Supporting Information

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

Fig. S1. Schematic of experimental design of Experiment 1. Replicates at each level shown in parentheses.
Fig. S2. Schematic of experimental design of Experiment 2. Replicates at each level shown in parentheses.
Fig. S3. Schematic of experimental design of Experiment 7. Replicates at each level shown in Parentheses.