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Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean

Lisa N. S. Shama*[,1], Anneli Strobel[2], Felix C. Mark[2] and K. Mathias Wegner[1]

[1]*Coastal Ecology Section, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wadden Sea Station Sylt, Wadden Hafenstrasse 43, 25992 List, Germany; and* [2]*Integrative Ecophysiology Section, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany*

Summary

1. Our study addresses the role of non-genetic and genetic inheritance in shaping the adaptive potential of populations under a warming ocean scenario. We used a combined experimental approach [transgenerational plasticity (TGP) and quantitative genetics] to partition the relative contribution of maternal vs. paternal (additive genetic) effects to offspring body size (a key component of fitness), and investigated a potential physiological mechanism (mitochondrial respiration capacities) underlying whole-organism growth/size responses.

2. In very early stages of growth (up to 30 days), offspring body size of marine sticklebacks benefited from maternal TGP: offspring of mothers acclimated to 17 °C were larger when reared at 17 °C, and offspring of mothers acclimated to 21 °C were larger when reared at 21 °C. The benefits of maternal TGP on body size were stronger and persisted longer (up to 60 days) for offspring reared in the warmer (21 °C) environment, suggesting that maternal effects will be highly relevant for climate change scenarios in this system.

3. Mitochondrial respiration capacities measured on mature offspring (F1 adults) matched the pattern of TGP for juvenile body size, providing an intuitive mechanistic basis for the maternal acclimation persisting into adulthood. Size differences between temperatures seen at early growth stages remained in the F1 adults, linking offspring body size to maternal inheritance of mitochondria.

4. Lower maternal variance components in the warmer environment were mostly driven by mothers acclimated to ambient (colder) conditions, further supporting our tenet that maternal effects were stronger at elevated temperature. Importantly, all parent–offspring temperature combination groups showed genotype × environment (G × E) interactions, suggesting that reaction norms have the potential to evolve.

5. To summarize, TGP and G × E interactions work in concert to mediate impacts of ocean warming on metabolic capacity and early growth of marine sticklebacks. TGP can buffer short-term detrimental effects of climate warming and may buy time for genetic adaptation to catch up, therefore markedly contributing to the evolutionary potential and persistence of populations under climate change.

Key-words: climate change, evolutionary potential, *Gasterosteus aculeatus*, genotype by environment interaction, heritability, maternal variance components, mitochondrial respiration capacity

Introduction

The need to include evolution into models predicting population responses to climate change in the world's oceans has recently come into focus. Empirical evidence is accumulating that marine species might be able to adapt to rapid environmental change if they have sufficient standing genetic variation (the raw material for evolutionary change) and/or phenotypic plasticity to mount fast responses (Munday *et al.* 2013; Sunday *et al.* 2014). The two are not mutually exclusive, as evolutionary potential will be influenced by both genetic adaptation and non-genetic inheritance mechanisms such as transgenerational plasticity (TGP). Combined approaches are, therefore,

*Correspondence author. E-mail: lisa.shama@awi.de

necessary to understand the link between these processes and to quantify the total adaptive potential of marine populations (Munday *et al.* 2013; Sunday *et al.* 2014).

Transgenerational plasticity has been shown to be a highly effective mechanism that can buffer populations against rapid environmental change such as ocean warming (Donelson *et al.* 2012; Salinas & Munch 2012) and acidification (Miller *et al.* 2012; Parker *et al.* 2012). TGP occurs when the environment experienced by parents influences offspring reaction norms (different phenotypes expressed by the same genotype in different environments; Mousseau & Fox 1998). When maternal environment is the predominant force influencing offspring responses, TGP can be considered an environment-dependent maternal effect (Charmantier & Garant 2005; Räsänen & Kruuk 2007). However, as paternal environment also has been shown to affect various offspring traits (e.g. Etterson & Galloway 2002; Crean, Dwyer & Marshall 2013), the more general term TGP is now commonly used (Salinas & Munch 2012).

Transgenerational plasticity has been detected in a wide range of organisms subjected to various selection pressures, and influences a diverse array of traits (Jablonka & Raz 2009; Salinas *et al.* 2013). In the marine realm, TGP in response to experimentally simulated ocean acidification and warming sea surface temperature has recently been shown in numerous invertebrates (Burgess & Marshall 2011; Parker *et al.* 2012; Vehmaa, Brutemark & Engström-Öst 2012; Kelly, Padilla-Gamino & Hofmann 2013) as well as several species of fish (Donelson *et al.* 2012; Miller *et al.* 2012; Salinas & Munch 2012). In most cases, elevated PCO_2 and/or water temperature had negative impacts on offspring traits such as growth (Burgess & Marshall 2011), development rate (Parker *et al.* 2012), egg production (Vehmaa, Brutemark & Engström-Öst 2012), aerobic scope (Donelson *et al.* 2012) and survival (Miller *et al.* 2012). However, exposing adults to stressful environments during reproductive conditioning had positive transgenerational effects on offspring traits, often showing full compensation of trait performance via TGP (Burgess & Marshall 2011; Donelson *et al.* 2012; Miller *et al.* 2012; Parker *et al.* 2012; Salinas & Munch 2012; Vehmaa, Brutemark & Engström-Öst 2012).

The consensus from these studies is that TGP is an important mechanism in marine systems that can buffer populations against environmental stressors associated with rapid climate change. Yet, underlying mechanisms on both physiological and cellular levels have received less attention (but see Donelson *et al.* 2012; Kelly, Padilla-Gamino & Hofmann 2013; Miller *et al.* 2012). In the context of ocean warming, the capacity to meet increased oxygen demands and maintain aerobic scope at increased temperatures will determine population persistence across locations, as a decline in aerobic scope would affect critical biological functions including growth, reproduction and behaviour (Pörtner & Farrell 2008). In fish, the heart has a crucial role in this context, as it is the most thermally sensitive organ, but at the same time has to sustain whole-organism aerobic scope by supplying oxygen to tissues (Pörtner, Mark & Bock 2004). Acclimation of oxygen transport to warmer temperatures has been shown in temperate marine fishes (Guderley & Johnston 1996), and further evidence exists for adaptation of cardiac capacity between populations with different thermal regimes (Gamperl & Farrell 2004). This suggests that adjustments to oxygen delivery may be critical in coping with climate change. Yet, at present, we have a limited understanding of how marine species might be able to alter their physiology over multiple generations to facilitate population persistence in a warming ocean (Donelson *et al.* 2012). Mitochondria, for example, are likely to play an important role, as they define a great part of cardiac capacity and occupy up to 40% of the volume and channel 90% of the energy in cardiomyocytes (Hochachka 1994). Due to predominant maternal inheritance of mitochondria (Brown 2008), mitochondrial respiration capacities will likely carry a strong maternal signal, and provide an ideal tool to investigate sex-specific transgenerational effects.

The recent surge of findings demonstrating non-genetic responses of organisms to environmental change (Bonduriansky, Crean & Day 2012) highlights the need to better understand the role of TGP and its underlying mechanisms as well as their relative contribution in relation to genotypic effects in shaping the ability of populations to cope with rapid climate change. Non-genetic inheritance mechanisms such as TGP decouple phenotypic change from genotypic change, circumventing some of the limitations of genetic inheritance, and by doing so can influence the rate and direction of adaptation (Bonduriansky & Day 2009). For instance, TGP could buffer populations against immediate impacts of climate change and provide time for genetic adaptation to catch up (Chevin, Lande & Mace 2010). But, parental contributions to offspring phenotypic variance and the adaptive benefits of parental effects can differ across environments (Hoffmann & Merilä 1999). Parent environment by offspring environment ($E_p \times E_o$) interactions and genotype by environment ($G \times E$) interactions are prominent features of parental effects, and can strongly influence the evolutionary trajectories of populations (Räsänen & Kruuk 2007). Thus, selection acting via parental effects is a complex interplay between non-genetic and genetic inheritance as well as environmental dependency (Marshall & Uller 2007), making predictions regarding the direction of evolutionary change challenging.

In the case of TGP, environmentally induced parental effects can increase offspring fitness in heterogeneous environments by promoting adaptive plasticity (Mousseau & Fox 1998). However, many findings of TGP cannot disentangle the relative contribution of maternal vs. paternal effects to TGP. Where these effects could be partitioned, it was most often found that maternal effects had the largest influence on offspring traits (e.g. Burgess & Marshall 2011; Vehmaa, Brutemark & Engström-Öst 2012; Kelly,

Padilla-Gamino & Hofmann 2013 but see Crean, Dwyer & Marshall 2013). However, changes in genetic and maternal variance components (which contain both genetic and environmental effects) across environments have rarely been tested in the context of TGP in general (Räsänen & Kruuk 2007), and even less so in marine systems (but see Sunday *et al*. 2011; Kelly, Padilla-Gamino & Hofmann 2013). Very few studies to date have explicitly taken a combined experimental approach considering both TGP and genetic adaptation in adaptive responses to environmental change (Munday *et al*. 2013; Sunday *et al*. 2014). Hence, the evolutionary potential of marine populations under climate change remains poorly understood.

Here, we tested for TGP in response to a warming ocean by acclimating adult marine sticklebacks to either ambient (17 °C) or elevated summer water temperatures (21 °C) that were previously shown to have detrimental effects on stickleback growth rates (Ramler *et al*. 2014), and are in accordance with a 2100 scenario (Sheppard 2004). We then produced crosses within and reciprocal crosses between acclimation environments to enable us to partition maternal and paternal contributions to TGP of offspring traits. In addition to measuring parental environment effects on offspring size (a key component of fitness), we also estimated standing genetic variation and maternal variance components across environments, as well as G × E interactions to test whether offspring reaction norms have the potential to evolve. Finally, we investigated if parental environment effects on mature offspring (F1 adult) mitochondrial respiration could be a potential cellular-level mechanism underlying whole-organism growth/size responses to rapidly warming ocean environments. With such an experimental approach, we can try to understand the mechanisms as well as the effects of non-genetic vs. genetic information transfer for offspring fitness during several stages of ontogeny, which will decisively shape the ability of populations to cope with rapid climate change.

Materials and methods

GROWTH EXPERIMENT

Adult sticklebacks from a coastal marine population were caught by trawling in the Sylt-Rømø Bight, Germany (55°05′ N, 8°41′ E) in February 2012. Fish were held in groups of *c*. 20 in 25-L aquaria set at 15 °C for 2 weeks. After this initial period, fish were divided equally amongst four replicate 25-L aquaria at each of the two experimental acclimation temperatures (17 and 21 °C; eight aquaria in total) and held at these temperatures for 60 days. Throughout the experiment, adult fish were fed daily with chironomid larvae *ad libitum*.

Crosses were performed over a 2-week period in May 2012 to produce F1 families in four groups: 17 °C male × 17 °C female, 17 m × 21 f, 21 m × 17 f and 21 m × 21 f. Producing the reciprocal crosses allowed us to partition the influence of maternal and paternal thermal environment. Within the four crossing groups, 11, 10, 11 and nine families, respectively, were produced (n = 41 families in total). The 41 families were produced from 22 crosses, where each female was mated to two different males: a 17 °C male and a 21 °C male (note: three crosses were made with only one male due to a lack of males). Egg clutches from each family were split and reared at 17 and 21 °C (n = 82 split-clutches/families in total). Briefly, crosses were performed by strip-spawning, and eggs were divided between two Petri dishes containing moist paper towel. Female size was measured as standard length (±1 mm). We sacrificed two males in an excess of MS-222 (Tricaine methanesulfonate; an anaesthesia agent) and removed the testes. Testes from each male were crushed separately in isotonic non-activating medium (Fauvel *et al*. 1999) and the solutions were applied to eggs. Fertilized eggs were left for 30 min before assigning them to temperature treatments (further dividing egg clutches into halves).

Each split-clutch was photographed under a dissecting microscope for digital analyses of clutch size and mean egg size (using LEICA QWIN IMAGING software Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). Clutch size was estimated as the total number of eggs per female, for example the sum of all split-clutches per female. Mean egg size was estimated as the mean diameter (±0·01 mm) of 10 eggs from each clutch. The 10 measured eggs were chosen based on the clarity of their outer perimeter in the photograph, that is eggs with edges that were not distorted by contact with neighbouring eggs. Each split-clutch was placed individually in a 1-L glass beaker containing filtered seawater and an air supply. Beakers were placed into water baths heated with aquarium heaters at either 17 °C or 21 °C. Hatching success was estimated as the proportion of hatched eggs in each split-clutch (the number of hatchlings divided by the number of eggs). Hatchlings were held in beakers for the first 30 days. Water was changed in the beakers every week. At 30 days post-hatch, 10 randomly chosen offspring from each split-clutch were photographed under a dissecting microscope for digital analysis of size (standard length ± 0·01 mm; using LEICA QWIN). At this point, the 10 offspring were transferred to a 2-L aquarium connected to a flow-through seawater system set at either 17 °C or 21 °C for another 30 days. At 60 days post-hatch, standard length was again measured on the 10 offspring per family by digital photography. Throughout the experiment, juvenile fish were fed daily with live *Artemia* nauplii *ad libitum*.

MITOCHONDRIAL RESPIROMETRY

At the end of the growth experiment, families were pooled within each sire-dam-offspring temperature combination group (eight groups in total). Each of the eight groups was then divided amongst two to four replicate 25-L aquaria to reach a final density of 25–30 fish per aquaria (i.e. the total number of fish per group ranged between *c*. 50 and 100). Groups were maintained at their offspring rearing temperature, for example four groups at 17 °C, four groups at 21 °C. Fish were fed daily with chironomid larvae *ad libitum* until they reached adulthood to ensure that sufficient amounts of heart tissue for the respiration assays were available. Assays were performed over two rounds: 25 June 2013 and 20–22 August 2013. In both rounds, 4–6 F1 adult fish were randomly selected from each group, fish were sacrificed in an excess of MS-222, standard length was measured (±1 mm) and hearts were dissected out under a binocular microscope. Hearts were pooled for each of three replicates per group. Heart tissue from each replicate was assayed at 17 and 21 °C, summing up to 48 assays in total (16 assays in round 1, and 32 assays in round 2).

For each group, heart tissue was mechanically disrupted in ice-cold biopsy buffer using fine forceps, and stored on ice in biopsy buffer until used for the different respiration assays. Directly before each assay, heart fibre bundles were split into halves and each half was permeabilized in a solution of 10 μg mL^{-1} saponin by gentle mixing on ice for 30 min. Afterwards, the fibres were removed and washed three times on ice for 10 min each in 2 mL

of modified assay medium (MiRO5, for buffer compositions, see Strobel *et al.* 2013). The subsample was then blotted, weighed and immediately used for respirometry analyses. One half was used to measure respiration at 17 °C, the other half at 21 °C. Respiration was measured in 2-mL assay medium plus 300 U mL^{-1} catalase (MiRO6, for reoxygenation of the respiration medium with hydrogen peroxide during the assays) in glass chambers of two Oroboros Oxygraph-2k™ respirometers (Oroboros Instruments, Innsbruck, Austria). Heart fibre respiration was converted to pmol O_2 s^{-1} $mg_{fresh\ weight}{}^{-1}$.

Resting respiration (state II) was measured with Complex I (CI) and Complex II (CII) substrates, 10 mM glutamate, 2 mM malate, 10 mM pyruvate and 10 mM succinate, respectively. State III respiration was induced by the addition of 1 mM ADP, followed by further titration with 0·5 mM ADP until maximum respiration was reached, and maximum capacity of the phosphorylation system (max. OXPHOS) was determined. Leak respiration (state IV^+, LEAK), that is the basal mitochondrial substrate turnover while ADP phosphorylation by F_1F_o ATPase is inhibited, was evaluated by adding 4 μg mL^{-1} oligomycin. Stepwise titration with the uncoupler carbonylcyanide-*p*-(trifluoromethyl) phenylhydrazone (FCCP) (2 mM stock) revealed maximum capacity of the electron transport system [uncoupled flux (*u*), maximum ETS capacity *'E'*]. After inhibition of CI with 5 μM rotenone (E_{Rot}, state III*u* of CII), non-mitochondrial respiration (ROX) was detected by adding 2·5 μM antimycin A (for further details, see Strobel *et al.* 2013).

DATA ANALYSES

Offspring body size

We fitted general linear models using ANOVA for significance testing in our analyses, and concentrated on body size as a decisive component of fitness for sticklebacks. To show that the relationship between size and fitness also holds for the parental fish we used here, we analysed the effect of female size on egg traits related to reproductive output (clutch size, mean egg size and hatching success). Hatching success was analysed as a generalized linear model with a binomial error distribution. In all analyses of body size of juvenile fish, we used family means of untransformed standard length at a defined age (30 and 60 days) to eliminate confounding effects of common environment experienced by each family. We accounted for differences in initial hatchling densities in the beakers (0–30 days) by including initial density in the 30-day model. The same was applied to body size at 60 days to account for any families that had <10 individuals, and for any mortality during the experiment. While density had a significant effect on body size in full models both at 30 ($F_{1,59}$ = 118·28; $P < 0·001$) and 60 days ($F_{1,59}$ = 141·85; $P < 0·001$), there were no differential effects of temperature on the relationship between density and body size (density × temperature 30 days: $F_{1,59}$ = 0·77; P = 0·385 and 60 days: $F_{1,59}$ = 0·54; P = 0·467), and no systematic bias towards higher or lower density in either temperature (30 days: $F_{1,59}$ = 0·06; P = 0·805, and 60 days: $F_{1,59}$ = 0·004; P = 0·951, Fig. S1, Supporting information). Thus, any potentially confounding density effects in the results were the same for both temperature treatments. Mean egg size was included as a covariate in all body size models, as well as the egg size × temperature interaction. We analysed body size at 30 and 60 days first using full models, and then used stepwise model selection (using *F* tests between fits of nested models) until minimal adequate models remained. This procedure was repeated separately for each rearing temperature to assess any rearing temperature-specific effects. For graphical representation, we chose to display residuals of standard length corrected for density (where residuals were calculated from models of standard length as a function of density to eliminate the overall effect of density).

Mitochondrial respirometry

Minimal adequate models for respirometry analyses (OXPHOS: state III maximum respiration, ETS: maximum capacity of the electron transport system and LEAK: mitochondrial idling) were fitted using the same model terms as for standard length (except for density). To account for any systematic differences between measurement rounds (age of fish, chemical batches etc.), we included experimental round as an additional factor. Since measurement values from the second round were substantially higher than those from the first round, we repeated the respirometry analyses with data from round 2 only (see Table S1, Supporting information). To maintain scale in the respiration plots, we chose to display least square means eliminating the effect of experimental round. All analyses were run in the R statistical environment (R Development Core Team 2011).

Variance components

We chose a character state approach (treating the same trait measured in different environments as separate traits; Falconer 1952; Via & Lande 1985) to assess genetic and maternal variance components and their genetic correlations (between experimental temperatures). Only those families that hatched in both temperatures (n = 30) were included in these analyses. We fitted animal models as multivariate generalized linear mixed models with body size in both temperatures as Gaussian response variables using the MCMCglMM package (Hadfield 2010) of the R statistical environment (R Development Core Team 2011). We fitted dam and sire temperature, egg size and density as fixed effects, and animal and dam as random effects, where the V_{animal} term reflects the additive effects explained by the pedigree-based relatedness of individuals (additive genetic variance) and the V_{Dam} term reflects maternal effects, that is the additional phenotypic effects of the mother on offspring phenotypes, beyond the additive effects of her genotype (Kruuk, Slate & Wilson 2008). Model fits were assessed by their respective Deviance Information Criterion (DIC) scores (Spiegelhalter *et al.* 2002), including random effects. We used weak but informative priors of half the observed phenotypic variance and set the covariance between temperature-specific body sizes to zero to account for measurements stemming from separate individuals. Markov chains were run for 500 000 iterations and we kept every 100th value after removing 300 000 iterations of burn-in to generate posterior distributions of random and fixed parameters. Genetic correlations (r_G) were calculated as the covariance between traits (size in the two temperatures) divided by the square root of the product of both traits, and significance was assessed by estimating the proportion of estimates from the posterior distributions that overlapped with zero (Wilson *et al.* 2010). An r_G differing significantly from 1 represents a G × E interaction (Windig 1997). Genetic variances and correlations were assessed first by combining all crossing groups within each rearing temperature, and then by each crossing group separately.

Results

EGG TRAITS AND HATCHING SUCCESS

Clutch size did not differ between female acclimation temperatures ($F_{1,15}$ = 0·019; P = 0·891). Females acclimated to 17 °C produced clutches ranging between 33 and 184 eggs, with a mean clutch size of 119·25. Females acclimated to 21 °C produced clutches ranging between 31 and 180 eggs, with a mean clutch size of 124·7. Clutch size was also not significantly influenced by egg size ($F_{1,15}$ = 2·783; P =

0·116), female size ($F_{1,15}$ = 2·383; P = 0·144) or the interaction between female size and acclimation temperature ($F_{1,15}$ = 0·518; P = 0·483). There was no significant difference in female size between acclimation temperatures ($F_{1,11}$ = 2·957; P = 0·114). Mean egg size also did not differ between female acclimation temperatures ($F_{1,15}$ = 1·331; P = 0·267). Overall mean egg size of females acclimated to 17 °C was 1·58 mm (range: 1·50–1·68 mm), and 1·54 mm (range: 1·47–1·60 mm) for females acclimated to 21 °C. Clutch size ($F_{1,15}$ = 3·314; P = 0·089), female size ($F_{1,15}$ = 1·271; P = 0·277) and the interaction between female size and acclimation temperature ($F_{1,15}$ = 3·737; P = 0·072) did not have significant effects on mean egg size.

Hatching success varied by female size, egg size, offspring temperature, the interaction between offspring and dam temperature and the three-way interaction between offspring, sire and dam temperature (Table 1). Hatching success was lower at 21 °C for all four crossing groups, and comparatively lower at 21 °C for crosses originating from 21 °C females (Fig. 1). The lowest mean hatching success occurred at 21 °C in the 17 °C sire × 21 °C dam parental temperature group (Fig. 1), which is reflected by the significant three-way interaction term. However, the significance of the three-way interaction term is wholly dependent on one family in the 17 × 21 group that had the largest difference overall in hatching success between temperatures (0·87 at 17 °C and 0·10 at 21 °C). Smaller eggs had lower hatching success, and hatching success was lower for larger females (but female size did not differ between acclimation temperatures: see above). Of the 41 families produced (and then split by temperature), all 41 hatched at 17 °C, and 30 hatched at 21 °C (hence, n = 71 families in the body size analyses). The number of clutches that failed at 21 °C was distributed equally amongst the four parental temperature crossing groups (n = 3; 2; 3; 3), and can thus be considered an effect of offspring (hatching) environment.

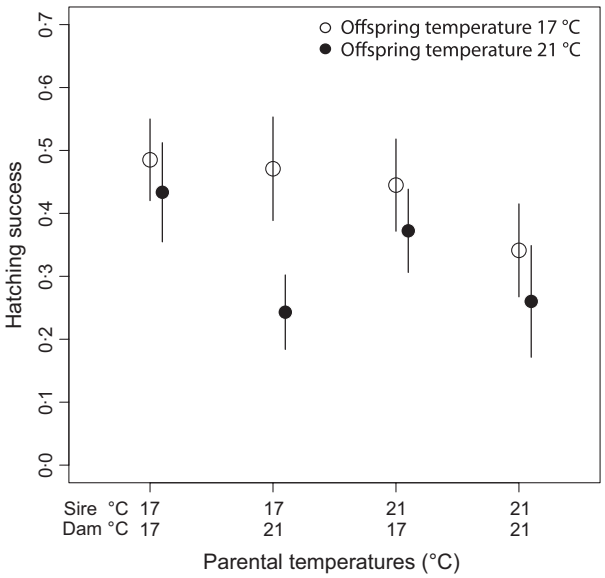

Fig. 1. Hatching success of clutches produced from crosses within and between parental acclimation temperatures and reared at 17 °C (open circles) and 21 °C (closed circles). Parental temperature combinations are shown as male (sire) temperature (upper × axis) and female (dam) temperature (lower × axis). Points depict mean hatching success (±SE) for all families within a temperature combination group.

OFFSPRING BODY SIZE

Offspring body size at 30 days was strongly influenced by the interaction between dam temperature and offspring temperature, that is transgenerational (maternal) plasticity (Table 2a; Fig. 2a). Offspring of 21 °C females were significantly larger at 21 °C than 17 °C, and offspring of 17 °C females were larger at 17 °C than 21 °C (Table 2b; Fig. 2a). Paternal (sire) temperature did not significantly influence offspring size (model term never significant). Overall, there was no significant difference in mean body size between 17 and 21 °C at 30 days (Fig. 2a). At 60 days, however, size was primarily determined by offspring rearing temperature (Table 2a), with all four crossing groups reaching a much larger size at 17 °C than 21 °C (Fig. 2b). Transgenerational (maternal) plasticity persisted at 21 °C, with offspring of 21 °C females maintaining (relatively) larger size at 21 °C than offspring of 17 °C females (Table 2b; Fig. 2b). Transgenerational effects were not significant at

Table 1. Generalized linear model showing the influence of offspring temperature (offspring °C) and parental (sire °C and dam °C) acclimation temperatures on *Gasterosteus aculeatus* hatching success

Source	d.f.	Deviance	Res. d.f.	Res. deviance	Pr (>Chi)
NULL			73	620·11	
Female size	1	11·549	72	608·56	***0·001***
Egg size	1	64·708	71	543·85	***< 0·001***
Offspring °C	1	21·078	70	522·77	***< 0·001***
Sire °C	1	2·366	69	520·40	0·124
Dam °C	1	0·086	68	520·32	0·769
Offspring × sire °C	1	3·632	67	516·69	0·057
Offspring × dam °C	1	6·357	66	510·33	***0·012***
Sire × dam °C	1	0·041	65	510·29	0·839
Offspring × sire × dam °C	1	5·448	64	504·84	***0·020***

Res. d.f. and Res. deviance indicate residual degrees of freedom and residual variance, respectively. Significant terms are highlighted in bold italics.

Table 2. Minimum adequate models for *Gasterosteus aculeatus* size at 30 and 60 days post-hatch depicting the influence of offspring (offspring °C) and parental (dam °C) thermal environments analysed as (a) both offspring rearing temperatures combined and (b) separately for each temperature (17 and 21 °C)

	Size 30 days				Size 60 days			
Source	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
(a)								
Density	1	85·300	128·370	***< 0·001***	1	136·528	151·734	***< 0·001***
Offspring °C	1	0·013	0·019	0·891	1	45·149	50·177	***< 0·001***
Dam °C	1	0·065	0·097	0·756				
Dam °C × offspring °C	1	5·148	7·748	***0·007***				
Error	66	0·664			68	0·900		
(b)								
17 °C								
Density	1	49·315	59·593	***< 0·001***	1	90·969	80·054	***< 0·001***
Dam °C	1	2·655	3·209	*0·081*				
Error	38	0·828			39	1·221		
21 °C								
Density	1	36·218	81·406	***< 0·001***	1	47·533	86·743	***< 0·001***
Dam °C	1	2·850	6·405	***0·018***	1	2·979	5·708	***0·024***
Egg size	1	0·303	0·681	0·417	1	1·253	2·286	0·142
Error	26	0·445			26	0·548		

Analyses were performed on family means. Size was measured as the standard length (mm) at 30 and 60 days post-hatch. Egg size was measured as mean egg diameter (mm). Significant terms are highlighted in bold italics.

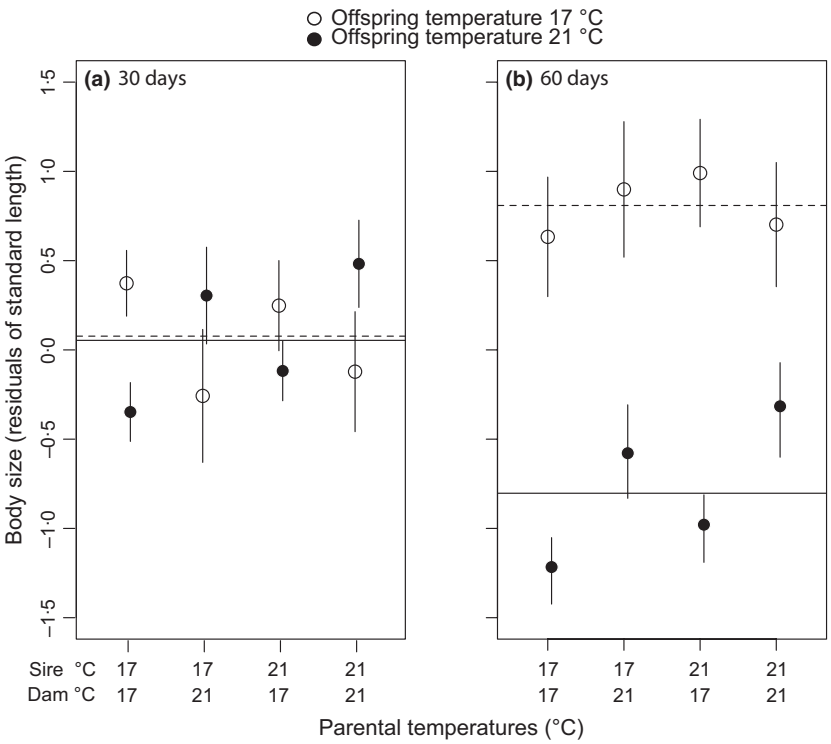

Fig. 2. Offspring size corrected for density (shown as residuals of standard length) at (a) 30 days and (b) 60 days post-hatch for each parental temperature combination, and reared at 17 °C (open circles) and 21 °C (closed circles). Parental temperature combinations are shown as male (sire) temperature (upper × axis) and female (dam) temperature (lower × axis). Points depict mean residuals (±SE) for all families within a temperature combination group. Overall means per rearing temperature are given as horizontal dashed (17 °C) and solid (21 °C) lines.

17 °C at 60 days (Table 2b). Egg size and the interaction between egg size and temperature were included in all models, but were never significant.

OFFSPRING MITOCHONDRIAL RESPIRATION

Body size of F1 adults differed between offspring temperatures and dam temperatures. F1 adults were larger at 17 °C than at 21 °C ($F_{1,108} = 48{\cdot}815$; $P < 0{\cdot}001$), and larger when mothers were acclimated to 21 °C ($F_{1,108} = 4{\cdot}780$; $P = 0{\cdot}031$). For all three respiration parameters (OXPHOS, ETS and LEAK), both round and assay temperature were highly significant (ETS: round $F_{1,31} = 33{\cdot}722$; $P < 0{\cdot}001$; assay temperature $F_{1,31} = 7{\cdot}381$; $P = 0{\cdot}011$; OXPHOS and LEAK shown in Table 3). Although there were significant differences between

Table 3. Minimum adequate models for *Gasterosteus aculeatus* respiration parameters (OXPHOS and LEAK) showing the influence of assay temperature (°C), and offspring (offspring °C) and parental (sire and dam °C) thermal environments

Source	d.f.	MS	*F*	*P*
(a) OXPHOS				
Round	1	9609·7	56·274	***< 0·001***
Assay temperature (°C)	1	914·6	5·356	***0·026***
Sire temperature (°C)	1	181·4	1·062	0·309
Dam temperature (°C)	1	1165·0	6·822	***0·013***
Offspring temperature (°C)	1	180·5	1·057	0·311
Sire °C × dam °C	1	434·9	2·547	0·119
Dam °C × offspring °C	1	916·7	5·368	***0·026***
Dam °C × assay °C	1	2·2	0·013	0·911
Offspring °C × assay °C	1	14·4	0·084	0·773
Dam °C × offspring °C × assay °C	1	298·5	1·748	0·194
Error	37	170·8		
(b) Leak				
Round	1	128·6	19·805	***< 0·001***
Assay temperature (°C)	1	89·4	13·764	***0·001***
Sire temperature (°C)	1	0·2	0·024	0·879
Dam temperature (°C)	1	80·9	12·459	***0·001***
Offspring temperature (°C)	1	0·7	0·100	0·753
Sire °C × dam °C	1	2·1	0·329	0·570
Sire °C × offspring °C	1	3·9	0·594	0·446
Dam °C × offspring °C	1	34·6	5·334	***0·027***
Sire °C × assay °C	1	6·0	0·929	0·342
Offspring °C × assay °C	1	0·4	0·066	0·799
Sire °C × dam °C × offspring °C	1	15·5	2·383	0·132
Sire °C × offspring °C × assay °C	1	16·7	2·576	0·118
Error	35	6·5		

Significant terms are highlighted in bold italics.

rounds, we found qualitatively similar results when only data from round 2 were analysed, indicating that significant model effects were consistent between rounds (Table S1). Therefore, we used all data in our analyses to maintain adequate sample sizes in our factorial design.

At 21 °C assay temperature (Fig. 3b,d), respiration was generally higher than when assayed at 17 °C (Fig. 3a,c). Although thermal sensitivity of mitochondrial capacities was somewhat lower than expected (Q_{10} for OXPHOS: 1·71 ± 0·27, mean ± SE), such an increase is predicted by Q_{10} relationships (an increase in biological reactions with temperature, for example by a factor of 2–3 per 10 °C). LEAK respiration stayed constant at around 23% of OXPHOS (0·231 ± 0·011, mean ± SE), and the LEAK/OXPHOS ratio was not affected by maternal, offspring or assay temperature (all $P > 0·05$; data not shown). Both OXPHOS and LEAK respiration were significantly influenced by maternal temperature (Table 3, Fig. 3), with offspring of mothers acclimated to 17 °C having higher maximum respiration (OXPHOS), which entailed correspondingly higher LEAK respiration – that is, more waste of mitochondrial substrates. OXPHOS and LEAK respiration were lower for offspring of mothers acclimated to 21 °C, and this effect was more pronounced when respiration was assayed at 21 °C (Fig. 3). For both OXPHOS and LEAK, we observed significant interactions between maternal and offspring temperatures, suggesting TGP for mitochondrial respiration (Table 3).

VARIANCE COMPONENTS AND HERITABILITIES

The multivariate animal models for offspring size showed better model fits when dam and egg size was included. At 30 days, model fits were: animal model DIC = −2810·6; animal + egg size DIC = −2801·5; animal + egg size + dam DIC = −2767·7. And at 60 days, model fits were: animal model DIC = −3168·5; animal + egg size DIC = −3160·1; animal + egg size + dam DIC = −3133·7. These results indicate that a considerably higher proportion of variance can be explained by maternal identity than by egg size, suggesting that maternal effects were not egg size mediated. Overall, maternal variance components tended to be lower at 21 °C than 17 °C

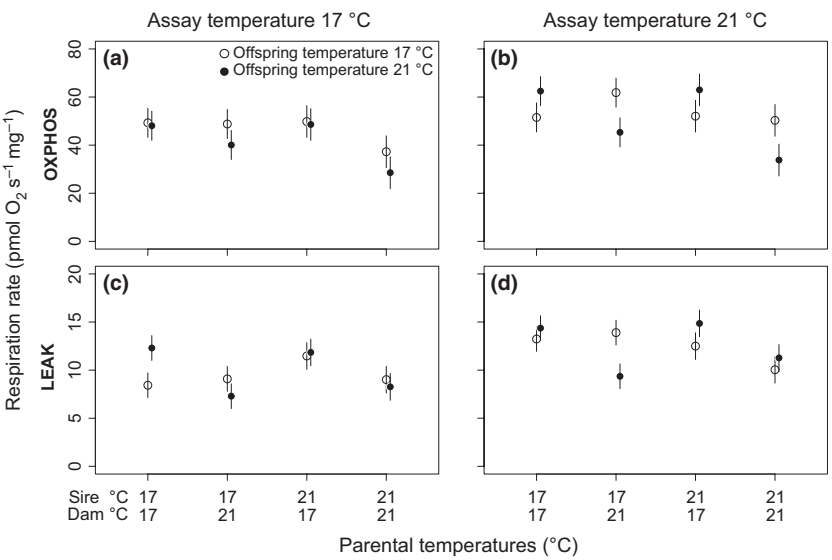

Fig. 3. Offspring respiration rates (pmol O_2 s^{-1} mg^{-1}) measured as OXPHOS assayed at (a) 17 °C and (b) 21 °C, and LEAK assayed at (c) 17 °C and (d) 21 °C for each parental temperature combination. Offspring reared at 17 °C are shown as open circles and offspring reared at 21 °C as closed circles. Parental temperature combinations are shown as male (sire) temperature (upper × axis) and female (dam) temperature (lower × axis). Points depict least square means (±SE) for all families within a temperature combination group.

Table 4. Genetic variance-covariance matrices of *Gasterosteus aculeatus* offspring size at 30 and 60 days analysed by multivariate animal models taking temperature-specific size (character states) as response variables

	17 °C	21 °C
Size 30 days		
17 °C	V_{animal} $6{\cdot}44e^{-4}$ ($3{\cdot}34e^{-4}$–$9{\cdot}73e^{-4}$) V_{DAM} $5{\cdot}11e^{-4}$ ($2{\cdot}96e^{-4}$–$1{\cdot}78e^{-3}$) V_E $4{\cdot}31e^{-4}$ ($1{\cdot}95e^{-4}$–$5{\cdot}52e^{-4}$) h^2 0·34 (0·17–0·55)	CoV $3{\cdot}74e^{-4}$ ($-1{\cdot}48e^{-5}$ to $6{\cdot}34e^{-4}$) r_G 0·52* ($-3{\cdot}58e^{-3}$ to $7{\cdot}40e^{-1}$)
21 °C		V_{animal} $8{\cdot}37e^{-4}$ ($5{\cdot}04e^{-4}$–$1{\cdot}24e^{-3}$) V_{DAM} $3{\cdot}96e^{-4}$ ($1{\cdot}79e^{-4}$–$1{\cdot}46e^{-3}$) V_E $2{\cdot}71e^{-4}$ ($1{\cdot}43e^{-4}$–$5{\cdot}07e^{-4}$) h^2 0·50 (0·27–0·67)
Size 60 days		
17 °C	V_{animal} $3{\cdot}57e^{-4}$ ($2{\cdot}29e^{-4}$–$5{\cdot}35e^{-4}$) V_{DAM} $1{\cdot}66e^{-4}$ ($9{\cdot}00e^{-5}$–$5{\cdot}74e^{-4}$) V_E $1{\cdot}26e^{-4}$ ($7{\cdot}03e^{-5}$–$2{\cdot}26e^{-4}$) h^2 0·47 (0·27–0·66)	CoV $4{\cdot}06e^{-5}$ ($-1{\cdot}13e^{-4}$ to $1{\cdot}90e^{-4}$) r_G 0·17 ^{ns} ($-0{\cdot}36$ to 0·57)
21 °C		V_{animal} $2{\cdot}14e^{-4}$ ($9{\cdot}09e^{-5}$–$4{\cdot}15e^{-4}$) V_{DAM} $1{\cdot}48e^{-4}$ ($5{\cdot}79e^{-5}$–$4{\cdot}23e^{-4}$) V_E $2{\cdot}00e^{-4}$ ($1{\cdot}01e^{-4}$–$2{\cdot}98e^{-4}$) h^2 0·31 (0·15–0·60)

Elements on the diagonal give estimated variance components for V_{animal}, V_{DAM} and V_E plus the resulting heritability with 95% CI. Off-diagonal elements give genetic covariances and genetic correlations (r_G) between character states. Significance of genetic correlations was tested as the proportion of posterior values overlapping zero.
*$P < 0{\cdot}05$; ns = not significant.

for both 30 and 60 days (although confidence intervals overlap), but heritabilities did not show a clear pattern with temperature for the two time points (Table 4). When each crossing group was assessed separately, maternal variance components showed significantly lower values at 21 °C than 17 °C (in seven of the eight cross × temperature combinations; paired *t* test, $t_7 = 3{\cdot}43$; $P = 0{\cdot}011$). This pattern was mostly driven by greater decreases in V_{DAM} at 21 °C for females acclimated to 17 °C, indicating that maternal effects of 17 °C females decreased more under stressful conditions than maternal effects of 21 °C females (Table S2, Supporting information). At day 60, heritabilities tended to be lower at 21 °C than 17 °C for all crossing groups, likely reflecting increases in V_E and decreases in V_{animal} at 21 °C for each group. Heritabilities for each crossing group at day 30 showed less clear patterns with temperature (Table S2).

Temperature-specific character states of body size showed a significant positive genetic correlation (r_G) at day 30 but not day 60. G × E interactions were detected for both time periods (Table 4; Fig. S2, Supporting information). When each crossing group was analysed separately, no significant genetic correlations were detected, possibly due to the lower number of families (per group) in the analysis. G × E interactions, on the other hand, were found in all eight groups (Table S2; Fig. S2).

Discussion

Our study demonstrates environment-specific maternal TGP of offspring body size. In early growth stages (up to 30 days), offspring reached a larger size when reared in their maternal environment, and the transgenerational benefits were stronger and persisted longer (seen at 60 days and into adulthood) under stressful (warmer) conditions. In later growth stages, offspring environment became the major determinant of growth trajectories, and these size differences persisted into adulthood. Since maternal environment effects were also imprinted on the metabolic capacities of offspring heart mitochondria, we can mechanistically link the underlying acclimation mechanism of body size to metabolic modification of maternally inherited mitochondria. Transgenerational body size benefits were not egg size mediated, nor do they reflect a higher contribution of maternal variance to offspring phenotypic variance under stress. Rather, our results suggest a complex interaction between maternal and offspring environments (TGP) as well as G × E interactions in shaping thermal reaction norms of offspring traits and the adaptive potential of marine stickleback populations in a warming ocean.

TRANSGENERATIONAL EFFECTS ON OFFSPRING TRAITS

Our results using a paired, split-clutch experimental design indicate that eggs of acclimated mothers harbour some type of maternally-derived, temperature-dependent mechanism that promotes larger size, that is better growth in the specific maternal environment. In essence, mothers are programming offspring to grow better in the predicted future environment, and this programming is particularly strong at elevated (stressful) temperatures. Interestingly, for the first 30 days post-hatch, overall (mean) offspring body size was similar at 17 and 21 °C, indicating that

environmental temperature played a minor role in comparison with TGP in very early life stages. By 60 days, however, this pattern was reversed: environmental temperature was the predominant force shaping body size trajectories, with offspring reaching a substantially larger size at 17 °C than 21 °C.

Unlike other recent studies of TGP in fish (Donelson *et al*. 2012; Miller *et al*. 2012; Salinas & Munch 2012), we did not find complete compensation of offspring traits via TGP. Although the effects of TGP were stronger and persisted longer at 21 °C, they could only partially compensate for the fitness (size) loss at elevated temperature. Previous studies on this population have shown that short-term as well as long-term exposure to 21 °C is suboptimal for growth (F. M. Schade, L. N. S. Shama and K.M. Wegner, unpublished data) and development (Ramler *et al*. 2014). Here, we can additionally show the consequences of elevated temperature on reproductive output, as hatching success was in general lower at 21 °C, and comparatively lower for crosses originating from 21 °C mothers. Yet, offspring body size showed a clear (relative) benefit of TGP, suggesting that transgenerational effects could be highly effective in mediating some of the impacts of ocean warming if this population experiences a gradual increase in temperature over several generations (Miller *et al*. 2012). Moreover, transgenerational effects persisted only under stressful conditions, suggesting that at ambient, non-stressful temperatures, other genetic and environmental effects may override transgenerational effects (Charmantier & Garant 2005).

Possible mechanisms of TGP that have been discussed in recent literature include: differential gene expression, for example heat shock proteins in the context of thermal TGP, differing mitochondrial densities, hormone and mRNA levels, maternal provisioning, for example yolk contents, modifications to oxygen delivery pathways, and selection for tolerant genotypes (Sultan, Barton & Wilczek 2009; Donelson *et al*. 2012; Miller *et al*. 2012), most of which should mediate a general advantage but not a temperature-specific response like we observed here. We found that transgenerational effects were transmitted predominantly by mothers and not fathers, suggesting an egg- or mitochondria-mediated mechanism. While we can rule out egg size (i.e. maternal provisioning) as a possible mechanism, acclimation of mitochondrial energy metabolism (Oellermann, Pörtner & Mark 2012) seems to be a promising mechanism for whole-organism growth/size responses.

Mitochondria are a primary target for thermal compensation (Guderley & St-Pierre 2002), and our results for mitochondrial OXPHOS capacities between the different offspring groups support this. Although mitochondrial efficiency remained constant in all offspring groups (LEAK respiration was always around 23% of OXPHOS), maximum capacities differed depending on maternal temperature. Offspring of 21 °C mothers that were reared at 21 °C showed full metabolic compensation with respect to heart mitochondrial capacities (Precht Type 2, see Precht 1958); their maximum capacities were equal to those of fish reared and assayed at 17 °C. Lower OXPHOS capacities of offspring of 21 °C mothers reared and assayed at 21 °C point to optimized metabolic rates, which also have been observed for tropical reef fishes acclimated to warmer temperatures over consecutive generations (Donelson *et al*. 2012). Optimized metabolic rates can be a consequence of symmorphosis (the concept that structural design is matched to functional demand; Weibel, Taylor & Hoppeler 1991). Here, this would imply that an animal's physiological capacities are tailored to its respective environmental needs, which has been discussed for sticklebacks (Dalziel, Ou & Schulte 2012). However, as the acclimation response of offspring groups reared at 21 °C differed depending on maternal temperature, we can exclude pure symmorphosis effects, and attribute the compensated mitochondrial capacities of offspring of 21 °C mothers to environment-dependent maternally inherited thermal compensation.

An optimized metabolism would generate a higher scope for growth, which is in line with our findings for offspring body size – that is, although offspring reared at 21 °C were generally smaller than offspring at 17 °C, offspring of 21 °C mothers reached a (relatively) larger size in the warmer environment than offspring of 17 °C mothers. Moreover, the size differences between temperatures seen in early growth stages remained in the F1 adults, indicating that maternal acclimation effects persisted into adulthood, and provide a direct link between mitochondrial respiration and growth. Essentially, our results suggest that mothers acclimated to 21 °C adjusted the metabolism of mitochondria that were then inherited by offspring, and differences in mitochondrial capacities and efficiency underlie the differences seen in offspring growth. Still, we cannot rule out a broader mechanistic basis of inheritance such as parental sex-dependent epigenetic marks that affect genes associated with mitochondrial function and thermal tolerance (e.g. by maternal transfer of mRNA). Here, we assessed differential metabolism on a phenotypic level that mechanistically links growth to mitochondrial metabolism, which does not exclude the possibility of an underlying epigenetic mechanism of inheritance.

Smaller offspring at 21 °C in general is likely due to energetic restrictions, which might manifest as reduced aerobic scope, but TGP benefits of 21 °C mothers still led to an improved energy metabolism that promoted better growth (larger size) because less energy may have been required to maintain standard metabolic rate (as seen in heart mitochondrial respiration). Interestingly, one would expect lower relative metabolic rates in larger fish, and thus, lower mitochondrial capacities of fish reared at 17 °C, which was clearly not the case. It may be that the population of sticklebacks used in our experiments has reached their upper limit of thermal acclimation at 21 °C, which also may be a reason for the relatively low Q_{10} observed for OXPHOS between 17 and 21 °C in all groups (cf. Mark *et al*. 2012). Ocean warming of only a few

degrees could have deleterious effects on stickleback populations that are already living at or near their thermal limit. Elevated temperatures generally affect condition factors and conversion efficiency in this species (Guderley, Leroy & Gagne 2001), entailing reduced growth, and also may lead to severe decreases in population size (Moran *et al.* 2010). Transgenerational (maternal) effects on stickleback metabolism may be a first step towards metabolic adaptation (*sensu* Chevin, Lande & Mace 2010) that could alleviate some of the physiological stress associated with warming waters.

EVOLUTIONARY POTENTIAL IN CHANGING ENVIRONMENTS

Our data suggest that selection gradients were steeper at elevated temperature, as we found smaller genetic variance components in the more stressful environment (see also Donelson *et al.* 2012; F. M. Schade, L. N. S. Shama and K.M. Wegner, unpublished data). Clutches that failed at 21 °C were equally distributed amongst combinations of parental rearing temperatures, indicating that a low number of families in any particular group or a systematic failure bias towards mothers of one temperature did not occur. Yet, lower hatching success of eggs at 21 °C and from mothers acclimated to 21 °C indicates that selection may have occurred at the egg level, and may be driving the pattern of lower genetic variance components at 21 °C. Alternatively, it may be that the 60-day acclimation period used in our study was not long enough for plasticity of reproductive output to occur. Here, we did not find a trade-off between egg size and clutch size; hence, no reproductive output adjustments by females depending on acclimation temperature. Studies using females that have spent their entire lives at a particular temperature would be needed to show whether females allocate resources to eggs differently depending on their environment (Mousseau & Fox 1998), and whether these adjustments result in differential hatching success.

It is important to note that selection at the egg level cannot explain our results for offspring body size (growth) that depend on the maternal environment. Our experimental design specifically addresses this aspect, because maternal (as well as paternal) genotypes were randomly distributed over acclimation treatments leading to a similar scope for selection in both acclimation groups. The fact that offspring in split-clutches from the same family grew better in their respective maternal environment cannot, thus, be attributed to genetic selection, but must depend on an environment-specific modification transferred from mothers to offspring. This modification may be epigenetic and therefore also heritable to a certain degree, but the specific mechanism of inheritance was beyond the scope of the current study.

Maternal variance components were lower at elevated temperature. Higher variance due to maternal effects may be expected in unfavourable conditions because individual differences amongst mothers should be exacerbated by environmental stress (reviewed in Charmantier & Garant 2005). However, the reviewed studies did not show a consistent trend across environments, with approximately half of the traits investigated showing the opposite direction response. In our study, lower maternal variance in the stressful environment was mostly driven by 17 °C mothers. Whereas 21 °C mothers contributed similar levels of maternal variance to offspring phenotypic variance across environments, 17 °C mothers showed higher erosion of maternal variance at 21 °C, further supporting our tenet that maternal programming was stronger under stressful conditions. Maternal effects, whether genetic or environmental, are increasingly recognized as a potentially powerful mechanism that can enhance the adaptive potential and persistence of populations in rapidly changing environments (Kirkpatrick & Lande 1989; Mousseau & Fox 1998; Räsänen & Kruuk 2007; Burgess & Marshall 2011). In our study, several lines of evidence (TGP of body size and mitochondrial respiration; maternal variance components) showed that mothers acclimated to stressful conditions transmitted stronger and more persistent maternal effects to offspring than mothers acclimated to ambient, non-stressful conditions, indicating that maternal effects will be an important component of the total adaptive potential of this population under climate change that may eventually lead to genetic assimilation and true adaptation (Badyaev 2005).

Observed heritabilities of body size tended to be lower in the stressful environment. Stressful conditions are known to contribute to changes in heritability across environments (reviewed in Hoffmann & Merilä 1999) via changes in additive genetic (V_A) and environmental (V_E) variance, non-additive effects including maternal effects and G × E interactions (Charmantier & Garant 2005; Kruuk, Slate & Wilson 2008). Here, we found a clear trend of lower heritabilities under stress at 60 days, likely reflecting reduced V_A and increased V_E (that was not driven by increased maternal variance) at 21 °C, and non-significant genetic correlations between size at the two experimental temperatures, indicating strong G × E interactions. Changes in variance components (V_A and V_E) and heritabilities across environments were less pronounced at 30 days, but a genetic correlation between temperature environments for early growth stages indicates a more similar contribution of genetic (V_A) and non-genetic (TGP) effects across environments (Charmantier & Garant 2005). This might indicate that in very early stages of life, transgenerational (maternal) effects may have buffered some of the changes in heritabilities across environments, while at 60 days, overriding effects of the stressful environment led to a clear breakdown in genetic architecture (Via & Lande 1985; Hoffmann & Merilä 1999; Visser 2008). Overall, G × E interactions likely contribute most strongly to the changes in genetic variances and heritabilities across environments found here, and most importantly indicate that, at least for this population, thermal reaction norms for

offspring body size have the potential to evolve (Via 1993; Charmantier & Garant 2005).

Conclusion

Parental effects will likely play an important role in mediating some of the impacts of climate change. Our study demonstrates a clear benefit of maternal TGP on offspring body size in stressful environments. With the persistence into adulthood of matching patterns of TGP for mitochondrial energy metabolism, we can link a mechanistic basis showing maternal inheritance to a crucial component of fitness. The responses to simulated ocean warming shown here indicate that selection gradients may be steeper at elevated temperature, but that stronger maternal programming under stressful conditions may help to alleviate some of the fitness consequences. This suggests that the sign and magnitude of evolutionary change (i.e. the population's evolutionary potential) will be influenced by both genetic and non-genetic inheritance with a strong component of environmental dependency (Marshall & Uller 2007). To better understand selection-driven change associated with phenomena of climate change in an evolutionary context, more studies taking combined genetic and non-genetic (e.g. TGP) approaches that cover multiple generations as well as the mechanistic basis of TGP are needed.

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Data accessibility

Data are deposited in PANGAEA (access no. PDI-5627; DOI: 10.1594/PANGAEA.816963-816966831652).

References

Badyaev, A.V. (2005) Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **272**, 877–886.

Bonduriansky, R., Crean, A.J. & Day, T. (2012) The implications of nongenetic inheritance for evolution in changing environments. *Evolutionary Applications*, **5**, 192–201.

Bonduriansky, R. & Day, T. (2009) Nongenetic inheritance and its evolutionary implications. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 103–125.

Brown, K.H. (2008) Fish mitochondrial genomics: sequence, inheritance and functional variation. *Journal of Fish Biology*, **72**, 355–374.

Burgess, S.C. & Marshall, D.J. (2011) Temperature-induced maternal effects and environmental predictability. *Journal of Experimental Biology*, **214**, 2329–2336.

Charmantier, A. & Garant, D. (2005) Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **272**, 1415–1425.

Chevin, L., Lande, R. & Mace, G.M. (2010) Adaptation, plasticity and extinction in a changing environment: towards a predictive theory. *Plos Biology*, **8**, e1000357.

Crean, A.J., Dwyer, J.M. & Marshall, D.J. (2013) Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology*, **94**, 2575–2582.

Dalziel, A.C., Ou, M. & Schulte, P.M. (2012) Mechanisms underlying parallel reductions in aerobic capacity in non-migratory threespine stickleback (*Gasterosteus aculeatus*) populations. *Journal of Experimental Biology*, **215**, 746–759.

Donelson, J.M., Munday, P.L., McCormick, M.I. & Pitcher, C.R. (2012) Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, **2**, 30–32.

Etterson, J.R. & Galloway, L.F. (2002) The influence of light on paternal plants in *Campanula americana* (Campanulaceae): pollen characteristics and offspring traits. *American Journal of Botany*, **89**, 1899–1906.

Falconer, D.S. (1952) The problem of environment and selection. *The American Naturalist*, **86**, 283–298.

Fauvel, C., Savoye, O., Dreanno, J. & Suquet, M. (1999) Characteristics of sperm of captive sea bass in relation to its fertilization potential. *Journal of Fish Biology*, **54**, 356–369.

Gamperl, A.K. & Farrell, A.P. (2004) Cardiac plasticity in fishes: environmental influences and intraspecific differences. *The Journal of Experimental Biology*, **207**, 2539–2550.

Guderley, H. & Johnston, I.A. (1996) Plasticity of fish muscle mitochondria with thermal acclimation. *Journal of Experimental Biology*, **199**, 1311–1317.

Guderley, H., Leroy, P.H. & Gagne, A. (2001) Thermal acclimation, growth, and burst swimming of threespine stickleback: enzymatic correlates and influence of photoperiod. *Physiological and Biochemical Zoology*, **74**, 66–74.

Guderley, H. & St-Pierre, J. (2002) Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *Journal of Experimental Biology*, **205**, 2237–2249.

Hadfield, J. (2010) MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, **33**, 1–22.

Hochachka, P.W. (1994) *Muscles as Molecular and Metabolic Machines*. CRC Press, Boca Raton, USA.

Hoffmann, A.A. & Merilä, J. (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution*, **14**, 96–101.

Jablonka, E. & Raz, G. (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, **84**, 131–176.

Kelly, M.W., Padilla-Gamino, J. & Hofmann, G.E. (2013) Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Global Change Biology*, **19**, 2536–2546.

Kirkpatrick, M. & Lande, R. (1989) The evolution of maternal characters. *Evolution*, **43**, 485–503.

Kruuk, L.E.B., Slate, J. & Wilson, A.J. (2008) New answers for old questions: the evolutionary quantitative genetics of wild animal populations. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 525–548.

Mark, F.C., Lucassen, M., Strobel, A., Barrera-Oro, E., Koschnick, N., Zane, L. *et al.* (2012) Mitochondrial function in Antarctic Nototheniids with *ND6* translocation. *PLoS ONE*, **7**, e31860.

Marshall, D.J. & Uller, T. (2007) When is a maternal effect adaptive? *Oikos*, **116**, 1957–1963.

Miller, G.M., Watson, S., Donelson, J.M., McCormick, M.I. & Munday, P.L. (2012) Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Climate Change*, **2**, 858–861.

Moran, R., Harvey, I., Moss, B., Feuchtmayr, H., Hatton, K., Heyes, T. *et al.* (2010) Influence of simulated climate change and eutrophication on three-spined stickleback populations: a large scale mesocosm experiment. *Freshwater Biology*, **55**, 315–325.

Mousseau, T.A. & Fox, C.W. (1998) The adaptive significance of maternal effects. *Trends in Ecology and Evolution*, **13**, 403–407.

Munday, P.L., Warner, R.R., Monro, K., Pandolfi, J.M. & Marshall, D.J. (2013) Predicting evolutionary responses to climate change in the sea. *Ecology Letters*, **16**, 1488–1500.

Oellermann, M., Pörtner, H.O. & Mark, F.C. (2012) Mitochondrial dynamics underlying thermal plasticity of cuttlefish (*Sepia officinalis*) hearts. *Journal of Experimental Biology*, **215**, 2992–3000.

Parker, L.M., Ross, P.M., O'Connor, W.A., Borysko, L., Raftos, D.A. & Pörtner, H.O. (2012) Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, **18**, 82–92.
Pörtner, H.O. & Farrell, A.P. (2008) Physiology and climate change. *Science*, **322**, 690–692.
Pörtner, H.O., Mark, F.C. & Bock, C. (2004) Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. *Respiratory Physiology & Neurobiology*, **141**, 243–260.
Precht, H. (1958) Concepts of the temperature adaptations of unchanging reaction systems of cold-blooded animals. *Physiological Adaptation*, (ed C. L. Prosser) pp. 50–78. American Physiological Society, Washington, DC, USA.
R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
Ramler, D., Mitteroecker, P., Shama, L.N.S., Wegner, K.M. & Ahnelt, H. (2014) Non-linear effects of temperature on body form and developmental canalization in the threespine stickleback. *Journal of Evolutionary Biology*, **27**, 497–507.
Räsänen, K. & Kruuk, L.E.B. (2007) Maternal effects and evolution at ecological time scales. *Functional Ecology*, **21**, 408–421.
Salinas, S. & Munch, S.B. (2012) Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecology Letters*, **15**, 159–163.
Salinas, S., Brown, S.C., Mangel, M. & Munch, S.B. (2013) Non-genetic inheritance and changing environments. *Non-Genetic Inheritance*, **1**, 38–50.
Sheppard, C. (2004) Sea surface temperature 1871–2099 in 14 cells around the United Kingdom. *Marine Pollution Bulletin*, **49**, 12–16.
Spiegelhalter, D., Best, N., Carlin, B. & Van Der Linde, A. (2002) Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society. Series B, Statistical Methodology*, **64**, 583–639.
Strobel, A., Graeve, M., Pörtner, H.O. & Mark, F.C. (2013) Mitochondrial acclimation capacities to ocean warming and acidification are limited in the Antarctic Nototheniid fish, *Notothenia rossii* and *Lepidonotothen squamifrons*. *PLoS ONE*, **8**, e68865.
Sultan, S.E., Barton, K. & Wilczek, A.M. (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology*, **90**, 1831–1839.
Sunday, J.M., Crim, R.N., Harley, C.D.G. & Hart, M.W. (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE*, **6**, e22881.
Sunday, J.M., Calosi, P., Dupont, S., Munday, P.L., Stillman, J.H. & Reusch, T.B.H. (2014) Evolution in an acidifying ocean. *Trends in Ecology and Evolution*, **29**, 117–125.
Vehmaa, A., Brutemark, A. & Engström-Öst, J. (2012) Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. *PLoS ONE*, **7**, e48538.
Via, S. (1993) Adaptive phenotypic plasticity-target or by-product of selection in a variable environment. *The American Naturalist*, **142**, 352–365.
Via, S. & Lande, R. (1985) Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution*, **39**, 505–522.
Visser, M.E. (2008) Keeping up with a warming world: assessing the rate of adaptation to climate change. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **275**, 649–659.
Weibel, E.R., Taylor, C.R. & Hoppeler, H. (1991) The concept of symmorphosis: a testable hypothesis of structure–function relationship. *Proceedings of the National Academy of Sciences of the USA*, **88**, 10357–10361.
Wilson, A.J., Reale, D., Clements, M.N., Morrissey, M.M., Postma, E., Walling, C.A. *et al.* (2010) An ecologist's guide to the animal model. *Journal of Animal Ecology*, **79**, 13–26.
Windig, J.J. (1997) The calculation and significance testing of genetic correlations across environments. *Journal of Evolutionary Biology*, **10**, 853–874.

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Supporting Information

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

Table S1. Minimum adequate models using data only from round 2 for *Gasterosteus aculeatus* respiration parameters (OXPHOS, ETS and LEAK) depicting the influence of assay temperature (°C), and offspring (°C) and parental (sire and dam temperature °C) thermal environments.
Table S2. Genetic variance-covariance matrices of *Gasterosteus aculeatus* offspring size at 30 and 60 days analysed separately for the four paternal-maternal thermal environment groups (shown as sire °C × dam °C).
Fig. S1. Relationship between density and size (measured as standard length ± 0·01 mm) for offspring reared at 17 °C (open symbols; dashed lines) and 21 °C (closed symbols; solid lines).
Fig. S2. Family-level reaction norms of offspring size (estimated using residuals of standard length) at 17 and 21 °C for each parental temperature combination at 30 days (upper panel) and 60 days (lower panel).

