

# Bet hedging in a warming ocean: predictability of maternal environment shapes offspring size variation in marine sticklebacks

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## Abstract

Bet hedging at reproduction is expected to evolve when mothers are exposed to unpredictable cues for future environmental conditions, whereas transgenerational plasticity (TGP) should be favoured when cues reliably predict the environment offspring will experience. Since climate predictions forecast an increase in both temperature and climate variability, both TGP and bet hedging are likely to become important strategies to mediate climate change effects. Here, the potential to produce variably sized offspring in both warming and unpredictable environments was tested by investigating whether stickleback (*Gasterosteus aculeatus*) mothers adjusted mean offspring size and within-clutch variation in offspring size in response to experimental manipulation of maternal thermal environment and predictability (alternating between ambient and elevated water temperatures). Reproductive output traits of F1 females were influenced by both temperature and environmental predictability. Mothers that developed at ambient temperature (17 °C) produced larger, but fewer eggs than mothers that developed at elevated temperature (21 °C), implying selection for different-sized offspring in different environments. Mothers in unpredictable environments had smaller mean egg sizes and tended to have greater within-female egg size variability, especially at 21 °C, suggesting that mothers may have dynamically modified the variance in offspring size to spread the risk of incorrectly predicting future environmental conditions. Both TGP and diversification influenced F2 offspring body size. F2 offspring reared at 21 °C had larger mean body sizes if their mother developed at 21 °C, but this TGP benefit was not present for offspring of 17 °C mothers reared at 17 °C, indicating that maternal TGP will be highly relevant for ocean warming scenarios in this system. Offspring of variable environment mothers were smaller but more variable in size than offspring from constant environment mothers, particularly at 21 °C. In summary, stickleback mothers may have used both TGP and diversified bet-hedging strategies to cope with the dual stress of ocean warming and environmental uncertainty.

**Keywords:** climate change, diversified bet hedging, egg size plasticity, environmental variability, *Gasterosteus aculeatus*, maternal effects, paternal effects, transgenerational plasticity

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## Introduction

Population persistence in changing environments requires that organisms adjust their phenotypes to match local conditions. This can be achieved by rapid evolution (genetic tracking) and/or adaptive phenotypic plasticity (Chevin *et al.*, 2010). Adaptive phenotypic plasticity is expected to evolve when environmental cues of future conditions are predictable or recurring (Via, 1993; Simons, 2011). Plasticity can occur both within generations (organisms respond to their immediate environment) and across generations via transgenerational plasticity (TGP). In the case of

TGP, anticipatory parental effects (*sensu* Marshall & Uller, 2007; Burgess & Marshall, 2014) on offspring phenotypes will only be favoured if the parental environment is a good predictor of the environment offspring will experience (Mousseau & Fox, 1998; Räsänen & Kruuk, 2007). When environmental conditions vary unpredictably, and parents cannot predict their offspring's environment, the evolution of bet hedging is expected, for instance, parents should produce a range of offspring phenotypes with at least some having the optimal phenotype (Einum & Fleming, 2004; Marshall *et al.*, 2008; Fischer *et al.*, 2011; Simons, 2011). Given that recent climate predictions forecast an increase in weather variability (IPCC 2012), such as more frequent and severe storms and other extreme events including heat waves, droughts and flooding (Thornton *et al.*,

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2014), bet hedging is likely to become an increasingly important strategy to mediate some of the impacts of rapid climate change (Crean & Marshall, 2009). Indeed, it was recently shown that projections based on mean temperature change alone differ substantially from those incorporating changes to temperature variation, and that increased variation might pose an even greater risk to species than climate warming (Vasseur *et al.*, 2014). Nevertheless, bet hedging is currently under-represented in treatments of evolutionary responses to environmental variance (Simons, 2011).

Theoretical evidence for bet hedging at reproduction as an adaptive strategy in unpredictable environments has been established for half a century (Cohen, 1966; Seger & Brockmann, 1987; Philippi & Seger, 1989). Bet hedging should be advantageous because it increases geometric-mean fitness (e.g. cumulative fecundity across generations), but does so at the expense of expected fitness within a generation. Thus, bet-hedging strategies appear to be detrimental over the short-term (Slatkin, 1974; Simons, 2011). Two main forms of bet hedging are commonly recognized: conservative and diversified (Seger & Brockmann, 1987; Philippi & Seger, 1989; but see Olofsson *et al.*, 2009 for a third form). Conservative bet hedging or 'playing it safe' occurs when, for example, mothers produce larger offspring than would be the optimum in a stable environment with the same long-term mean quality. This strategy can result in greater reproductive success during poor years if larger offspring are more than successful than smaller offspring in poor conditions (Einum & Fleming, 2004). Diversified bet hedging occurs when mothers increase the phenotypic variance among individual offspring, thereby spreading the risk of reproductive failure in uncertain environments among an array of offspring phenotypes (Crean & Marshall, 2009). There is theoretical support for both forms (Einum & Fleming, 2004; Marshall *et al.*, 2008), but ultimately, the relative benefits of one or the other strategy will depend on the specific offspring trait-fitness function (Smith & Fretwell, 1974; Marshall *et al.*, 2008). Empirical evidence for bet hedging, on the other hand, is rather scant. While there are numerous correlative studies invoking bet hedging as an explanation for within-clutch variation in offspring phenotypes (reviewed in Crean & Marshall, 2009 and Simons, 2011), direct tests that manipulate environmental predictability are rare (but see Halpern, 2005; Manenti *et al.*, 2014; Furness *et al.*, 2015). Recent comparative studies of populations along gradients of environmental predictability (Morrongiello *et al.*, 2012; Garcia-Roger *et al.*, 2014) and direct tests of Cohen's predictions regarding propagule dormancy (Graham *et al.*, 2014; Gremer & Venable, 2014; Simons, 2014) highlight the importance of bet hedging as a

potential mechanism for organisms to cope with increasing climate variability.

Bet hedging entails an inherent trade-off between maximizing within-generation fitness and minimizing among-generation variation in reproductive success (Marshall *et al.*, 2008). Although evidence for paternal environment effects on offspring phenotypes is accumulating (e.g. Crean *et al.*, 2013; Shama & Wegner, 2014), the majority of studies have investigated how mothers deal with the challenge of managing this trade off. Within a generation, mothers maximize fitness via plasticity in the mean offspring phenotype, whereas across generations, they manage the variance in fitness via bet hedging (Crean & Marshall, 2009). Because environmental variance is composed of both predictable and unpredictable components, the concurrent evolution of both plasticity and bet hedging is expected, although little studied (but see Simons, 2014; Furness *et al.*, 2015). The number of studies showing changes in the mean offspring phenotype as a result of TGP has risen dramatically over the past few years (reviewed in Bonduriansky *et al.*, 2012; Salinas *et al.*, 2013), but very few have explicitly estimated the predictability of environmental cues (but see Galloway & Etterson, 2007), hence the adaptive significance of this plasticity remains unclear (Burgess & Marshall, 2011). Moreover, although correlative and comparative studies of bet hedging suggest that females can adjust the variability of offspring phenotypes (see references listed above), experiments that manipulate maternal environmental predictability are needed to determine if mothers can dynamically modify this variation (Crean & Marshall, 2009).

Optimality models predict that under constant environmental conditions, a single, optimal offspring size will be favoured, and different sizes will be optimal in different environments depending on the offspring size-fitness relationship in each environment (Marshall *et al.*, 2008). Consistent with this, there are numerous studies showing that mothers can shift the phenotype of their offspring to match local conditions (reviewed in Burgess & Marshall, 2014), that is, they exhibit adaptive phenotypic plasticity. When environmental conditions are good, some models predict that mothers should produce smaller, but more offspring to maximize fecundity, whereas under harsh conditions, they should produce fewer, larger offspring that are presumed to be of higher quality (Einum & Fleming, 2004). Yet, evidence is accumulating to support the argument that 'bigger is not always better in harsh environments' (Kaplan, 1992). For example, smaller offspring may be favoured under size-selective predation, or when larger size is associated with higher physiological demands under heat stress (see Morrongiello *et al.*, 2012 for more

examples). When environmental conditions are unpredictable, mothers should maximize fitness by producing a range of offspring sizes in the hope that at least some will have the optimal size for local conditions. By using such a diversified bet-hedging strategy, mothers decrease the variance in fitness across years, but do so at a cost to fitness within a year, since the more offspring size varies, the more offspring that are away from the optimal size (Crean & Marshall, 2009).

The aim of this study was to test the potential to produce variably sized offspring in both warming and unpredictable environments by investigating whether stickleback (*Gasterosteus aculeatus*) mothers adjust mean offspring size and within-clutch variation in offspring size in response to experimental manipulation of maternal thermal environment and predictability, in this case, varying sea surface temperatures (SST). Previous studies of this population found that elevated water temperatures (21 °C) simulated in accordance with a 2100 climate scenario (Sheppard, 2004) had detrimental effects on growth (Schade *et al.*, 2014) and development (Ramler *et al.*, 2014), in comparison to water temperatures that reflected local, ambient summer conditions (17 °C). Yet, when mothers were acclimated to elevated temperature during reproductive conditioning, offspring reached (relatively) larger sizes in the warmer environment as a result of maternal TGP (or anticipatory maternal effects) in response to predictable environmental cues of future water temperatures (Shama *et al.*, 2014). But what happens when environmental cues are unpredictable, for example, when seas surface temperatures change by several degrees over short time periods in response to variable weather patterns and extreme events such as heat waves (Thornton *et al.*, 2014; Vasseur *et al.*, 2014)? When environmental cues are unpredictable, mothers should adopt a bet-hedging strategy and produce a range of offspring sizes (Marshall *et al.*, 2008).

In the current study, the prediction was that not only the mean but also the variance in offspring phenotypes should differ depending on maternal thermal environment and predictability. Given that previous work has also shown that stickleback mothers that developed at elevated temperature (21 °C) produced more, but smaller eggs than mothers that developed under ambient conditions (Shama & Wegner, 2014), the specific prediction here was that mothers acclimated to constant, but different temperatures (17 °C or 21 °C) should produce offspring of a mean optimal size to match local conditions. Under variable environmental conditions (switching between ambient and elevated temperature), mothers should produce a range of offspring sizes, with some matching the optimal mean size for both experimental temperatures. In other words, when mothers

can predict the thermal environment their offspring will experience, TGP in mean offspring size should be favoured, whereas environmental uncertainty should promote increased variance in offspring size (Crean & Marshall, 2009; Simons, 2011).

## Materials and methods

### *Environmental predictability in the wild*

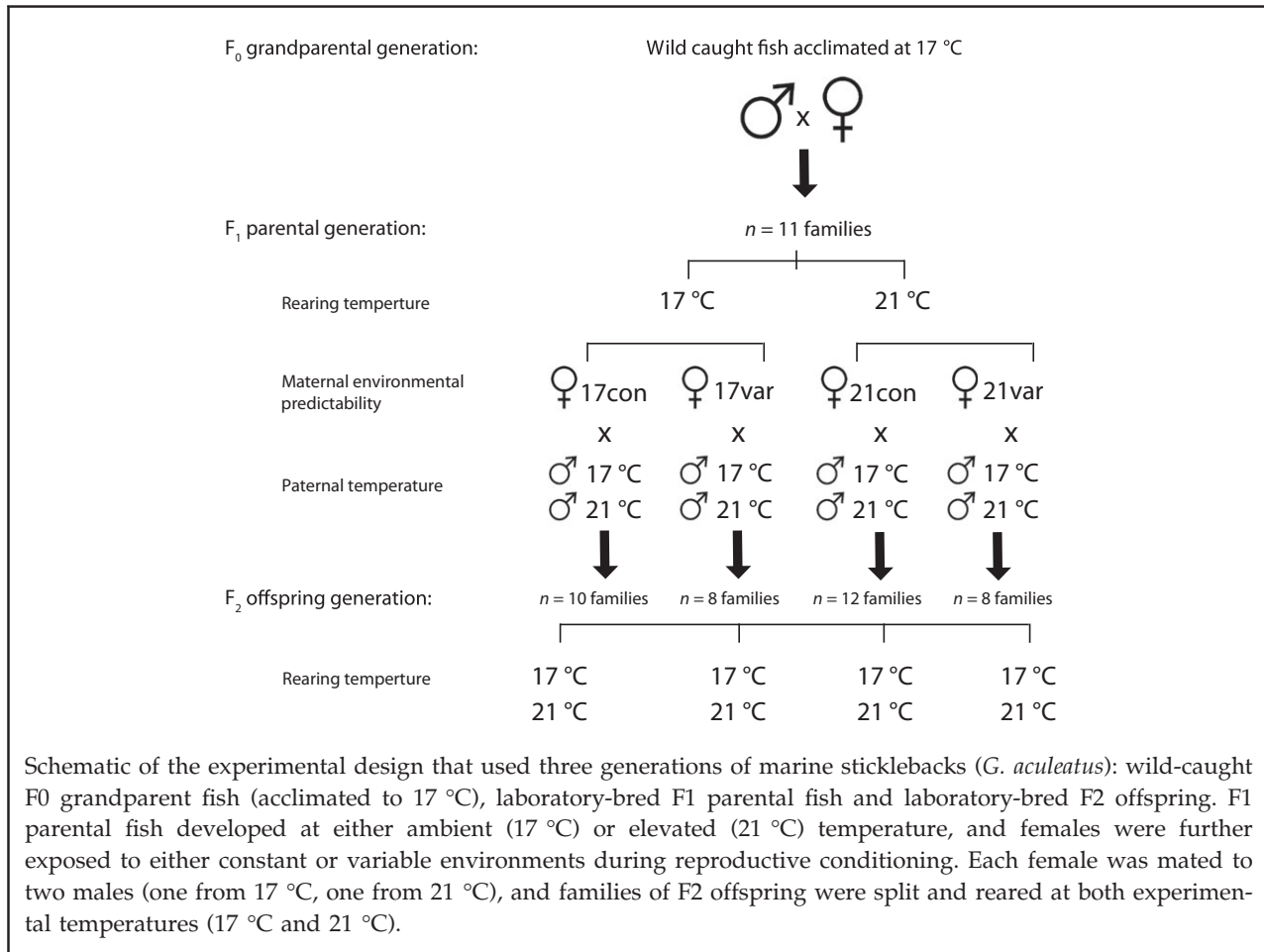
To characterize the predictability of environmental conditions that mothers and offspring experience in the wild, SST from the study area were obtained from the Coastal Observing System for Northern and Arctic Seas data web portal of the Helmholtz-Zentrum Geesthacht Zentrum für Material-und Küstenforschung (<http://www.cosyna.de>). Temperatures were recorded every 30 min from 15 March to 31 December 2012 using a Directional Waverider Buoy (Datawell bv, Haarlem, the Netherlands) located off the west coast of Sylt, Germany (54°79'N, 8°27'E).

### *Fish crosses and experimental design*

The experiment used marine three-spine sticklebacks from three generations: wild-caught grandparental fish (F0), laboratory-reared parental fish (F1) and laboratory-reared F2 offspring (Box 1). Grandparent fish originated from an oceanic population in the Sylt-Rømø Bight (55°05'N, 8°39'E) that were caught by trawling in February 2012, brought to the laboratory, and held at 17 °C for 2 months during reproductive conditioning. Crosses were made over a 2-week period in May 2012 to produce 11 F1 families with a 17 °C male × 17 °C female thermal acclimation history. Offspring from each family were split and reared at 17 and 21 °C (Box 1). Families were reared separately for the first 60 days posthatch. After this, juvenile F1 fish from all families within each rearing temperature were pooled, divided amongst several 25-L aquaria to reach a final density of 25–30 fish per aquarium, and reared to adulthood (see Shama *et al.*, 2014 for details).

When the F1 fish were 10 months old, (now) adult fish from within each rearing temperature were randomly split into environmental predictability treatments and held under these conditions for another 2 months, corresponding to the period of F1 reproductive conditioning (Box 1). Specifically, fish reared at 17 °C were either maintained at a constant temperature of 17 °C (17con), or switched weekly between 17 and 21 °C (17var). Similarly, fish reared at 21 °C were either maintained at 21 °C (21con) or switched weekly between temperatures (21var). Fish in constant temperature treatments were also switched among aquaria of the same temperature each week to account for possible confounding effects of physically moving to different aquaria. F2 crosses were performed over a 2-week period in May 2013 to produce 38 families. Each of 19 F1 females was mated to two F1 males, one from 17 °C and one from 21 °C (crossing methods as in Shama *et al.*, 2014). As the focus of the study was maternal environment predictability, only males from the constant temperature treatments were used in crosses. F2 egg clutches from each family were then

**Box 1** Experimental design using 3 generations of marine sticklebacks to investigate potential transgenerational plasticity (TGP) and bet hedging in response to ocean warming and increased climate variability.



split and reared at 17 °C and 21 °C ( $n = 76$  split clutches/families in total; Box 1).

#### Egg traits and offspring body size

Each split clutch of eggs was photographed under a dissecting microscope for digital analyses of egg size and clutch size (using LEICA QWIN imaging software; Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). Egg size was estimated by measuring the diameter ( $\pm 0.01$  mm) of 20 eggs per female. The 20 measured eggs were chosen based on the clarity of their outer perimeter in the photographs. Clutch size was estimated as the total number of eggs per female. Split-clutches were placed individually in 1-L glass beakers containing filtered seawater and an air supply, and beakers were held in water baths heated to either 17 °C or 21 °C. Hatching success was estimated as the proportion of hatchlings from each split clutch (no. hatchlings/no. eggs). Hatchlings were held in beakers for the first 30 days, and water in the beakers was changed every week. At 14 days posthatch, hatchling densities were reduced to approximately 10 offspring per beaker.

At 30 days posthatch, 10 randomly chosen offspring from each split clutch were photographed under a dissecting microscope for digital analysis of body size (standard length  $\pm 0.01$  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 60 days. At 60 and 90 days posthatch, 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* sp. nauplii *ad libitum*.

#### Data analyses

Correlograms were used to assess the predictability of SST in the wild (see also Burgess & Marshall, 2011). Correlograms are plots of the autocorrelation between successive terms in a data series, in this case, a time series of water temperature data. Autocorrelation measures the dependence of values in a series on the values before it at a distance of  $k$  lags, and can be used to show, for example, whether water temperature on a given day is a good predictor of water temperature at some

time point in the future (Burgess & Marshall, 2011, 2014). Daily means for SST from 01 May through 31 August 2012 ( $n = 123$  days) were used in the analyses, and reflect the time period that fish from the grandparental population were present and potentially reproductive in the Sylt-Rømø Bight (L.N.S. Shama, personal observation). The raw SST data did not exhibit stationarity (Shapiro-Wilk normality test,  $W = 0.914$ ,  $P < 0.001$ ), so were first detrended by taking the residuals of a linear model of SST as a function of time (adjusted  $R^2 = 0.927$ ; Legendre & Legendre, 1998). After detrending, the data exhibited stationarity (Shapiro-Wilk normality test,  $W = 0.987$ ,  $P = 0.319$ ) and autocorrelations were calculated using the *acf* function within the R package 'TSA'.

Generalized linear mixed effects models (GLMMs) were used to quantify the effects of temperature and environmental predictability on traits related to reproductive output of F1 parental fish (egg size, clutch size and hatching success) and F2 offspring body size. Body size is a decisive component of fitness for sticklebacks, influencing, for example, fecundity in females and success in fights for territories and sperm competition in males (Dufresne *et al.*, 1990). To show that the relationship between size and fitness also holds for the parental fish used here, the effect of female size on egg traits related to reproductive output was also analysed. Specifically, egg size and clutch size were modelled with female identity as a random effect, female size and clutch size (or egg size) as covariates, and maternal developmental temperature and environmental predictability (plus their interaction) as fixed effects. Offspring body size at 30, 60 and 90 days was modelled with family as a random effect, density, egg size and female size as covariates, and offspring rearing temperature, parental (sire and dam) developmental temperatures and environmental predictability (and their interactions) as fixed effects. Since only sires from the constant temperature treatments were used in crosses, no sire by environmental predictability term was included in the models. Egg size, clutch size and offspring body size were modelled with Gaussian errors using Maximum Likelihood within the *lme* function of the R package 'nlme'.

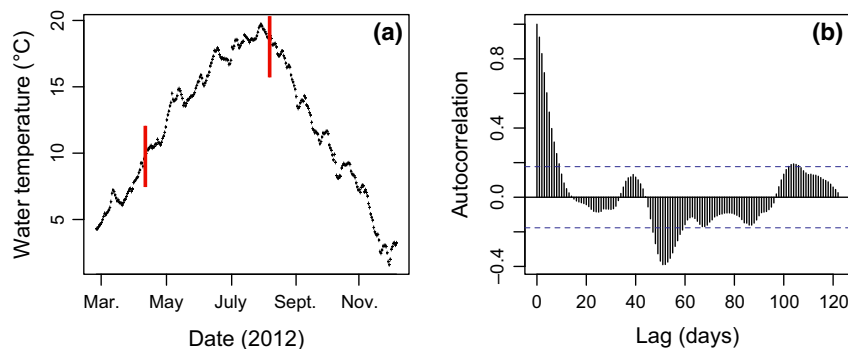
Given the potential for maternal environment unpredictability to lead to increased variation in offspring size,

and hence, heterogeneity of variances among predictability treatment groups, homogeneity of variances was first checked using Levene's test. Each response variable with significant heterogeneity of variances was then modelled using the *weights* function (variance classes) that allows unequal variances across groups in *lme*. Moreover, given that the focus of the study was not only response means but also their variance, and that standard deviations and variances typically show strong relationships with means (see Crean & Marshall, 2009), the coefficient of variation (CV) of egg size and offspring body size were also modelled using *lme*. Two levels of egg size variability were estimated using the CV: within-female variability (within-female egg size CV averaged across all females in a treatment) and among-female variability (CV of mean egg size per female in each treatment; see also Morrongiello *et al.*, 2012). Note: only within-female egg size variability was modelled using *lme*, as there was only one value per treatment (i.e. no error) for among-female variability. Differences between within- and among-female variability per treatment were tested with *t*-tests. Hatching success was modelled with the same fixed effects as above, but with binomial errors, family as a random effect, and an individual-level random effect to account for overdispersion using *glmer* implemented in the R package 'lme4'. All models were fit using individuals, that is, individual eggs for analyses of egg size and hatching success, and individual fish for offspring body size. For graphical display, offspring body size at 30, 60 and 90 days are shown as residual body size (standard length corrected for density). All analyses were run in the R statistical environment (R Development Core Team, 2011).

## Results

### *Environmental predictability*

Sea surface temperature on any particular day was a good predictor of water temperature up to 9 days in the future (Fig. 1). The correlogram shows a significant positive correlation between SSTs for 9 days, but the



**Fig. 1** Predictability of temperature in the wild reflected as (a) sea surface temperature (SST) profile, and (b) correlogram showing the autocorrelation of values in the series on values found at a distance of  $k$  lags (1 lag = 1 day). Red bars (a) delineate the time span within the annual SST profile (May through August) from which daily means were used in the analysis. Dashed blue lines in the correlogram (b) approximate 95% confidence limits for an independent data series for which the autocorrelation is zero.

strength of the autocorrelation decreases after 6 days. The significant negative correlation at 50 days and nearly significant correlations at approximately 70, 90 and 105 days likely reflect abrupt changes in temperature direction (step changes; Legendre & Legendre, 1998) within the data series (Fig. 1b). For this population of sticklebacks, eggs that develop at 17 °C hatch after 7 days, and those that develop at 21 °C hatch after 5 days (see below). A significant autocorrelation of SST for 6–9 days indicates that the environment into which mothers lay eggs is a good predictor of the conditions larvae will experience during very early life.

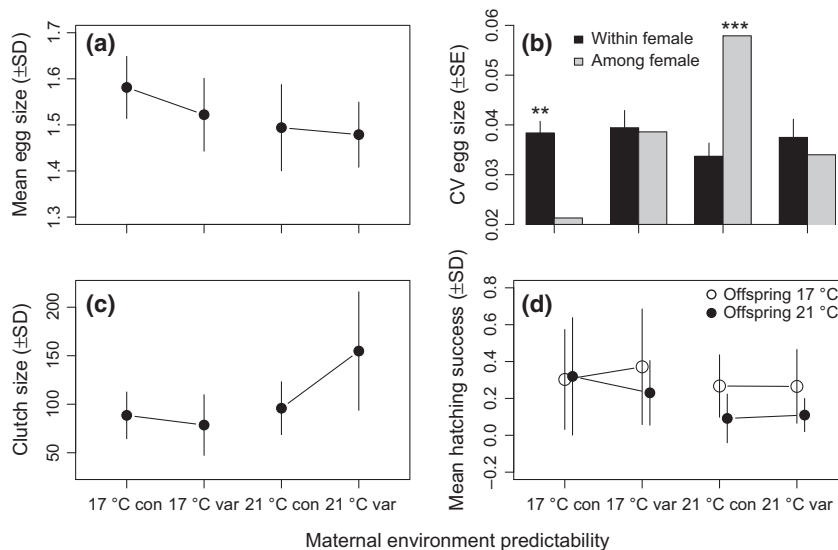
### Maternal environment predictability and reproductive output

Mean egg size was significantly influenced by clutch size and the interaction between maternal development temperature and environmental predictability (Table 1). Mothers acclimated to 17 °C variable conditions (17var) produced smaller eggs than mothers in 17con, whereas eggs from 21 °C mothers had similar mean sizes regardless of environmental variability (Fig. 2a). Overall, eggs of 17 °C mothers were larger than those from 21 °C mothers (ANOVA: dam °C

**Table 1** Linear mixed effects models of mean egg size, the coefficient of variation (CV) of egg size (within-female variability), and clutch size of stickleback (*Gasterosteus aculeatus*) females depicting the influence of maternal developmental temperature (dam °C) and environmental predictability (predictability)

Source	Mean egg size			CV of egg size			Clutch size		
	denDF	F	P	denDF	F	P	denDF	F	P
Intercept	361	20549.198	<0.001	14	596.132	<0.001	13	198.503	<0.001
Female size	13	0.258	0.620	14	0.802	0.386	13	0.443	0.517
Clutch size	13	13.432	<b>0.003</b>						
Egg size							13	10.531	<b>0.006</b>
Dam °C	13	3.830	0.072	14	0.973	0.341	13	1.283	0.278
Predictability	13	0.687	0.422	14	0.552	0.470	13	1.015	0.332
Dam °C × Predictability	13	5.236	<b>0.040</b>	14	0.161	0.694	13	6.996	<b>0.020</b>

Numerator degrees of freedom were 1 in all cases. denDF denotes denominator degrees of freedom. Significant terms are highlighted in bold.



**Fig. 2** Reproductive output traits of female sticklebacks (*Gasterosteus aculeatus*) for each maternal temperature and predictability combination. Treatment combinations are depicted as 17con (17 °C constant), 17var (17 °C variable), 21con (21 °C constant) and 21var (21 °C variable). (a) mean egg size, (b) coefficient of variation (CV) of egg size, shown as within-female variability (black bars) and among-female variability (grey bars), (c) clutch size and (d) mean hatching success (hatching proportion) for offspring reared at 17 °C (open circles) and 21 °C (closed circles). Points or bars reflect means for all females within a treatment (±SD or SE); lines join maternal thermal environments; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 2** Generalized linear mixed effect model (GLMM) for *Gasterosteus aculeatus* hatching success showing the influence of offspring rearing temperature (offspring °C), parental developmental temperature (dam °C, sire °C) and maternal environment predictability (predictability)

Random effects	Variance		SD	
Family (intercept)	5.589		2.364	
Hatch (intercept)	0.007		0.086	
Fixed effects	Estimate	SE	z value	Pr (> z )
(Intercept)	6.267	14.817	0.423	0.672
Egg size	-2.849	7.125	-0.400	0.689
Female size	-0.057	0.146	-0.387	0.699
Offspring °C	-0.049	0.058	-0.857	0.392
Dam °C	-0.757	1.486	-0.510	0.610
Sire °C	-1.233	1.135	-1.086	0.277
Predictability	0.137	1.225	0.112	0.911
Offspring × Dam °C	-1.893	0.087	-21.809	<b>&lt; 0.001</b>
Offspring × Sire °C	0.444	0.075	5.899	<b>&lt; 0.001</b>
Dam × Sire °C	1.249	1.562	0.800	0.424
Offspring °C × Predictability	-1.408	0.075	-18.649	<b>&lt; 0.001</b>
Dam °C × Predictability	-0.963	1.630	-0.591	0.555
Offspring × Dam × Sire °C	-0.005	0.095	-0.052	0.958
Offspring × Dam °C × Predictability	1.859	0.099	18.736	<b>&lt; 0.001</b>

Model fit with individual-level variation (accounting for overdispersion) by the Laplace approximation and a binomial error distribution using *glmer* implemented in the R package *lme4* (R Development Core Team, 2011). SD and SE denote standard deviation and standard error, respectively. Significant terms are highlighted in bold.

$F_{1,17} = 5.308$ ;  $P = 0.034$ ). The variance in mean egg size differed among treatment groups (dam °C × predictability: Levene's Test  $F_{3,376} = 6.910$ ;  $P < 0.001$ ), so mean egg size was modelled using the variance classes function in *lme*.

Within-female egg size variability (CV of egg size), however, was not significantly influenced by maternal temperature or environmental predictability (Table 1). Yet, there was a nonsignificant trend of higher within-female CVs of egg size in variable environments, especially for eggs of 21 °C mothers (Fig. 2b). A *post hoc* power analysis using the 'pwr' package in R revealed that Cohen's  $d$  for both maternal temperatures combined was 0.423 (calculated using the mean and standard deviation of egg size CV in constant and variable environments), which represents a large effect size. Splitting egg size CVs by maternal temperature, however, showed that Cohen's  $d$  at 17 °C was 0.1718, but was 0.5506 at 21 °C. Nevertheless, a linear model

approach using three model terms (dam °C × predictability) showed that the power of the experiment using  $n = 19$  females was 0.5333, and  $n = 30$  females would have been necessary to reach a power level above 0.800 (Cohen, 1988). Among-female egg size variability differed significantly from within-female variability in constant environments, but not in variable environments ( $t$ -tests: 17con  $t = 7.303$ ;  $P = 0.002$ , 21con  $t = -9.199$ ;  $P < 0.001$ ). Among-female variability for egg size was highest for 21con mothers, and lowest for 17con mothers, with mothers in variable environments showing similar CV values (Fig. 2b).

Mean clutch size differed significantly among maternal temperature and predictability treatments, and traded-off with egg size (Table 1). Mothers in 21var environments produced larger clutches than mothers in 21con, whereas 17 °C mothers produced clutches of similar size in both predictability treatments (Fig. 2c). Mean clutch size was inversely related to mean egg size, although 21var mothers produced much larger clutches without a correspondingly large reduction in mean egg size (Fig. 2a). In other words, 21var mothers produced eggs of approximately the same size as their constant environment counterparts despite having larger clutches, and this difference was not driven by female size ( $P > 0.05$  for all egg traits; Table 1). There was also no significant difference in female size among acclimation treatments (ANOVA: dam °C  $F_{1,14} = 1.611$ ;  $P = 0.225$ , predictability  $F_{1,14} = 0.058$ ;  $P = 0.814$ , dam °C × predictability  $F_{1,14} = 0.034$ ,  $P = 0.857$ ).

Hatching success varied with offspring environment, maternal and paternal developmental temperature and environmental predictability (Table 2). Mean time to hatching was  $7.32 \pm 0.48$  days at 17 °C and  $5.23 \pm 0.51$  days at 21 °C. Eggs from 17var mothers had lower hatching success at 21 °C (offspring temperature), but higher hatching success at 17 °C, whereas eggs from 17con mothers had similar hatching success at both temperatures (Fig. 2d). Overall, eggs from 21 °C mothers had higher hatching success at 17 °C than at 21 °C (26.65% vs. 9.87%), but there was no influence of maternal environmental predictability at either offspring temperature. That is, environmental variability only affected the hatching success of eggs from 17 °C mothers, and eggs from 21 °C mothers were more robust to temperature variability.

#### Maternal environment effects on offspring body size

Density had significant effects on offspring body size (Table 3). Offspring in families with higher densities reached smaller body sizes. Mean density per family was 8.97 fish at 17 °C and 7.20 fish at 21 °C, but the

**Table 3** Linear mixed effects models for stickleback (*Gasterosteus aculeatus*) mean body size at 30, 60 and 90 days posthatch depicting the influence of offspring rearing temperature (offspring °C), parental developmental temperatures (dam °C, sire °C) and maternal environment predictability (predictability)

Source	Size 30 days			Size 60 days			Size 90 days		
	denDF	F	P	denDF	F	P	denDF	F	P
Intercept	314	25742.25	<0.001	314	64844.75	<0.001	306	83721.79	<0.001
Density	314	27.79	<0.001	314	305.01	<0.001	306	396.74	<0.001
Egg size	24	14.17	<b>0.001</b>	24	4.82	<b>0.038</b>	25	0.73	0.400
Female size	24	2.65	0.116	24	5.22	<b>0.031</b>	306	4.35	<b>0.038</b>
Offspring °C	314	4.84	<b>0.029</b>	314	71.16	<0.001	306	182.12	<0.001
Dam °C	24	5.98	<b>0.022</b>	24	23.68	<0.001	25	16.94	<0.001
Sire °C	24	5.27	<b>0.031</b>	24	0.05	0.824	25	1.16	0.291
Predictability	24	1.48	0.236	24	14.16	<b>0.001</b>	25	9.76	<b>0.005</b>
Offspring × Dam °C	314	0.61	0.436	314	2.19	0.140	306	5.53	<b>0.019</b>
Offspring × Sire °C	314	0.14	0.714	314	4.45	<b>0.036</b>	306	2.14	0.144
Dam × Sire °C	24	0.03	0.862	24	0.06	0.810	25	1.12	0.300
Offspring × Predictability	314	2.77	0.097	314	2.29	0.131	306	0.74	0.390
Dam °C × Predictability	24	4.76	<b>0.039</b>	24	6.68	<b>0.016</b>	25	2.57	0.122
Offspring × Dam × Sire °C	314	0.40	0.527	314	0.41	0.523	306	0.72	0.397
Offspring × Dam °C × Predictability	314	1.62	0.204	314	2.23	0.136	306	0.24	0.625

Body size was measured as standard length (mm) at 30, 60 and 90 days posthatch. Numerator degrees of freedom were 1 in all cases. denDF denotes denominator degrees of freedom. Significant terms are highlighted in bold.

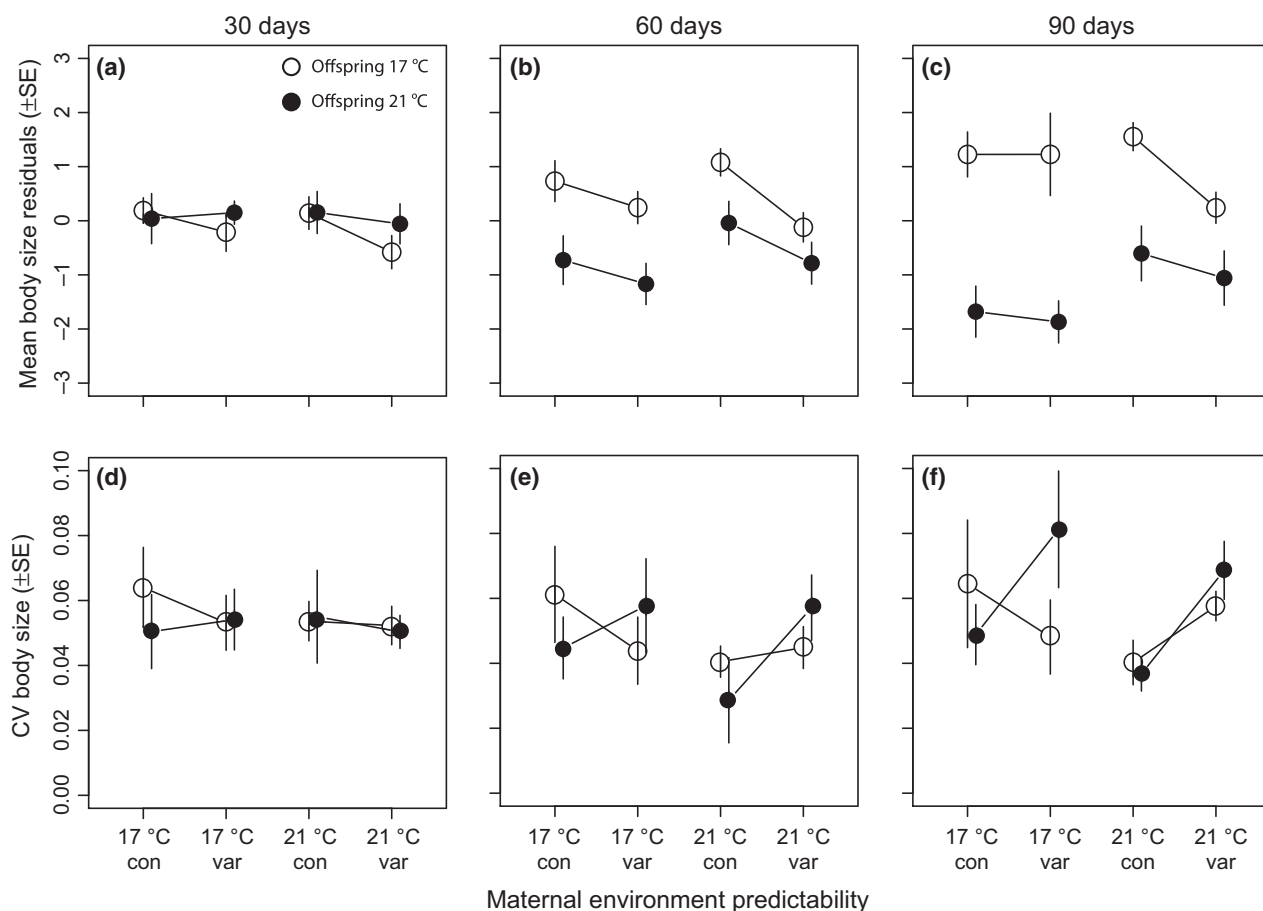
range of densities per family in the different rearing temperatures overlapped (Fig. S1). Differences in densities between rearing temperatures stem from differences in hatching success (lower hatching success at 21 °C). Nevertheless, any growth advantages of lower densities at 21 °C would only dampen the size differences between rearing temperatures (see below). Moreover, density × offspring °C interactions were not significant at 30 days ( $F_{1,349} = 3.406$ ;  $P = 0.066$ ), 60 days ( $F_{1,349} = 1.479$ ;  $P = 0.225$ ) or 90 days ( $F_{1,342} = 0.004$ ;  $P = 0.949$ ), indicating that any potential effects of density on offspring body size were the same in both temperatures (Fig. S1). Egg size and female size influenced offspring body size (Table 3), but these effects were the same in both maternal and offspring temperatures (egg size/female size × dam °C/offspring °C always  $P > 0.05$ ).

Variances among treatment groups were not homogeneous for offspring body size at 30, 60 or 90 days (Levene's test 30 days:  $F_{7,345} = 3.896$ ;  $P < 0.001$ , 60 days:  $F_{7,345} = 5.207$ ;  $P < 0.001$ , 90 days:  $F_{7,338} = 5.415$ ;  $P < 0.001$ ), so size was modelled using the variance classes function in *lme*. At 30 days, offspring from variable environment mothers grew better at 21 °C than at 17 °C (Fig. 3a), and this likely drove the small, overall size difference between offspring temperatures (21 °C > 17 °C) at this early stage (Table 3). The significant dam °C × predictability interaction stems from the same pattern, with smaller offspring of 21var mothers when reared at 17 °C. At

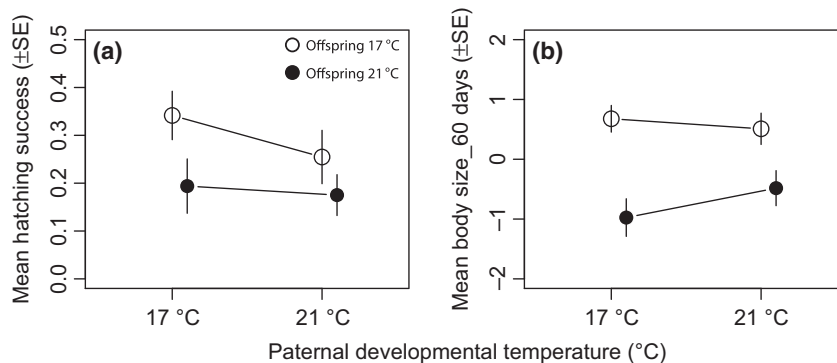
60 days, offspring were now larger at 17 °C than 21 °C (Table 3), and offspring from 21 °C mothers were larger than offspring from 17 °C mothers (Fig. 3b). Overall, offspring of mothers from variable environments were smaller than those from constant environment mothers, and this difference was greater for offspring of 21var mothers that were reared at 17 °C (Fig. 3b). At 90 days, differences between offspring rearing temperatures were even more pronounced (Table 3), and the pattern of larger offspring from 21 °C mothers and larger offspring from constant environment mothers remained (Fig. 3c). Again, this was mainly driven by smaller offspring of 21var mothers that were reared at 17 °C. Essentially, offspring of variable environment mothers suffered in terms of mean body size, and this effect was strongest for offspring of 21var mothers when they were reared in a nonmatching environment as their mothers (at 17 °C). These effects were already present at early growth stages (30 days), and increased over time. At 90 days, there was also evidence for maternal TGP of offspring body size in the stressful environment (offspring °C × dam °C interaction; Table 3). That is, at 21 °C, offspring of 21 °C mothers were larger than offspring of 17 °C mothers, but at 17 °C, offspring reached similar sizes for both maternal developmental temperatures (Fig. 3c).

The CV of offspring body size at 30, 60 and 90 days was modelled using the same random and fixed effects as mean body size (Table S1). A significant interaction between offspring °C and predictability was detected at





**Fig. 3** *Gasterosteus aculeatus* offspring body size and coefficient of variation (CV) of offspring body size for each maternal temperature and predictability combination and reared at 17 °C (open circles) and 21 °C (closed circles). Treatment combinations are depicted as 17con (17 °C constant), 17var (17 °C variable), 21con (21 °C constant) and 21var (21 °C variable). (a–c) mean body size residuals (standard length corrected for density) at 30, 60 and 90 days posthatch, (d–f) CV of body size at 30, 60 and 90 days posthatch. Points reflect means ( $\pm$ SE) for all families within a treatment; lines join maternal thermal environment treatments.



**Fig. 4** Paternal developmental temperature effects on *Gasterosteus aculeatus* offspring (a) hatching success and (b) body size at 60 days posthatch when reared at 17 °C (open circles) and 21 °C (closed circles). Hatching success is shown as hatching proportion, and body size as residuals (standard length corrected for density). Points depict means ( $\pm$ SE) for all families within a treatment.

90 days ( $F_{1,10} = 5.663$ ;  $P = 0.039$ ). At 90 days, offspring reared at 21 °C had higher CVs of body size when their mother was acclimated to a variable environment (both

17var and 21var), whereas at 17 °C, only offspring of 21var mothers had a higher CV of body size (Fig. 3f). In other words, offspring of 21var mothers had higher

CVs of body size regardless of rearing temperature, whereas offspring of 17°C mothers had higher CVs only when reared in the stressful environment. A similar pattern was also evident at 60 days (Fig. 3e), although the interaction term in the model was not significant ( $P = 0.152$ ).

#### *Paternal environment effects*

Significant offspring  $\times$  sire  $^{\circ}\text{C}$  interactions for hatching success (Table 2) and offspring body size at 60 days (Table 3) suggest paternal TGP (Fig. 4). For hatching success, positive paternal TGP benefits were only present at 17  $^{\circ}\text{C}$ : hatching success was higher at 17  $^{\circ}\text{C}$  when fathers developed at 17  $^{\circ}\text{C}$ , whereas hatching success at 21  $^{\circ}\text{C}$  was similar for both paternal temperatures (Fig. 4a). For offspring body size at 60 days, positive paternal TGP benefits were seen at both rearing temperatures – offspring reached a larger size at 21  $^{\circ}\text{C}$  if their fathers developed at 21  $^{\circ}\text{C}$ , and a larger size at 17  $^{\circ}\text{C}$  if their fathers developed at 17  $^{\circ}\text{C}$  – although the effects were stronger at 21  $^{\circ}\text{C}$  (Fig. 4b).

#### **Discussion**

Bet hedging is expected to evolve when mothers are exposed to unpredictable cues for future environmental conditions, whereas TGP should be favoured when cues reliably predict the environment offspring will experience (Simons, 2011). In line with theoretical expectations, when stickleback mothers could predict the thermal environment of their offspring, TGP benefits led to greater mean offspring sizes at elevated temperature. In varying thermal conditions, mothers may have dynamically modified the variability in offspring size, but at a cost to mean offspring size. By directly manipulating maternal thermal environment and predictability, this study demonstrates that both TGP and potential bet hedging at reproduction can play important roles in mediating some of the impacts of ocean warming and increasing climate variability. Additionally, the findings highlight the importance of considering both the mean and variance of traits responding to climate change, as these are likely to vary independently depending on the predictability of environmental cues.

#### *Environmental predictability*

Local SSTs that stickleback mothers and offspring experience in the wild are predictable for up to 9 days in the future. Since the time to hatching for this population ranges from 5 to 7 days under summer conditions, the environment into which mothers lay eggs is a good

predictor of the conditions offspring will experience during embryogenesis and larval development, upon hatching, and for the first days of free-swimming life. After this initial phase, the temperature of the offspring environment becomes increasingly independent of the temperature of the maternal environment (see also Burgess & Marshall, 2011). Here, the strength of the effects of offspring and maternal thermal environment on offspring body size was similar for the first 30 days of growth, after which offspring environment had a much stronger influence. Given that maternal environment cues are reliable predictors of the early offspring environment for this population, anticipatory maternal effects on trait means associated with reproductive output are likely to evolve (Mousseau & Fox, 1998; Räsänen & Kruuk, 2007; Burgess & Marshall, 2014). Yet, variability and abrupt changes in temperature direction within the local SST profile indicate that mothers may also experience unpredictable environmental conditions (i.e. <9 days predictability) within the reproductive season, leading to the evolution of bet-hedging strategies (Einum & Fleming, 2004; Marshall *et al.*, 2008; Crean & Marshall, 2009; Burgess & Marshall, 2011; Fischer *et al.*, 2011; Simons, 2011). Switching females between experimental temperatures every 7 days corresponded to the profile of temperature changes seen in the wild, and led to greater variance in offspring size, suggesting that mothers may be capable of dynamically adjusting the variability of offspring sizes depending on environmental predictability.

#### *Maternal environment predictability and reproductive output*

Stickleback mothers allocated resources to eggs differently depending on the temperature and predictability of the environment they experienced during development and reproductive conditioning. Overall, females produced larger, but fewer eggs at 17  $^{\circ}\text{C}$  and smaller, but more eggs at 21  $^{\circ}\text{C}$ , in line with other findings of egg size plasticity in response to oviposition temperature (Bownds *et al.*, 2010; Liefting *et al.*, 2010; Shama & Wegner, 2014). Since reproductive output varied with maternal environment independent of female size, the plasticity of mean egg size shown here was not simply due to physiological constraints (Heath & Blouw, 1998), but rather, more likely reflects selection for different-sized offspring in different environments (Marshall *et al.*, 2008). If the relationship between offspring size and performance differs in each environment (Smith & Fretwell, 1974), for example, if smaller eggs at elevated temperature are advantageous due to their lower oxygen demands (Kaplan, 1992; Kolm & Ahnesjö, 2005; Bownds *et al.*, 2010), then the egg size plasticity found

here would constitute an anticipatory maternal effect or adaptive TGP (Mousseau & Fox, 1998; Marshall & Uller, 2007; Räsänen & Kruuk, 2007). Still, size-related oxygen demands will depend on the proportion of yolk vs. higher respiring embryo tissue in eggs at different temperatures (Hendry & Day, 2003), which remains to be tested for sticklebacks.

Mothers in unpredictable environments had smaller mean egg sizes and tended to have greater within-female egg size variability, especially at 21 °C (see also Morrongiello *et al.*, 2012), suggesting that mothers may have used a diversified bet-hedging strategy to spread the risk of incorrectly predicting future environmental conditions (Crean & Marshall, 2009) by producing a broader range of egg sizes (Marshall *et al.*, 2008). Developmental instability in stressful environments has been proposed as an alternate explanation for increased within-clutch size variance (Crean & Marshall, 2009), but was likely not the case here as mothers exposed to constant stress (21con, see below) had the lowest within-female egg size variance overall. Interestingly, mean egg size of 21var mothers was only slightly smaller than that of 21con mothers, despite originating from much larger clutch sizes. Optimality models predict that mean egg size should decrease for 17var mothers if smaller eggs are favoured at elevated temperature, because producing a range of egg sizes that includes smaller eggs will lower the overall mean. At 21 °C, however, the mean should increase because more, larger eggs that are favoured at 17 °C should be produced (Marshall *et al.*, 2008; Bownds *et al.*, 2010). Here, mean egg size of 21var mothers was not greater than that of 21con mothers; hence, there was only partial size compensation at elevated temperature. Nevertheless, given the much larger clutch sizes of 21var mothers, (relative) mean egg size was indeed larger. Moreover, hatching success at elevated temperature of 21var eggs did not show the same decrease relative to 21con as did eggs from 17 °C mothers, suggesting that possible bet hedging at elevated temperature led to (relatively) larger eggs that were more robust to stressful conditions in terms of hatching environment.

Among-female variability in egg size is expected to be higher than within-female variability when mothers experience predictable environments and can therefore provision offspring according to local optima, whereas within-female variability should be higher than among-female variability in unpredictable environments (Marshall *et al.*, 2008; Morrongiello *et al.*, 2012). The variability in egg size found here fits well to these predictions when mothers developed at 21 °C, but less so when mothers developed at 17 °C. Selection can act differently on the two sources of female variation depending on maternal thermal environment (Marshall

*et al.*, 2008), but this cannot be confirmed by the current data, as the study cannot make adaptive inference for the variance expression found (see below). Nevertheless, females in 17con environments had the lowest among-female variability in egg size, and females in 21con had the highest, with females in variable environments intermediate between the two. Given that prolonged exposure to 21 °C has been shown to be a stressful environment for this population having negative effects on several traits (e.g. hatching success (Shama & Wegner, 2014), growth (Schade *et al.*, 2014) and development (Ramler *et al.*, 2014), it may be that females in 21con had the greatest among-female variability in egg size because the stressful environment exacerbated individual differences among females (Charmantier & Garant, 2005). Females in 17con experienced the least thermal stress, and females in variable environments switched between stressful and non-stressful environments. In any case, females that developed at 21 °C fit well to theoretical predictions of diversified bet-hedging, indicating that stickleback mothers may use this as a strategy to cope with the dual stress of ocean warming and environmental uncertainty.

It is important to note that the results presented here demonstrate the expression of plasticity that can be adaptive and variance expression that can be interpreted as diversified bet hedging. Additional work would be needed to make adaptive inference in both cases. For instance, egg size-performance relationships at different temperatures and fitness benefits of smaller size at elevated temperature (e.g. higher reproductive success) need to be established. Similarly, whether offspring of mothers from variable environments grow or reproduce better in variable vs. constant environments could be determined (L.N.S. Shama, unpublished data). The finding of increased offspring size variation in unpredictable environments conforms to three of Simons' six criteria for evidence of bet hedging (Simons, 2011; see also Furness *et al.*, 2015). Namely, a trait showing a high degree of variance was identified (offspring size), an environmental factor that leads to habitat unpredictability was identified (varying SST), and variation in the trait of interest was expressed within the same cohort while controlling for genetic and environmental sources of variation (e.g. common garden experiment using F2 families). The other three of Simons' (2011) criteria for evidence of bet hedging could not be demonstrated here: establishment of variable fitness consequences, demonstrating the advantages of these consequences under fluctuating selection, and finding a match between bet hedging trait expression and fluctuating selection. Nevertheless, the pattern found here is in the predicted direction, but more

experiments would be needed to establish the variance expression as adaptive bet hedging.

*Managing the mean and variance of offspring size (TGP and bet hedging)*

Several recent climate change-related studies have demonstrated changes in the mean offspring phenotype as a result of TGP (reviewed in Munday *et al.*, 2013; Salinas *et al.*, 2013; Sunday *et al.*, 2014). In most cases, offspring reared in an environment matching that of their parents showed better trait performance in terms of mean response of the variable being measured. Here, offspring reared at 21 °C had larger mean body sizes if their mother also developed at 21 °C, whereas this TGP benefit was not present under ambient (17 °C) conditions, suggesting that anticipatory maternal effects will be highly relevant for climate change scenarios in this system. A similar result was found in a previous study using wild caught females that experienced acute acclimation only during reproductive conditioning (Shama *et al.*, 2014). In that study, maternal TGP effects on offspring size were strongest at elevated temperature, but were not mediated by egg size, implying that mothers were not simply producing larger or better-provisioned offspring. Rather, mothers exposed to elevated temperatures adjusted their mitochondrial metabolic capacity, and this acute acclimatory response was transferred to offspring resulting in (relatively) better growth at higher temperatures (Munday, 2014; Shama *et al.*, 2014).

In the present study, egg size plasticity in response to maternal developmental temperature resulted in smaller eggs at 21 °C, yet surprisingly, these smaller eggs grew to become larger offspring – offspring from 21 °C mothers were larger than offspring of 17 °C mothers overall – indicating that the TGP benefits on offspring growth were not egg size-mediated despite the occurrence of egg size plasticity. Paternal TGP benefits on offspring growth may have played a role (see also Crean *et al.*, 2013; Shama & Wegner, 2014), since offspring grew better in their respective paternal environment (at 60 days), especially at elevated temperature. Acclimation to elevated temperature has been shown to influence plasticity of sperm phenotype (Adriaenssens *et al.*, 2012), and methylomes – DNA methylation patterns that can regulate gene expression – are paternally inherited, as recently demonstrated in zebra fish (Jiang *et al.*, 2013). Still, the mechanism(s) underlying the larger sizes of offspring from mothers that developed at 21 °C remain unclear, and further studies are needed to determine if plasticity of mitochondrial respiration (Shama *et al.*, 2014) and/or other epigenetic marks that affect genes associated with thermal tolerance (Herman

*et al.*, 2014) underlie the TGP benefits on mean offspring size.

Similar to the pattern for egg size, offspring of variable environment mothers were smaller but more variable in size than offspring from constant environment mothers, particularly at elevated temperature. In terms of mean size, offspring of 21var mothers suffered most when reared in the nonmatching environment of their mothers (17 °C), indicating that TGP benefits at 21 °C may have offset some of the losses in mean offspring size in the unpredictable environment. In terms of increased variance in offspring size, it is likely that the effects of diversification employed at reproduction persisted into later developmental and growth stages. That the strength of these effects increased over time, especially under a warming ocean scenario, possibly indicates different mechanisms of response operating over different time spans or life history stages (see also Furness *et al.*, 2015). For instance, offspring of mothers exposed to both stressors (21var) had higher CVs of body size regardless of rearing temperature, whereas offspring of 17var mothers had higher CVs only when reared at 21 °C, indicating that the phenotypic variance resulting from possible bet hedging at reproduction by 17var mothers was only expressed under stressful conditions (Herman *et al.*, 2014). By rearing split-clutches of offspring in different environments, the test of expression of trait variance used here was actually a test of plasticity of the expression of variance. In other words, for classical bet hedging, one would expect the expression of trait variance regardless of rearing environment. The fact that variance was higher at elevated temperature indicates context-dependent expression of variance, that is, plastic expression of variance, and may be more consistent with the idea of plastic fine-tuning of a bet-hedging strategy (see also Sadeh *et al.*, 2009; Furness *et al.*, 2015), whereby responses to both maternal and offspring environments interact, resulting in a combination of diversification (by mothers) and plasticity (by offspring).

Since climate change is expected to bring an increase in environmental variability (IPCC 2012), the likelihood that populations will persist under these conditions may depend on the evolution of bet hedging (Simons, 2011). Here, stickleback mothers exposed to variable environments may have spread the risk of future environment uncertainty by producing a range of offspring sizes. At the same time, mothers exposed to predictable, but elevated temperatures primed their offspring via TGP to grow better under warmer conditions. When both predictable and unpredictable environmental variance influence offspring phenotype, and hence, fitness, both plasticity and bet hedging are expected to evolve, and diversified bet hedging may

occur around the norm of reaction as suggested here (Simons, 2011; Furness *et al.*, 2015). In this study, when the environmental cue was applied was critical in determining its effects. For instance, mean egg size 'decisions' by mothers likely developed based on lifetime or possibly early-life exposure (Donelson *et al.*, 2012; Burton & Metcalfe, 2014; Shama & Wegner, 2014), whereas the diversification of egg sizes resulted from the cue of environmental variability experienced only during reproductive conditioning. That is, egg size plasticity leading to a mean egg size that is appropriate for future environmental conditions is likely established at an early developmental stage (constant environment at this time point), but is fine-tuned later during reproductive conditioning to account for environmental variability.

Striking a balance between adaptive plasticity and bet hedging in a warming and increasingly variable climate will be necessary to optimize fitness as environmental conditions change (Simons, 2011, 2014). Here, stickleback mothers responded differently depending on whether warming alone, or warming in conjunction with increased climate variability occurred (Vasseur *et al.*, 2014). With warming alone, offspring size decreased, but TGP benefits offset some of the size losses resulting in partial size compensation. Smaller size at elevated temperature is a common finding in climate change studies (Daufresne *et al.*, 2009), but may or may not reduce fitness, as discussed above. With gradual increases to the mean temperature, many species, especially those at mid-latitudes, are expected to benefit due to lengthening of the growing season. On the other hand, increased variability could counteract any benefits due to greater magnitudes and durations of heat waves (Vasseur *et al.*, 2014). In this study, the effects of possible bet hedging at reproduction on offspring size were exacerbated by warming, that is, variance was more strongly expressed at 21 °C. Here again, TGP benefits at 21 °C offset some of the losses in offspring size in the variable environment. With only increased climate variability (17var), there was less change to mean offspring size, but context-dependent expression of variance depending on the environment offspring ultimately experienced. A greater understanding of the roles of both TGP and bet hedging will be crucial for predicting how populations will respond to climate change, and if these strategies are sufficient to buffer the ecological and evolutionary implications of warming and increasing climate variability (Crean & Marshall, 2009; Gremer & Venable, 2014).

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### Supporting Information

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

**Figure S1.** Relationship between density and body size (measured as standard length  $\pm 0.01$  mm) for *Gasterosteus aculeatus* offspring reared at 17 °C (open symbols; dashed lines) and 21 °C (closed symbols; solid lines) at (a) 30 days, (b) 60 days and (c) 90 days posthatch.

**Table S1.** Linear mixed effects models for *Gasterosteus aculeatus* coefficient of variation (CV) of body size at 30, 60 and 90 days posthatch depicting the influence of offspring rearing temperature (offspring °C), parental developmental temperatures (dam °C, sire °C) and maternal environment predictability (predictability).