

## Why do colder mothers produce larger eggs? An optimality approach

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Accepted 25 August 2010

### SUMMARY

One of the more common patterns of offspring size variation is that mothers tend to produce larger offspring at lower temperatures. Whether such variation is adaptive remains unclear. Determining whether optimal offspring size differs between thermal environments provides a direct way of assessing the adaptive significance of temperature-driven variation in egg size. Here, we examined the relationship between offspring size and performance at three temperatures for several important fitness components in the zebra fish, *Danio rerio*. The effects of egg size on performance were highly variable among life-history stages (i.e. pre- and post-hatching) and dependent on the thermal environment; offspring size positively affected performance at some temperatures but negatively affected performance at others. When we used these data to generate a simple optimality model, the model predicted that mothers should produce the largest size offspring at the lowest temperature, offspring of intermediate size at the highest temperature and the smallest offspring at the intermediate temperature. An experimental test of these predictions showed that the rank order of observed offspring sizes produced by mothers matched our predictions. Our results suggest that mothers adaptively manipulate the size of their offspring in response to thermally driven changes in offspring performance and highlight the utility of optimality approaches for understanding offspring size variation.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/213/22/3796/DC1>

Key words: egg size, maternal effect, offspring size, plasticity.

### INTRODUCTION

Offspring size is a fundamentally variable trait with important consequences for both mothers and their offspring. Among species, populations, individuals and broods, offspring size is highly variable, and for over 60 years biologists have sought to understand the selective forces that drive this variation (Bagenal, 1969; Einum and Fleming, 2002; Lack, 1947; Marshall and Keough, 2008; Williams, 1994). A common pattern in offspring size variation is the relationship between offspring size and temperature – mothers produce larger offspring at lower temperatures across a wide variety of taxa in both terrestrial and aquatic environments (Atkinson et al., 2001; Azevedo et al., 1996; Blanckenhorn, 2000; Christians, 2002; Fischer et al., 2006; Fischer et al., 2003a; Fischer et al., 2003b; Marshall et al., 2008a; Van der Have and de Jong, 1996; Voorhies, 1996). The prevalence of this relationship raises a fundamental question: why do lower temperatures often result in the production of larger offspring?

Theory predicts that mothers should produce offspring of a size that maximises maternal fitness (Smith and Fretwell, 1974). Because mothers have a finite amount of resources available for reproduction, they can make either many small offspring or fewer large offspring. According to theory, mothers should make smaller offspring if the relationship between offspring size and offspring performance is shallow – because large increases in offspring size yield little fitness return for mothers, they should maximise their own fitness by producing very small (and therefore, more numerous) offspring (Smith and Fretwell, 1974). Conversely, if the relationship between offspring size and performance is very steep, the fecundity costs of producing larger offspring are more than offset by the fitness benefits, such that mothers should produce fewer, larger offspring. The relationship between offspring size and performance therefore determines the optimal offspring size, i.e. the size that perfectly

balances fecundity and performance (McGinley et al., 1987). Because the environment into which an offspring is released will strongly affect the offspring size–performance relationship (and thus the optimal offspring size), theory predicts that mothers should alter the size of their offspring according to local environmental conditions (McGinley et al., 1987). There is good empirical support for this prediction. For example, seed beetles produce larger offspring when they can only lay eggs on well-defended food sources, but produce smaller offspring when food sources are poorly defended (Fox et al., 1997). Similarly, when the sessile marine invertebrate *Bugula neritina* is exposed to high levels of intraspecific competition, mothers produce larger, more competitive offspring (Allen et al., 2008). Such changes in offspring size in response to different environmental conditions have been termed ‘transgenerational phenotypic plasticity’ or ‘anticipatory maternal effects’ (Agrawal, 2001; Marshall and Uller, 2007). In a range of organisms, transgenerational plasticity in offspring size does appear to occur in response to changes in temperature, but it is unclear whether this plasticity is adaptive. Several studies have exposed mothers to different thermal regimes and examined offspring performance, with mixed results. For example, Blanckenhorn found that offspring do not perform better when placed in the same temperature regime as their mothers (Blanckenhorn, 2000), but others have found offspring do perform better in the same regime as their mothers (Fischer et al., 2006; Fischer et al., 2003a; Landa, 1992).

With regards to offspring size, selection acts to maximise maternal rather than offspring fitness, so it is important to understand the fitness benefits of any allocation strategy from the perspective of the mother (Bernardo, 1996; Einum and Fleming, 2000). In an adaptive framework, mothers should only produce larger offspring under lower temperatures if the optimal offspring size is larger at these temperatures (Marshall and Uller, 2007). Long-term,

experimental evolutionary studies have been used to great effect to understand the adaptive significance of offspring size variation under different temperature regimes (Angilletta, 2009), but such studies are not feasible for many organisms and so alternative approaches are required. One such approach is to use optimality models. Optimality models combine offspring size–number trade-offs with empirically estimated offspring size–performance relationships to predict the optimal offspring size that mothers should produce. Optimality models have the advantage of estimating maternal fitness rather than offspring fitness (Marshall and Uller, 2007), and have been used to great effect in other situations to understand the selection pressures acting on mothers (Einum and Fleming, 2000; Marshall et al., 2006). In a recent review of the incidence of small offspring at higher temperatures, Angilletta noted that current theory depends ‘greatly on the underlying assumption about the relationship between the size and performance of offspring’ under different temperatures, but that data on such relationships were lacking (Angilletta, 2009). No studies have attempted to model for optimal offspring size under different thermal conditions or test the resulting predictions. This lack of optimality studies is surprising given there are a number of reasons to suspect that temperature will strongly affect the relationship between offspring size and performance (Angilletta, 2009; Woods, 1999). For example, because both developmental rate (and, thus, temperature) and egg size can affect size at hatching, the relative of benefits of small and large eggs can change with temperature (Kaplan, 1992).

Here, we examined the effect of temperature on the relationship between egg size and subsequent offspring performance (estimated as fertilisation success, hatching success, hatchling length and swimming velocity) in the zebra fish, *Danio rerio*. We used our estimates of the offspring size–performance relationship at different temperatures to produce a simple offspring size optimality model to predict the optimal offspring size at different temperatures. We then tested the model predictions by placing mothers into different thermal environments (which corresponded to those temperatures used in our first experiment) and determined whether mothers changed the size of their offspring in accordance with our model predictions.

## MATERIALS AND METHODS

### Experimental animals

Adult zebra fish (*Danio rerio*, Hamilton 1822) were obtained from a commercial aquarium supplier and maintained in 401 aquaria in groups of 10 females and 3 males. All fish were fed daily with a commercial flake food and maintained on a 14h:10h light:dark cycle. To maximise the number of viable eggs obtained from females in each experiment, males were removed from the communal aquaria 4 days before spawning was attempted to prevent predation. Hisaoka and Firlit reported a spawning interval of 5–10 days as optimal for egg viability (Hisaoka and Firlit, 1962). We measured a variety of performance traits as components of fitness, including: fertilisation success, hatching success, hatchling length and swimming velocity (the maximum swimming speed of the larvae as a measure of swimming performance). Both fertilisation and hatching success are direct measures of viability, and thus highly relevant and commonly measured components of fitness. Both hatchling size and swimming velocity are commonly estimated ecologically important measures of performance as they predict survival during encounters with predators (Watkins, 1996; Miles, 2004; Walker et al., 2005; Husak, 2006).

All experiments were approved by the University of Queensland Animal Welfare and Ethics committee.

### Experiment 1: effect of temperature on offspring size and performance

Females were transferred from their communal aquaria into isolated spawning tanks 1 day before spawning. Each spawning tank contained two layers of glass marbles to prevent adults cannibalising eggs (Laale, 1977; Hisaoka and Battle, 1958). Males were placed into the spawning tanks on the afternoon before spawning to stimulate the production and transfer of pheromonal cues necessary for oviposition (Gerlach, 2006). *Danio rerio* spawn in response to light (Hisaoka and Firlit, 1962; Laale, 1977), and are estimated to spawn within 15–30 min of daybreak (Eaton and Farley, 1974). Therefore, spawning pairs were removed from spawning containers after 30 min of initial morning light. Females were photographed using an Olympus  $\mu$ 780 digital camera. Standard length (from the tip of the snout to the origin of the caudal fin, also called the peduncle) was measured from these photographs using digitising software (SigmaScan 8.0, www.sigmascan.org). Female mass was recorded using an electronic balance ( $\pm 0.001$  g). The newly spawned eggs were pipetted onto a Petri dish containing fresh water after carefully removing the glass marbles lining the spawning containers. Eggs were photographed under a dissecting microscope using PixelINK Capture SE (version 1.0; www.pixelink.com) at 1.5 $\times$  magnification and individually measured from photos using SigmaScan 8.0. Total clutch size was recorded as the number of eggs spawned, and egg size was measured as yolk diameter, which is a commonly used proxy for the energetic content of eggs (Bernardo, 1996; Duarte and Alcaraz, 1989; Elgar, 1990; Fischer et al., 2006).

The equatorial diameter of each egg was measured three times and the average of these measures was recorded as egg size. After fertilisation, the chorion of *D. rerio* eggs is spherical; however, the egg cell itself is ellipsoidal (Hisaoka and Battle, 1958). All eggs were photographed before the gastrula stage as after this time the blastoderm commences to overgrow the yolk mass (Hisaoka and Battle, 1958). Before this stage, there is no change in the shape or size of the yolk mass, as cell division occurs only in the blastodisc at the animal pole. As is the case for most teleostean eggs, the yolk does not undergo any division (Hisaoka and Battle, 1958), hence the equatorial diameter of the yolk is a suitable measure of the maternal investment in the eggs. Seventy-two eggs from each female were photographed and divided between three 24-well plates with each well filled with 1 ml of water. One plate was placed into each of three water baths set at 20°C, 25°C and 30°C. These temperatures represent the lowest, intermediate and highest temperatures that *D. rerio* would experience under natural conditions (Hisaoka and Battle, 1958). The temperature of the water baths was maintained using standard aquarium heaters and air was bubbled into the baths to circulate the water. The developing embryos remained at the respective incubation temperature for the duration of development (maximum of 5 days).

*Danio rerio* eggs develop rapidly and take only 96 h from fertilisation to hatching when incubated at 26°C (Hisaoka and Battle, 1958). At 30°C, embryos hatched as early as 36 h post-fertilisation, and the developing embryos in each treatment were checked for hatching after this period. Eggs incubated at 20°C did not begin hatching until 4–5 days post-fertilisation. Once hatching started in a treatment, hatched larvae were pipetted into a Petri dish containing fresh water at their treatment temperature and photographed under a dissecting microscope using PixelINK Capture SE (version 1.0) at 1.5 $\times$  magnification as above. Standard length of each fish was measured using SigmaScan 8.0. The larvae were then pipetted back into the wells and returned to the water bath to continue development. After the larvae had absorbed all of their yolk sac,

we remeasured them to produce a 'size at yolk absorption' measure. The fish were photographed and standard length measured using the method described above. Larvae were not fed to ensure that any growth resulted from the consumption of energy stored in the residual yolk sac.

Swimming velocity was assessed by filming three startle responses using a high-speed digital camera (Redlake Imaging Corporation, Tucson, AZ, USA). Startle responses were elicited by directing a fine hair at the caudal region of the larvae, a method successfully used previously for *D. rerio* larvae (Thorsen et al., 2004). Each startle response was characterised by an initial C-start that results in the simultaneous contraction of all the muscle fibres on one side of the body (Kimmel et al., 1972). Swimming sequences were filmed in a 10 cm diameter Petri dish at 200 Hz by recording the image off a mirror that was suspended at 45 deg above the aquarium. The temperature of the Petri dish was maintained at each larva's incubation temperature by partially submersing it in a 30 l aquarium that was temperature controlled using an aquarium heater. The Redlake software package was used for analysis of the first 45 ms of a startle response – the first frame being the one immediately preceding the first detected movement. The centre point of each larva's head was digitised frame-by-frame to obtain the total distance travelled and swimming velocity over the 15 frames analysed (Condon and Wilson, 2006). The fastest of three sequences analysed was defined as an individual's maximum swimming performance.

#### Experiment 2: effect of temperature on maternal investment

Communal groups of 10 females and 3 males were acclimated to 20°C, 25°C or 30°C for 6 weeks. For each treatment, three replicate aquaria were kept under the same light and feeding regime as adults in experiment 1. After 6 weeks, all males were removed from each aquarium 4 days before spawning the females. Spawning containers were submersed in large water baths that were heated to the desired temperature (the temperature at which the female had been acclimated) using standard aquarium heaters ( $\pm 0.5^\circ\text{C}$ ). Each morning, within 1 h of day break, spawning pairs were removed and the containers checked for eggs. All eggs were photographed before the gastrula stage. If a pair had spawned, the female was photographed to obtain standard length (as described above) and mass was recorded using an electronic balance ( $\pm 0.001\text{ g}$ ). Total clutch size was recorded for successfully spawned females and 30 eggs from each clutch were photographed, as in experiment 1. Pairs that did not spawn were also removed from spawning containers, the water replaced and the female returned along with another randomly allocated male. Males can spawn repeatedly across multiple days (Eaton and Farley, 1974) and so all males, including those that had spawned that morning, were pooled and randomly allocated to a new female each day. Males were randomly distributed between the spawning temperatures and placed into spawning containers before nightfall to ensure time for the appropriate pheromones to be released and interactions to occur (Gerlach, 2006). As *D. rerio* have a spawning interval of between 2 and 9 days (Eaton and Farley, 1974), females were given 11 opportunities to spawn with a randomly selected male for each day.

#### Data analysis

To examine the effects of egg size on fertilisation success and hatching success at different temperatures, we used logistic regression with egg size as a continuous predictor. To examine the effects of egg size on hatchling length at different temperatures, we used analysis of covariance (ANCOVA), with egg size as a continuous predictor and

temperature as a categorical, fixed factor. We also used ANCOVA to test for the effects of egg size on hatchling swimming velocity and included size at yolk absorption as a covariate because this factor explained significant levels of variation.

Throughout our analyses of the relationship between egg size and offspring performance, we included 'maternal identity' as a random factor. However, there was no significant interaction between maternal identity and the factors of interest and, as such, interactions between maternal identity and egg size were excluded as a factor from the final analysis (Quinn and Keough, 2002).

To analyse the effects of temperature on egg size, mean egg size from each female was the unit of replication. After first testing for the random effects of aquaria temperature and measurement date (the main effects and interactions of each were non-significant), we ran a reduced model with temperature as a fixed factor.

#### Optimality model

We modified an existing optimality model to examine differences in the predicted optimal offspring size among experimental environments (see Marshall and Keough, 2008). Our model used our estimates of the significant effects of offspring size on fertilisation success, hatchling size and swimming velocity (see supplementary material Table S1), and we varied offspring size across a range of values encompassing those observed. Like previous models (e.g. Smith and Fretwell, 1974), ours incorporated a trade-off between offspring size and number:

$$N = \frac{M}{s}, \quad (1)$$

where  $N$  is the number of offspring produced by a mother with  $M$  resources (an arbitrary value kept constant throughout) and  $s$  is offspring size (estimated as egg size). To predict the fertilisation success ( $B$ ) of an egg of a given size ( $s$ ), we used:

$$B = \frac{e^{(\alpha s + \beta)}}{1 + e^{(\alpha s + \beta)}}, \quad (2)$$

where the constants  $\alpha$  and  $\beta$  were generated from a logistic regression of egg size on subsequent fertilisation. The relationship between egg size ( $s$ ) and subsequent performance ( $P$ ) was modelled as:

$$P = (\gamma s + \delta) (\kappa s + \lambda), \quad (3)$$

where the constants  $\gamma$  and  $\delta$  were generated from a linear regression of egg size on hatchling length and  $\kappa$  and  $\lambda$  were generated from a linear regression of egg size on swimming velocity. We generated these estimates for each thermal environment and so we were able to explore the impact of different temperatures on predictions of optimal offspring size. We combined Eqns 1–3 to estimate maternal fitness ( $\Psi$ ) as:

$$\Psi = NBP. \quad (4)$$

To calculate optimal offspring size, we then determined the offspring size at which maternal fitness was maximised for each performance metric of interest.

## RESULTS

### Fertilisation

There was no significant relationship between egg size and fertilisation success at 20 or 25°C (20°C:  $\chi^2=1.48$ ,  $P=0.223$ ; 25°C:  $\chi^2=0.82$ ,  $P=0.364$ ), but there was a significant, negative relationship between egg size and fertilisation success at 30°C ( $\chi^2=6.39$ ,  $P=0.0115$ ): larger eggs were much less likely to be successfully fertilised at this temperature (Fig. 1).

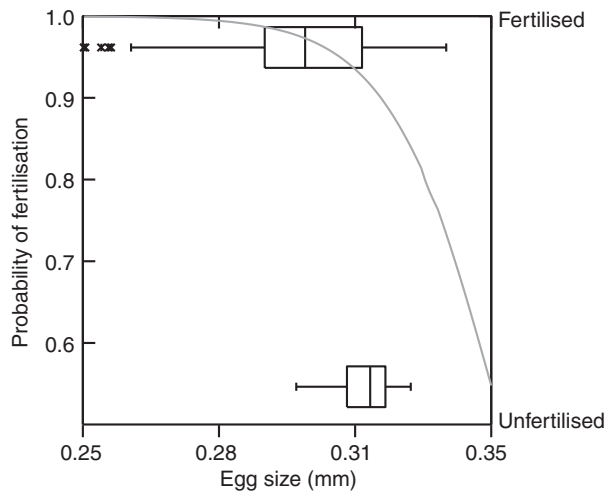


Fig. 1. Effect of egg size on fertilisation success in *Danio rerio* at 30°C. Probability of fertilisation is the line of best fit for a logistic regression. The box plots represent the distribution of sizes of eggs that were successfully fertilised (top box) and those that were not fertilised (bottom box).

#### Hatching success

There was no significant relationship between egg size and hatching success at any of the temperatures tested, although there was a trend for larger eggs to have lower hatching success at 30°C (20°C:  $\chi^2=0.028$ ,  $P=0.866$ ; 25°C:  $\chi^2=0.937$ ,  $P=0.332$ ; 30°C:  $\chi^2=2.848$ ,  $P=0.091$ ).

#### Hatching size

There was a strong interaction between temperature and egg size on hatchling length: the strongest relationship between egg size and hatchling length occurred at the lowest temperature and the weakest relationship occurred at the highest temperature (Fig. 2, Table 1).

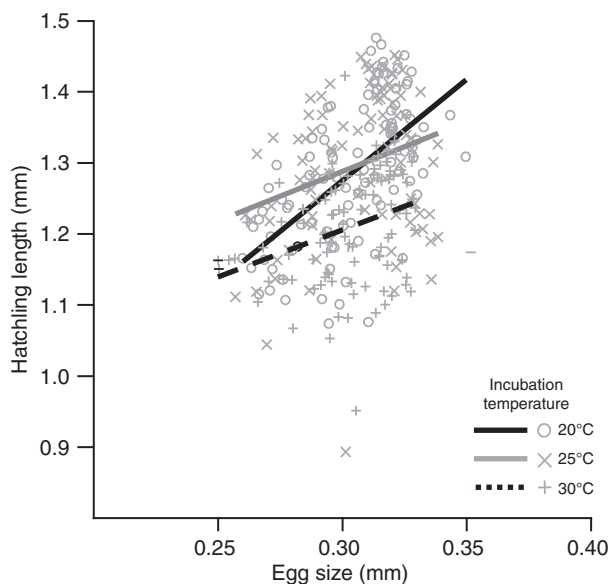


Fig. 2. Effect of egg size and temperature on hatchling length in *D. rerio*. Each point represents a single egg and lines of best fit are shown for each temperature.

Table 1. Effect of temperature and egg size on hatchling length in *Danio rerio*

| Source                        | d.f. | Mean square | F-ratio | P       |
|-------------------------------|------|-------------|---------|---------|
| Egg size                      | 1    | 0.0853      | 17.88   | 0.0000  |
| Temperature                   | 2    | 0.0313      | 6.56    | 0.0016  |
| Maternal identity             | 5    | 0.1628      | 34.16   | <0.0001 |
| Temperature $\times$ egg size | 2    | 0.0384      | 8.05    | 0.0004  |
| Error                         | 286  | 0.0048      |         |         |

Note that model is reduced after testing for non-significant random effects.

#### Size at yolk absorption

The egg size  $\times$  temperature  $\times$  size at yolk absorption interaction ( $F_{2,233}=0.2348$ ,  $P=0.791$ ) was not significant and so was removed from the model. We found a strong, significant interaction between egg size and temperature, and size at yolk absorption and temperature, but no interaction between egg size and size at yolk absorption (Table 2).

#### Swimming velocity

When we further explored the interactions between temperature and the variables measured using multiple regression within each temperature treatment, we found a positive effect of egg size on swimming velocity in both the 20°C ( $F_{2,88}=4.633$ ,  $P=0.0122$ ) and 30°C ( $F_{2,73}=5.62$ ,  $P=0.0053$ ) treatments, but a negative effect of egg size on swimming velocity in the 25°C treatment ( $F_{2,74}=40.85$ ,  $P<0.001$ ; Fig. 3).

#### Predicted optimal offspring size and observed offspring size

Our simple optimality model predicted that mothers kept at 20°C should produce the largest offspring possible ( $>0.35$  mm), mothers kept at 25°C should produce the smallest offspring possible ( $<0.2$  mm) and that mothers kept at 30°C should produce offspring of intermediate size (0.3 mm).

We found that temperature did have a strong, significant effect on egg size ( $F_{2,26}=7.44$ ,  $P=0.0028$ ) and that egg size closely matched the optimal size predicted across the three temperature treatments – at 20°C mothers produced the largest eggs, at 25°C mothers produced the smallest eggs and at 30°C eggs were slightly larger than those produced by mothers at 25°C (Fig. 4). While the observed offspring sizes were not precisely what were predicted, overall the rank order of observed and predicted offspring sizes was the same.

#### DISCUSSION

We found that the effects of egg size on offspring performance were variable across life-history stages and depended on the thermal

Table 2. Effect of egg size, yolk size and temperature on swimming velocity in *Danio rerio*

| Source                                       | d.f. | Mean square | F-ratio | P       |
|--|------|-------------|---------|---------|
| Egg size                                     | 1    | 4707        | 2.69    | 0.102   |
| Size at yolk absorption                      | 1    | 24569       | 14.04   | <0.0001 |
| Temperature                                  | 2    | 2882        | 1.64    | 0.194   |
| Temperature $\times$ egg size                | 2    | 12015       | 6.86    | 0.001   |
| Temperature $\times$ size at yolk absorption | 2    | 11555       | 6.60    | 0.001   |
| Error  | 235  | 1749        |         |         |

Note that the model is reduced after testing for homogeneity of slopes.

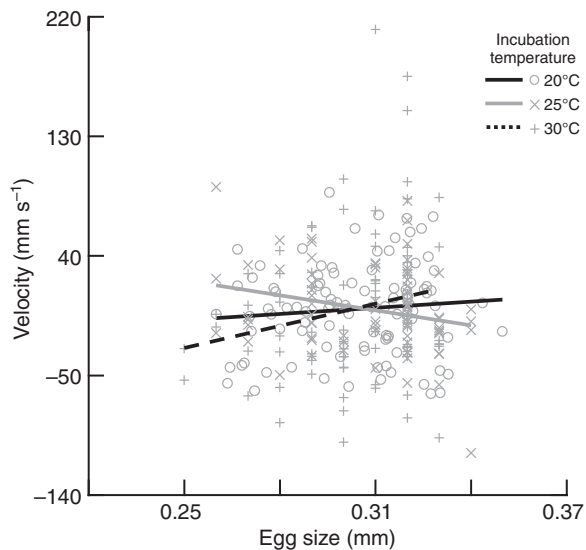


Fig. 3. Effect of egg size and temperature on swimming velocity in *D. rerio*. The y-axis shows residuals after removing the effect of size at yolk absorption on swimming velocity so as to show the effect of egg size alone. Each point represents a single egg and lines of best fit are shown for each temperature.

environment that offspring experienced. We also found evidence for anticipatory maternal effects (Marshall and Uller, 2007): mothers adjusted the size of their offspring in response to local thermal conditions, and this variation in offspring size corresponded to the rank order of predicted optimal offspring sizes in each thermal environment. Our findings suggest that, in *Danio rerio* at least, mothers adaptively adjust the size of their offspring in response to thermally driven changes in the offspring size–performance relationship.

We found that larger eggs suffered lower fertilisation success (defined here as the proportion of eggs that began developing) at the highest temperature in our study. In marine invertebrates with external fertilisation, larger eggs offer larger targets for sperm, so at high sperm concentrations, larger eggs can suffer higher levels of polyspermy (i.e. eggs become unviable when they are entered by multiple sperm simultaneously) (Marshall and Keough, 2003; Marshall et al., 2002). Therefore, polyspermy may have caused the reduction in fertilisation success of larger eggs and this would be compounded at higher temperatures because sperm typically swim faster at higher temperatures (Servedio, 2001), increasing the rate of contact between eggs and sperm (Vogel et al., 1982). The lower levels of fertilisation observed in larger eggs at higher temperatures may also have been due to developmental failures immediately after fertilisation but before cell cleavage. At this point in development larger eggs are more sensitive to higher temperatures, although there is mixed empirical support for such an effect (Einum et al., 2002; Woods, 1999). Whatever the mechanism for the observed effect, it is clear that there is a fitness cost to *D. rerio* mothers when they make larger offspring at higher temperatures because fertilisation success will be lower.

Some of the observed relationships between offspring size and our measures of offspring performance in the different thermal environments were unexpected. Temperature-mediated developmental plasticity appears to be present in most ectotherms (Fischer et al., 2006) and, here, we found the relationship between egg size and hatchling size was mediated by temperature but not in

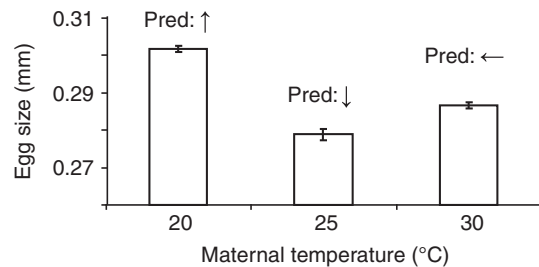


Fig. 4. Effect of temperature on the mean ( $\pm$ s.e.) size of eggs that *D. rerio* mothers produced. Arrows indicate prediction of optimality model based on our findings from experiment 1. Up arrow indicates maternal fitness is predicted to be maximised by producing the largest offspring size, down arrow indicates maternal fitness is predicted to be maximised by producing the smallest offspring and sideways arrow indicates maternal fitness is predicted to be maximised by producing offspring of intermediate size.

the stepwise fashion found elsewhere. An increase in egg size yielded a much larger increase in hatchling size at the lowest temperature (20°C) relative to the two higher temperatures (25°C and 30°C), but the shallowest egg size–hatching size relationship was at the intermediate, rather than at the highest, temperature. Our data suggest that the development of larger eggs is more efficient at lower temperatures, yielding much larger hatchlings for each increase in egg size, and it may be that the energy reserves in eggs are preferentially allocated to body construction rather than maintenance at lower temperatures in this species [but see Wapstra for a contrasting view (Wapstra, 2000)].

The variability in the effects of egg size on swimming velocity was also unexpected. While swimming velocity increased with egg size at the highest and lowest temperatures, swimming velocity decreased with egg size at the intermediate temperature. It has been shown that temperature can have strong effects on muscle development and muscle cell fates in fish (Johnston and Hall, 2004), but we are unaware of any studies that have specifically examined the interplay of egg size and temperature on muscle development. Regardless of the mechanisms responsible, it appears that the relationship between offspring size and subsequent performance (in terms of both hatchling size and swimming velocity) is highly mutable and context dependent in *D. rerio*. This context dependence of the offspring size–performance relationship means that the fitness benefits of maternal investment strategies will also be highly context dependent (Allen et al., 2008), and our results suggest that mothers provision their offspring accordingly.

We found that for two offspring performance measures (fertilisation success and swimming velocity), the relationship between offspring size and performance went from positive or absent to negative in some thermal environments. Other studies have found similar effects. For example, Kaplan found that at lower temperatures, tadpoles from larger eggs performed better than tadpoles from smaller eggs but at higher temperatures, larger tadpoles were more likely to suffer predation because greater amounts of yolk diminished their locomotor ability (Kaplan, 1992). We found that the offspring size–performance relationship changed from positive to negative depending on the temperature, and this presents a significant challenge to *D. rerio* mothers when allocating resources to offspring. A recent modelling study predicted that, when there are risks of producing offspring that are too small or too large for the local environment, mothers should employ a bet-hedging strategy and produce a broader range of offspring sizes (Marshall et al., 2008b). Whether such a strategy would be favoured here

depends strongly on the ability of mothers to anticipate the thermal environment of their offspring, as shown for other species (Crean and Marshall, 2009; Marshall et al., 2008b).

We found a high level of concordance between the predicted optimal offspring size and the rank order of the observed offspring sizes. Our model predicted that maternal fitness would be maximised by producing the smallest offspring at the intermediate temperature, the largest offspring at the lowest temperature and offspring of intermediate size at the highest temperature, and our experimental results match these predictions. It is important to note that our model predicted quantitatively different offspring sizes from those observed. Such imprecision is inevitable in studies such as ours where offspring fitness is only estimated using components of fitness and so the rank order of the predictions is more informative than the precise values themselves (Marshall and Keough, 2008; Marshall et al., 2008a). Interestingly, the effects of temperature on offspring size were non-linear. Our study suggests that mothers adaptively adjust the size of their offspring according to the local thermal conditions in order to increase their own fitness – the first such finding using an optimality modelling approach. This study joins a growing list showing that temperature-mediated changes in offspring size are due to adaptive plasticity effects (Angilletta et al., 2006; Fischer et al., 2006; Fischer et al., 2003a; Fischer et al., 2003b; Landa, 1992; Seko and Nakasuji, 2006) and that mothers exhibit adaptive plasticity in offspring size in response to environmental change more generally (Agrawal, 2001; Allen et al., 2008; Crean and Marshall, 2008; Fox et al., 1997).

#### ACKNOWLEDGEMENTS

We thank C. Cook, S. Burgess, R. Allen, S. Perry, E. Wapstra and one anonymous reviewer for helpful comments on the manuscript.

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Table S1. Appendix of estimates of effects of offspring size on various performance parameters across different temperatures for *Danio rerio*

| Measure          | Slope    | s.e.  | Intercept |
|------------------|----------|-------|-----------|
| Fertilisation    |          |       |           |
| 20°C             | 0        | n.a.  | 8.12      |
| 25°C             | 0        | n.a.  | 5.48      |
| 30°C             | -74.5437 | 35.94 | 26.27     |
| Hatchling length |          |       |           |
| 20°C             | 2.85     | 0.40  | 0.41      |
| 25°C             | 1.38     | 0.51  | 0.87      |
| 30°C             | 1.32     | 0.39  | 0.80      |
| Velocity         |          |       |           |
| 20°C             | 275      | 228   | 50        |
| 25°C             | -503     | 203   | 49.72     |
| 30°C             | 935      | 389   | -119.06   |