Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality

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The magnitude of fitness variation caused by maternal effects and, thus, the adaptive significance of maternal traits may depend on environmental quality, generating crossing reaction norms among offspring phenotypes that shape life-history evolution. By manipulating intraclutch variation in egg size and comparing siblings we examined the maternal effects of egg size on offspring performance and tested for the existence of reaction norms to environmental quality using the brown trout *Salmo trutta*. When sibling groups of small and large eggs were reared separately in a hatchery environment initial size differences disappeared rapidly. However, in semi-natural environments and under direct competition, juveniles from large eggs experienced growth and survival advantages over siblings from small eggs. Moreover, distinct reaction norms existed, with the differences in performance of juveniles from small and large eggs being most pronounced in the poorer growth environments. Our results provide the first direct evidence, to our knowledge, for a causal relationship between egg size and fitness-related traits in fishes, independent of potentially confounding genetic effects. Moreover, they indicate that previous studies have been biased by experimental conditions that excluded competitive asymmetries and environmental variability. The existence of reaction norms indicates a shift in optimal egg size across gradients of environmental quality that probably shapes the evolution of this trait.

Keywords: maternal effects; reaction norms; phenotypic plasticity; selection; evolution; egg size

1. INTRODUCTION

The adaptive significance of maternal traits is being increasingly recognized and it is suggested that they are shaped by natural selection to act as a mechanism for an adaptive phenotypic response to environmental heterogeneity (Mousseau & Fox 1998). For example, maternal influences on offspring size may translate into variation in offspring fitness (reviewed by Roff 1992; Mousseau & Fox 1998), with the shape of this relationship determining the optimal solution to the size–number trade-off (Smith & Fretwell 1974). However, the relationship is unlikely to be static with additive properties (reviewed by Rossiter 1998). Rather, it may be conceptually similar to genotype–environment interactions, with the relative performance of different offspring sizes depending on environmental conditions and resulting in different slopes of reaction norms. The effects of interactions between offspring size and environment on fitness are a basic assumption of many optimality models for the evolution of offspring size (e.g. Smith & Fretwell 1974; Parker & Begon 1986; McGinley *et al.* 1987; Sargent *et al.* 1987). As such, knowledge about the shapes of these relationships is critical to understanding their potential impacts on life-history evolution.

In fishes, egg size has been suggested to affect traits such as size at hatching and offspring survival (reviewed by Reznick 1991; Roff 1992). Yet, whenever these effects have been observed, they tend to be transient and, therefore, have been believed to be limited to the initial period following hatching (reviewed by Heath & Blouw 1998). The effects of egg size, however, are likely to depend on where on the environmental scale tests are performed, reflecting the existence of distinct reaction norms. For example, if the effects of egg size are only manifested in competitive environments, probably the conditions under which such traits evolve, observations in benign environments may not reveal any information about optimal values. Indeed, when testing for the effects of egg size, different groups have usually been reared separately and fed to satiation in artificial environments (cf. Reznick 1991). Furthermore, no attempts have been made to control for the potential influence of genetic or non-genetic variation in other traits, although this is crucial to understanding the evolutionary dynamics expected from the phenotype–fitness relationship (Lande & Arnold 1983; Price *et al.* 1988; Sinervo & Doughty 1996). It thus remains unclear how well previous studies reflect the direction and strength of evolutionary forces shaping egg size in natural fish populations (Reznick 1991).

We therefore undertook a study to examine the effects of egg size on offspring fitness using the brown trout (*Salmo trutta*). Egg size was manipulated by artificially

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rearing their parents to adulthood, a procedure that can result in some females producing more variable egg sizes than in the wild. Such variability is related to the position of the egg relative to blood vessels in the female ovary during development and appears to be independent of genetic or other characteristics of the oocytes themselves (Mejen (1940) cited in Kamler (1992)). Incubation time and growth and survival following emergence from gravel nests were then examined in pairs of full sib groups which differed only in egg size to test directly for a causal relationship between egg size and offspring fitness and for interactions with environmental quality.

2. MATERIAL AND METHODS

Eight female and eight male brown trout, reared to maturity in captivity at the Norwegian Institute for Nature Research (NINA) Research Station, south-western Norway (58°59'N, 5°58'E), were artificially stripped. The females were chosen based on having pronounced intraclutch variation in egg size (range in clutch variation = 8.1–18.8%, cf. 2.8–4.7% for wild females; I. A. Fleming, unpublished data). To test for correlations between egg mass, chemical composition and energy content, ten eggs were sampled from each female prior to fertilization and analysed according to Berg & Bremset (1998).

To produce paired groups of differently sized but on average genetically identical eggs, equal samples (approximately 100–200) of small and large eggs were obtained from each female. Small eggs were on average 23.3% lighter than their large siblings (mean $\pm$ s.d. small eggs 60.5 ± 8.1 mg and large eggs 78.9 ± 6.3 mg). The paired groups from one female were then fertilized by one male, producing a total of eight pairs.

(a) *Timing of hatching and emergence*

To test for the effects of egg size on the egg development rate, samples of five eggs from the small and large size egg groups were taken at the eyed stage to record the date of hatching. Eggs from two of the females had experienced high mortality during earlier stages and were therefore not sampled in order to ensure sufficient numbers of juveniles for later parts of the study.

Then, to test for differences in emergence time and subsequent performance, equal numbers of small and large eggs within a pair were buried in one of 16 artificial gravel nests consisting of a Plexiglas cylinder, 25 cm high and 20 cm in diameter. At the onset of emergence, emerging juveniles were removed and counted once a day.

(b) *Semi-natural environment*

To test for the effects of egg size on juvenile performance in semi-natural environments, juveniles emerging from the artificial nests were weighed (± 0.1 mg) and measured (total length ± 0.5 mm) before being marked and released into one of 24 enclosures. The enclosures were 42 cm $\times$ 32 cm $\times$ 25 cm transparent plastic containers, with the front and back walls replaced with 2 mm mesh screens and the bottoms covered with 5 cm of gravel. These were placed in semi-natural streams with natural invertebrate communities providing food for the juvenile fishes (described in Fleming & Einum 1997).

Each enclosure contained juveniles from a single female, half originating from small and half from large eggs. Three enclosures were established for each female, having densities of eight, 16 and 24 uniquely marked (alcian blue dye) juveniles per enclosure, corresponding to low (168 cm² per fish), medium

(84 cm² per fish) and high (56 cm² per fish) densities, respectively (Elliott 1990). The different enclosures were filled successively during 26 April–14 May, with the first four emerging juveniles from both size groups being released into the low-density environment, the next eight into the medium-density environment and the next 12 into the high-density environment (on average all enclosures of each female were filled within two days of each other). This experiment was discontinued on 25–28 May, at which time the fishes had spent, on average, 18 days in the enclosures. All fishes were then reweighed and remeasured. The mean temperature during the experiment was 11.9 °C (range 7.5–16.5 °C).

(c) *Hatchery control*

To test for the effects of egg size on juvenile performance under hatchery conditions, five emergent juveniles from each nest of six of the females (the two females with high mortality during incubation were excluded) were anaesthetized, weighed, individually marked and placed into 31 tangential flow stream tanks, with juveniles from small and large eggs in separate tanks. These were then fed commercial food pellets (EWOS) *ad libitum* and were weighed again after 12–15 days. The mean temperature during the experiment was 13.6 °C (range 10.4–16.5 °C).

(d) *Statistical analysis*

Fish growth rates are influenced by body mass and temperature (e.g. Wootton 1990; Elliott & Hurley 1995). Thus, directly using observed growth rates as a measure of competitive performance introduces a bias when comparing groups of fishes that differ in initial body mass or temperatures experienced. The specific growth rate for each individual was therefore adjusted to a common body mass according to Elliott & Hurley (1995) and Elliott *et al.* (1995). Furthermore, to control for variation in the temperatures experienced among individuals, growth rates were transformed to a growth index, $G_{\text{obs}}/G_{\text{max}}$, where G_{obs} is the observed specific growth rate (Wootton 1990) and G_{max} is the maximum potential growth rate for the fish at the given body size and temperature regime. G_{max} was calculated as

$$G_{\text{max}} = \frac{\ln W_{\text{max}} - \ln W_0}{t} \times 100, \quad (1)$$

where W_0 is the initial observed body mass and W_{max} is the body mass attained after a period t when growing at the maximum potential rate. W_{max} was calculated by applying Elliott *et al.*'s (1995) model for maximum growth:

$$W_{\text{max}} = \left[W_0^b + bc \frac{(T - T_{\text{lim}})t}{\{100(T_{\text{M}} - T_{\text{lim}})\}} \right]^{1/b}. \quad (2)$$

The allometric constant b describes the change in growth rate with body mass ($b = 0.308$ for brown trout; Elliott *et al.* 1995) and c is the growth rate of a 1 g fish at the optimum temperature ($c = 5.28$ for our population (known as Tunhovd); Anonymous 1998). $T_{\text{lim}} = T_{\text{L}}$ if $T \leq T_{\text{M}}$ or $T_{\text{lim}} = T_{\text{U}}$ if $T > T_{\text{M}}$, where T_{L} and T_{U} are the lower and upper temperatures for growth and T_{M} is the temperature for optimum growth. For our population, these are given as 2.76, 27.76 and 17.42 °C, respectively (Anonymous 1998).

Daily mortality rates were calculated following Wootton (1990). To examine changes in body mass and length at emergence caused by selection in the semi-natural environment, univariate selection differentials (s) were calculated separately for each female as $\bar{z}_s - \bar{z}$, where $\bar{z}_s$ and $\bar{z}$ are the mean values

Figure 1. Mean body mass $\pm$ s.d. of juvenile brown trout at emergence (initial) and after growth experiments (end). Siblings originating from small (filled bars) and large (open bars) eggs were reared separately for 12–15 days in stream tanks (hatchery, $n = 6$ females) or in competition for 18 days in semi-natural environments (stream enclosures, $n = 8$ females). Differences between sibling groups tested by paired samples t -test. n.s., not significant, $p > 0.05$; *** $p \leq 0.001$.

prior to and after selection, respectively. Standardized selection differentials (s') were estimated as

$$s' = \frac{(\bar{z}_s - \bar{z})}{\sqrt{V_p}}, \quad (3)$$

where V_p is the phenotypic variance (Lande & Arnold 1983). Overall selection differentials for the population were then calculated as the means of the different females.

3. RESULTS

(a) Chemical composition

The dry mass and total energy contents of the eggs were highly correlated with wet egg mass (dry mass $r = 0.98$, $n = 80$ and $p < 0.001$ and energy content $r = 0.98$, $n = 80$ and $p < 0.001$; partial correlation controlling for females). Moreover, the fat (mean $\pm$ s.d. $7.0 \pm 1.7\%$) and protein percentages ($89.5 \pm 1.5\%$) and mass specific energy content ($24.2 \pm 0.6 \text{ kJ g}^{-1}$) did not vary significantly with wet egg mass (fat $r = -0.06$, $n = 80$ and $p = 0.616$, protein $r = -0.16$, $n = 80$ and $p = 0.162$ and energy $r = -0.21$, $n = 80$ and $p = 0.064$). Thus, the relative chemical and energetic compositions of small and large eggs were similar.

(b) Timing of hatching and emergence

Egg size did not affect the development rates during incubation. The mean time until hatching ($\pm$ s.d.) was 431.4 ± 6.6 (number of days multiplied by the mean temperature ($^{\circ}\text{C}$)) for small eggs and 430.7 ± 6.4 for large eggs ($t_5 = 0.428$ and $p = 0.687$, paired samples t -test). The mean time until emergence was 701.5 ± 13.9 for small eggs and 703.4 ± 11.4 for large eggs ($t_7 = 1.43$ and $p = 0.195$). Thus, there was no significant difference in the date of release into the enclosures ($t_7 = 0.22$ and $p = 0.836$).

Figure 2. Reaction norms for the maternal effects of egg size on growth of post-emergent brown trout. Relationship between (a) growth rates or (b) growth indexes (corrected for the effects of temperature) of juveniles originating from small (filled circles) or large (open circles) eggs and the overall mean growth rate/growth index within each of 24 semi-natural enclosures. Each data point represents the mean growth rate/growth index for one egg size group in one enclosure. Regression: (a) small eggs $Y = 1.41X - 2.61$, $r^2 = 0.92$, $F_{1,22} = 250.86$ and $p < 0.001$ and large eggs $Y = 0.67X + 2.10$, $r^2 = 0.77$, $F_{1,22} = 77.54$ and $p < 0.001$; comparison of slopes $Z = 6.33$ and $p < 0.001$. (b) Small eggs $Y = 1.53X - 0.53$, $r^2 = 0.92$, $F_{1,22} = 268.52$ and $p < 0.001$ and large eggs $Y = 0.60X + 0.40$, $r^2 = 0.68$, $F_{1,22} = 50.99$ and $p < 0.001$; comparison of slopes $Z = 7.34$ and $p < 0.001$.

(c) Semi-natural environments

In the enclosures, initial size differences between siblings from small and large eggs persisted throughout the experiment (figure 1). Density had no significant effect on the growth rate (mean $\pm$ s.d. low density $5.61 \pm 0.83\%$ per day, medium density $6.02 \pm 0.60\%$ per day and high density $5.91 \pm 0.64\%$ per day; $F_{2,21} = 0.96$ and $p = 0.401$) or growth index ($F_{2,21} = 0.66$ and $p = 0.527$). The reaction norm between the mean growth rate within an enclosure and the corresponding growth rates of the two egg size groups differed significantly (figure 2a). This was also true when controlling for the effects of temperature (figure 2b), suggesting that the interaction between environmental quality (i.e. the mean growth rate within an enclosure) and the effect of egg size on growth rates was independent of temperature variation and, therefore,

Figure 3. (a) The relationship between the initial difference in body mass at emergence of juvenile brown trout siblings from large and small eggs and their final difference in body mass at the end of the growth experiment in semi-natural enclosures 18 days later. (b) The relationship between the mean growth rate within the enclosures and the residuals from (a). Each data point represents a single enclosure.

probably reflected variation in food abundance throughout the stream channels.

Both the initial differences in body mass and subsequent growth contributed significant explanatory power to the observed variation between enclosures in the final difference in mass. Differences in body mass between large and small eggs at the end of the experiment were positively related to the initial differences between enclosures (figure 3a). Furthermore, the residuals from this regression were negatively related to the mean growth rate in the enclosures, such that, in enclosures with high growth rates, the difference in body mass by the end of the experiment was lower than expected based on the initial difference (figure 3b).

The daily mortality rates in the enclosures were in the range of 0.0000–0.0128 (mean $\pm$ s.d. 0.0037 ± 0.0063) and decreased with increasing mean growth rate among the enclosures ($R_s = -0.68$, $n = 24$ and $p < 0.001$, Spearman's rank correlation). Juveniles from small eggs experienced higher mortality than those from large eggs ($Z = 2.20$, $n = 8$ and $p = 0.028$, Wilcoxon signed-ranks test), with the difference in mortality tending to decrease with increasing growth rate ($R_s = -0.35$, $n = 24$ and $p = 0.091$,

Figure 4. The relationship between the mean growth rate and the mortality rates of post-emergent juvenile brown trout among experimental enclosures. Siblings originating from small (filled circles) and large (open circles) eggs were reared in competition for 18 days in 24 semi-natural enclosures. Each data point represents the mean mortality rates for one egg size group in one enclosure.

figure 4). The effect of egg size on the mortality rate resulted in significant directional selection for increased body size at emergence (table 1).

(d) Hatchery control

In contrast to the semi-natural environment, there was no significant difference in the growth rates between juveniles from small (mean $\pm$ s.d. $3.49 \pm 0.76\%$ per day) and large eggs ($2.92 \pm 0.71\%$ per day; $t_5 = 1.143$ and $p = 0.305$) when they were reared separately in the hatchery environment. Thus, the initial difference in body mass between siblings from small and large eggs had disappeared by the end of the experiment (figure 1).

4. DISCUSSION AND CONCLUSIONS

This study, to our knowledge, provides the first direct evidence of a causal relationship between egg size and fitness-related traits in fishes, independent of potentially confounding genetic effects. Juveniles from large eggs were larger at emergence, providing them with a competitive advantage over siblings from small eggs and resulting in growth and survival advantages under certain environmental conditions. Variation in performance during the first days after emergence is likely to be of high evolutionary significance in juvenile salmonids, as this is a period of high mortality and probable intense selection in natural populations (Elliott 1989). Furthermore, the effects of the observed differences in the growth rate probably extend to later stages of life, influencing a range of fitness-related traits (Thorpe 1989; Jonsson 1993; Jonsson *et al.* 1996).

The effect of egg size on growth in the semi-natural environments depended on environmental quality, generating distinct reaction norms for small and large eggs during early post-emergence. Although the differences in growth between juveniles from small and large eggs decreased with increasing environmental quality, offspring from small eggs are not expected to have higher

Table 1. Directional selection differentials (s) for mass and length at emergence of juvenile brown trout resulting from mortality during 18 days in semi-natural enclosures

(Standardized coefficients (s') are in standard deviation units. Significance levels are for tests of deviation from zero by one sample t -test. $n = 8$ full-sib groups.)

	$s \pm \text{s.d.}$	t_7	p	$s' \pm \text{s.d.}$	t_7	p
mass (mg)	0.70 ± 0.50	3.88	0.006	0.05 ± 0.04	3.34	0.012
length (mm)	0.05 ± 0.03	4.43	0.003	0.04 ± 0.03	4.29	0.004

fitness than those from large eggs in high-quality environments. However, because egg size is a maternal trait, we need to consider the effects on maternal fitness to understand the evolution of this trait. Maternal fitness is a function of both fecundity and offspring fitness and these two traits are mutually constrained by a trade-off (Roff 1992). Thus, as offspring fitness differences between egg sizes decline with increasing environmental quality, females should produce more and smaller eggs to optimize fitness under favourable conditions.

Crossing reaction norms between maternally determined phenotypes may not be an uncommon feature (Rossiter 1998). Acknowledging the existence of crossing reaction norms makes it imperative to consider environmental quality when testing the ecological significance of maternal traits. Our findings suggest that experimental conditions may seriously influence results when the selective importance of phenotypic variation is examined. The high proportion of previous studies reporting only transient effects of egg size on offspring performance may be biased, as such concerns have largely been ignored and the groups compared have been reared separately in artificial, high-quality environments (cf. Reznick 1991).

Knowledge of reaction norms potentially improves our understanding of the evolutionary processes shaping maternal traits. The ones observed in this study predict a decrease in the optimal egg size with increasing environmental quality. However, environmentally induced components to maternal traits may complicate predictions of evolutionary responses to selection (Kirkpatrick & Lande 1989). Jonsson *et al.* (1996) observed a negative correlation between egg size, adjusted for body size and growth rate experienced as a juvenile among females in a population of Atlantic salmon (*Salmo salar*). They suggested that this was caused by adaptive phenotypic plasticity, with females experiencing high growth as juveniles producing higher numbers of smaller sized eggs. The basic assumption behind such a hypothesis would be that the optimal egg size decreases with increasing environmental quality, an assumption supported by our results. It is interesting to note that, according to this, females originating from relatively large eggs, experiencing high juvenile growth due to competitive superiority (this study), should produce relatively small eggs as adults. Such effects are likely to introduce 'evolutionary momentum' into the population, causing traits to evolve after selection ceases (Kirkpatrick & Lande 1989). Negative maternal effects may even cause the population to evolve away from fitness peaks initially, but conditions necessary for such processes to be stable are probably rare in nature (Kirkpatrick & Lande 1989). Thus, although evolutionary

rates are potentially affected, we expect egg size to evolve ultimately according to selection coefficients towards optimal values.

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