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Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo salar*)

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ABSTRACT

Question: Why does per-offspring investment (e.g. egg size) increase with maternal body size?

Theory: Fecundity also increases with maternal body size, and when egg/larva/juvenile dispersal is limited, offspring developing at higher densities (e.g. in larger clutches of eggs) experience more stressful conditions during development. Hence, an increase in per-offspring investment with maternal body size may be a form of maternal compensation for the negative effect of fecundity on offspring performance.

Hypothesis: In fishes with demersal eggs and larvae, offspring in larger clutches use more energy during development, resulting in a smaller subsequent juvenile size. This may occur because larvae in larger clutches are prone to mutual physical disturbance, and larvae may expend more energy seeking or creating oxygenated areas within the nest.

Organisms: Atlantic salmon (*Salmo salar*).

Location: Aquatron experimental research facility, Halifax, Nova Scotia, Canada.

Methods: We buried 12 full-sib families in 24 gravel egg pockets at high density (200 eggs per egg pocket, or 1.27 eggs per cm^3, $n = 12$) and low density (25 eggs per egg pocket, or 0.318 eggs per cm^3, $n = 12$) and monitored subsequent juvenile size, survival, time of emergence, and developmental stage at emergence.

Results: We found no evidence that density *per se* affects offspring phenotypes. However, offspring from larger eggs emerged later and at an earlier developmental stage than offspring from smaller eggs. We show that the fitness trade-off between size-at-emergence and emergence time can result in a lack of correlation between egg size and offspring fitness. Our results do not provide evidence that the positive correlation between egg size and maternal size is adaptive, but we do provide new evidence that bigger is not always better.

Keywords: density dependence, emergence time, maternal effects, optimal egg size, yolk sac.

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INTRODUCTION

In a given environment, there is a single level of per-offspring investment that will maximize maternal fitness (Smith and Fretwell, 1974). However, pronounced within-population variation in egg size is observed in many taxa, and much of this variation correlates positively with maternal body size (Roff, 1992; Hendry *et al.*, 2001). Although this pattern may reflect physiological or morphological constraints on egg size imposed by maternal size (Congdon and Gibbons, 1987; Sakai and Harada, 2001), it may also arise because the mother's phenotype predicts the rearing environment of her offspring (Hendry *et al.*, 2001; Hendry and Day, 2003). For example, fecundity increases with maternal size (Roff, 1992), so relatively intense competition for resources is expected among offspring from larger mothers, at least when offspring dispersal is limited (Parker and Begon, 1986; Plaistow *et al.*, 2007). In highly competitive environments, both maternal and offspring fitness increase with per-offspring investment (Hutchings, 1991, 1997; Einum and Fleming, 1999), so larger eggs may be favoured when juvenile density is high. Indeed, a theoretical framework in which the egg size–maternal size correlation can be understood as a form of maternal compensation for these density-related effects is well established (Parker and Begon, 1986; McGinley, 1989; Hendry *et al.*, 2001; Einum *et al.*, 2002; Hendry and Day, 2003).

In salmonids, both egg size and fecundity correlate positively with maternal size, so this group provides a good opportunity to test the adaptive significance of the egg size–maternal size correlation. Before spawning, salmonids migrate to their natal river, and mothers bury their eggs underneath the gravel in a series of egg pockets (collectively called a 'redd'). Both the number of egg pockets and the number of eggs per pocket increase with maternal fecundity, such that large eggs tend to occur in higher densities than small eggs (Fleming, 1998; Einum *et al.*, 2002). After the eggs hatch, the larvae (or 'alevins') remain beneath the gravel in the vicinity of the egg pocket to absorb their yolk sac, which takes approximately 40–90 days (Quinn, 2005). The larvae then navigate up through the small pore spaces and emerge as juveniles (or 'fry').

Part of the reason large salmonids produce large eggs may relate to density-dependent effects that occur after emergence (Einum and Nislow, 2005; Einum *et al.*, 2008; Leips *et al.*, 2009). However, the sub-gravel behaviour of salmonid larvae has been poorly studied, and recent evidence suggests a negative correlation between larval rearing density and yolk conversion efficiency on artificial substrates (Houde *et al.*, in press). This likely reflects mutual physical disturbance, whereby larvae stocked at high densities interact more frequently with their siblings and accrue higher metabolic costs during development. Sub-gravel movement of larvae also increases with increasing larval density, both as a direct consequence of mutual physical disturbance and as a behavioural response to high levels of carbon dioxide ('ventilation swimming'), which increases interstitial flow in high-density environments (Bams, 1969). Hence, part of the reason large salmonids lay large eggs may also reflect compensation for the effects of increased sub-gravel egg or larval density.

To test this hypothesis, we manipulate egg size and egg density of larval Atlantic salmon (*Salmo salar*). If density affects larval development by increasing larval movement and metabolic demand, then juveniles emerging from low-density treatments will be larger than juveniles emerging from high densities. However, if larger eggs are less sensitive to differences in environmental quality (Hutchings, 1991, 1997; Einum and Fleming, 1999), then we predict that body size of juveniles hatching from smaller eggs will be negatively affected by rearing density, but body size of juveniles hatching from large eggs will not. Finally, we explore relationships between emergence time, emergence success, rearing density, and egg size.

METHODS

Atlantic salmon were reared to maturity in the Aquatron facility at Dalhousie University, Nova Scotia, Canada. Twelve females and 11 males were stripped of their eggs or milt between 7 and 9 December 2009, and female fork length was measured to the nearest centimetre. A sample of 25 unfertilized eggs was collected from each female. These eggs were frozen at −4°C and subsequently weighed to the nearest milligram. Each clutch was then fertilized with milt from one male (1♂ : 1♀ mating), but due to a shortage of males, one particular male was used to fertilize the eggs of two different females. After fertilization, eggs were divided equally among two perforated plastic containers (10×12 cm or 10×22 cm), which were placed in one of eight 70-cm circular flow tanks, with the exception of one female (see below). Up to eight families were kept in a given flow tank, and water in all tanks was maintained at ambient temperature and was continuously aerated, using air stones. Dead eggs were removed from their containers every 3–4 days until the eyed stage. Egg trays were then briefly shaken ('shocked'), which kills the majority of eggs that fail to develop properly, and all dead eggs were removed. For one female, eggs were divided equally among four plastic containers and incubated separately in four flow tanks, two of which were not occupied by other eggs used in this study. Water in these extra two tanks was 0.34 ± 0.035°C (mean ± S.D.) higher than in the eight other tanks, and this temperature difference persisted until 10 March, which resulted in an a 7.5% difference in degree days (hot: 458.9 ± 0.64°D; cold: 424.15 ± 3.18°D). Mean temperature for all eggs during the entire incubation period was 6.57 ± 0.018°C.

We constructed water-tight containers designed to simulate natural egg pockets within salmon redds. Containers were constructed from 15.2-cm diameter PVC piping cut into 30-cm pieces. The base of each container was fixed with a plastic, water-tight cap, but the top was left open. Dechlorinated water was fed into the container through 0.25-cm tubing fixed in a small hole that was drilled 2 cm above the base of each container. Water flowed upward through the container, then out the top of the container through a 0.75-cm tube fixed to the lip. Emergence traps were created by cutting 1-cm diameter tubing into 0.75-cm sections and then fixing the sections into similar-sized perforations in transparent, circular, 15×5 cm plastic trays. The trays were then secured in the top of each container. Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia) and sieved into three size classes: 1.54 ± 0.19 cm, 2.12 ± 0.27 cm, and 2.82 ± 0.34 cm. These classes were then mixed in a 3:3:2 ratio resulting in a mean gravel diameter of approximately 2.01 cm, a spawning gravel size typical for Atlantic salmon (Louhi *et al.*, 2008).

Eyed eggs were transferred to the egg pocket replicates on 9 and 10 March 2010. For each of the 12 females, eggs were taken from all relevant incubation trays and pooled. To reduce the subsequent within-replicate variation in juvenile size, the very largest and very smallest eggs were removed (by eye) and discarded. A subsample of 28–32 eggs from each female was then weighed to the nearest milligram to obtain an estimate of egg wet mass, and unless otherwise noted, these values of egg wet mass were used in subsequent analyses. Twenty-five eggs were placed in one replicate (low density) and 200 were placed in the next replicate (high density). For the female with four incubation trays, 11 of the 25 eggs allocated to the low-density treatment were from the warmer two tanks, as were 87 of the 200 eggs allocated to the high-density treatment, such that an equal ratio of warm to cold eggs was maintained. A 1-cm layer of fine gravel (4–6 mm diameter) was placed on the bottom of each replicate, followed by a 1-cm layer of gravel mixture. Eggs were then carefully placed in

the centre of each replicate on the gravel mixture. Eggs were then covered with 23 cm of gravel, and the replicates of each female were subsequently placed side-by-side on steel racks to complete development. Flow rate was maintained at 3 $ml \cdot s^{-1}$, corresponding to an interstitial velocity of ~0.04 $cm \cdot s^{-1}$, which is typical of salmon redds (Lapointe *et al.*, 2004; Zimmerman and Lapointe, 2005). Flow was monitored and adjusted every 2 days, temperature was recorded daily, and a natural photoperiod was initiated after eggs were buried in gravel.

Based on our observations of egg dispersion after placement, we estimate that actual volume of our artificial egg pockets (the area in which eggs settled in each replicate) was 157 cm^3 for high-density treatments, such that egg density in high-density treatments was roughly 1.27 $eggs \cdot cm^{-3}$. Horizontal dispersion of eggs was similar between high and low densities, but there was no vertical stratification of eggs at low densities. Hence, we estimate that the volume of low-density egg pockets was roughly 78.5 cm^3, or 0.318 $eggs \cdot cm^{-3}$. After hatching, when larvae were capable of distributing themselves evenly throughout the entire column of gravel, the minimum density of larvae before the beginning of emergence was 0.15 larvae $\cdot cm^{-3}$ and 0.018 larvae $\cdot cm^{-3}$ for high- and low-density treatments, respectively. These values are likely to be underestimates, given that we would expect a more clumped larval distribution after hatching. (Note that values are corrected for the volume of gravel, whereby only 1370 ml of water was available to the larvae in each replicate.)

Juveniles began emerging from the gravel on 22 April 2010. When juveniles were detected in an emergence trap, they were captured with a turkey baster, over-anaesthetized in 0.1 $mg \cdot l^{-1}$ tricaine methanesulfonate, rinsed in fresh water, blotted dry, placed in labelled clear plastic bags, and frozen at −4°C. Between 23 May and 10 June 2010, juveniles were dried at 55°C in a drying oven and weighed to the nearest 0.001 g. When a visible yolk sac was observed on juveniles, it was removed (while the juveniles remained frozen), dried, and weighed separately. All samples were dried on parchment paper.

Paired samples *t*-tests were used to test for density-related effects, where samples were paired by female of origin. Paired differences were obtained by subtracting values of low-density treatments from values of high-density treatments. We tested the shape of the relationship between density-related effects and egg wet mass by regressing paired differences against egg wet mass, using least squares regression. Proportional data were logit-transformed before analysis, and all reported values are means ± standard errors, unless otherwise noted. Finally, when a paired *t*-test revealed no difference between treatments for a given juvenile trait, we took the average trait value to obtain one data point per replicate pair (i.e. one data point per female). However, given that the means generated in our high-density treatments were a more reliable estimate of the true mean (because n was much higher), we calculated weighted means across the high- and low-density treatment according to the formula:

$$W\bar{x} = \frac{((HD\bar{x} \times HDn) + (LD\bar{x} \times LDn))}{(HDn + LDn)}$$

where $W\bar{x}$ is the weighted mean, $HD\bar{x}$ and $LD\bar{x}$ are the means of the high-density and low-density treatment, respectively, and HDn and LDn are the number of juveniles contributing to the $HD\bar{x}$ and $LD\bar{x}$ means, respectively. Weighted means were then used in regression analyses. Note that in the Results section, HD is 'high density', LD is 'low density', and EWM is mean 'egg wet mass' of a particular female's eggs just before allocation to the experiment (i.e. at the eyed stage) in milligrams. All statistics were performed in Minitab v. 15.1.3 (Minitab Inc., 2007).

RESULTS

Mean egg wet mass of the 12 females ranged from 114.2 ± 1.6 to 184.2 ± 1.5 mg. Emergence occurred between 22 April and 8 June 2010. Median day of emergence did not differ between treatments (HD 14.29 ± 0.88, LD 14.14 ± 0.78; $t = 0.33$, $n = 12$, $P = 0.75$, paired t-test), but weighted median day of emergence increased with initial egg size ($y = 0.100(\text{EWM}) - 1.77$, $r^2 = 0.393$, $n = 11$, $P = 0.039$, linear regression; Fig. 1A). The female whose eggs were incubated at slightly warmer temperatures (see Methods) was

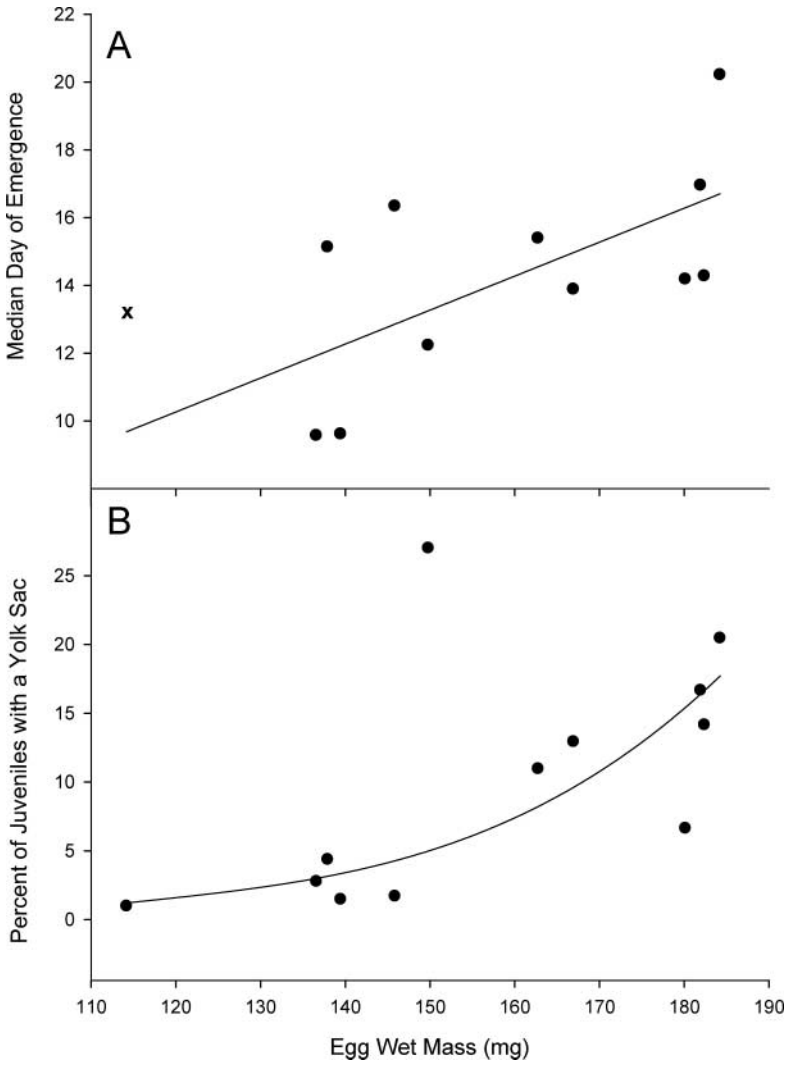

Fig. 1. (A) Weighted median day of emergence plotted against egg wet mass ($y = 0.100(\text{EWM}) - 1.77$, $r^2 = 0.393$, $n = 11$, $P = 0.039$). '×' is the omitted data point (see text). (B) Egg wet mass plotted against weighted mean percent of juveniles emerging from the nest environment with a visible yolk sac ($\text{logit}(y) = 0.0409(\text{EWM}) - 9.07$, $r^2 = 0.588$, $n = 12$, $P = 0.004$). Note that the line of best fit is a logistic function.

not included in this analysis (Fig. 1A), as incubation temperature influences development time (Teletchea *et al.*, 2009), but we note that the regression is marginally insignificant when this data point is included ($y = 2.99 + 0.0719$(EWM), $r^2 = 0.306$, $n = 12$, $P = 0.062$, linear regression). Mean dry mass of juveniles ranged from 22.8 to 44.0 mg and did not differ between treatments (HD 36.82 ± 1.93 mg, LD 36.70 ± 1.88 mg; $t = 0.65$, $n = 12$, $P = 0.53$, paired *t*-test). Mean paired differences in dry mass did not correlate with original egg size ($r^2 = 0.070$, $P = 0.41$, linear regression), indicating that offspring of all sizes were equally unaffected by density. Weighted mean dry mass of juveniles was, of course, highly correlated with initial egg size ($y = 0.287$(EWM) – 8.62, $r^2 = 0.971$, $n = 12$, $P < 0.001$, linear regression).

No difference in survival was detected between treatments (HD 90.96 ± 2.05%, LD 92.67 ± 2.02%; $t = 0.34$, $n = 12$, $P = 0.74$, paired *t*-test). Dry yolk weight expressed as a proportion of dry juvenile body mass did not differ between treatments (HD 1.91 ± 0.54%, LD 1.82 ± 0.49%; $t = 0.26$, $n = 12$, $P = 0.80$, paired *t*-test). However, dry yolk expressed as a proportion of juvenile body mass increased with egg mass (logit(y) = 0.0346(EWM) – 9.86, $r^2 = 0.512$, $n = 12$, $P = 0.009$, linear regression). Regression analysis, using untransformed data, generated the equation, yolk(%) = 0.0409(EWM) – 4.54, indicating that percent yolk increased by roughly 0.041% for every 1 mg increase in egg wet mass. Although the values for dry yolk expressed as a proportion of juvenile body mass were below 2% for both treatments (see above), we note that only 248 of 2468 juveniles actually emerged with a visible yolk. In fact, mean percent yolk was 17.7 ± 1.2% when excluding individuals that did not emerge with a visible yolk sac. In other words, the predictive equations immediately above are best applied to the group level, not an individual level.

The proportion of individuals emerging with a visible yolk did not differ among treatments (HD 10.12 ± 2.72%, LD 9.57 ± 2.11%; $t = –0.13$, $n = 12$, $P = 0.90$, paired *t*-test), but weighted mean proportion of individuals emerging with visible yolk correlated positively with egg size (logit(y) = 0.0409(EWM) – 9.07, $r^2 = 0.588$, $n = 12$, $P = 0.004$, linear regression; Fig. 1B). Regression analysis of the former variables, using untransformed percentages, indicates that the percent of individuals that emerged with a visible yolk increased by roughly 0.22% for every 1 mg increase in egg size ($y = 0.221$(EWM) – 24.64).

Finally, we tested for a positive correlation between female size and egg size. The mean egg wet mass of 25 unfertilized eggs, which were collected immediately after spawning, ranged from 111.7 mg to 163.7 mg. Egg size correlated with female fork length ($y = 1.78$(fork length, cm) + 44.3, $r^2 = 0.348$, $n = 12$, $P = 0.044$, linear regression).

DISCUSSION

Our results fail to support the hypothesis that an increase in egg or larval density in the egg pocket decreases juvenile size-at-emergence, such that the positive correlation between egg size and female body size is likely unrelated to sub-gravel egg or larval density *per se*. However, our results reveal two reasons why selection may favour smaller eggs. Specifically, we observed that individuals from small eggs emerged earlier than did individuals from large eggs and that offspring from large eggs emerged with more residual yolk than did their counterparts from small eggs. Below we outline why it may be undesirable for mothers to produce relatively large eggs if the consequences include relatively late emergence coupled with relatively large amounts of residual yolk.

A positive correlation between egg size and development time has been documented in

diverse taxa (Morgulis, 1909; Gillooly *et al.*, 2002; Teletchea *et al.*, 2009), and this pattern has been ascribed to the allometric effect of size on metabolic energy allocation at the cellular level (West *et al.*, 1997, 2001; Gillooly *et al.*, 2002). Indeed, Rombough (1985) predicted that eggs of Chinook salmon (*Oncorhynchus tshawytscha*) weighing 200 mg can complete development 14 days sooner than eggs weighing 500 mg at 10°C, and 31 days sooner at 5°C. Earlier emergence may increase fitness in salmonids, possibly because of increased time for growth (Cutts *et al.*, 1999; Einum and Fleming, 2000a), better selection of territories (Fausch, 1984; Hughes, 1992; but see Cutts *et al.*, 1999), and prior access to feeding territories, which confers an advantage in territorial disputes (Mason and Chapman, 1965; Cutts *et al.*, 1999).

Although the potential advantage of earlier emergence may be offset by increased predation (Brännäs, 1995) or lower environmental quality (Crecco and Savoy, 1985; Bailey *et al.*, 2010), realized advantages gained by earlier emergence can increase with increasing disparity in emergence time (Einum and Fleming, 2000a). In the present study, the predicted difference in emergence time between the largest and smallest eggs was 7.0 days. This difference is similar to the difference of 4.9 days predicted by fitting our data to the equation reported by Teletchea *et al.* (2009), which relates interspecific incubation time to egg diameter and temperature for freshwater fishes: $\log_{10}$ development time (days) = 3.002 + 0.599($\log_{10}$ egg diameter (mm)) – 1.91($\log_{10}$ incubation temperature (°C) + 2) ($r^2 = 0.92$). The predicted differences in emergence time, based solely on egg size, correspond to a difference in spawning time of 10–12 days (cf. Einum and Fleming, 2000a), and this may have an important effect on relative survival. For example, Einum and Fleming (2000a) found that selection on emergence date was stronger than selection on juvenile size: a 1 standard deviation delay in emergence date resulted in a 39% increase in mortality, whereas a 1 standard deviation decrease in juvenile length resulted in a 25% increase in mortality.

We applied these expected differences in mortality to our data set to test the relative benefits of emergence time and juvenile size. First, we standardized our weighted day of emergence data and our dry mass data (here we assume an equivalence of length and mass). Expected survival is then the product of Einum and Fleming's (2000a) values (–0.39 for day of emergence; 0.25 for mass) and our standardized values. We found a significant positive correlation between expected survival and egg wet mass for dry mass at emergence, and a significant negative correlation between expected survival and median day of emergence (Fig. 2). However, the sum of these expected survival values did not correlate with initial egg size ($y = -0.0004(\text{EWM}) + 0.0787$, $n = 11$, $r^2 < 0.001$, $P = 0.95$; Fig. 2), which suggests that the benefits of increasing egg size can be offset by the correlated effect of an increase in development time. We appreciate that the strength and direction of selection varies in space and time (e.g. Svensson and Sinervo, 2000 and references therein), and that spawning dates in a single population may span more than 2 months (Heggberget, 1988). Hence, we emphasize that this analysis is best considered a thought experiment designed to provoke future investigation.

Larvae hatching from larger eggs are bigger (Einum and Fleming, 1999), they have more energy (reviewed by Kamler, 2005), and they take longer to completely absorb their yolk (Killeen *et al.*, 1999). The latter point may help explain why juveniles from smaller eggs emerge sooner than those from larger eggs, though in the present study juveniles from larger eggs emerged relatively late and yet still had more yolk than those from smaller eggs. Similarly, there was a strong relationship between the percent of individuals emerging with a visible yolk sac and egg wet mass, where 20.5% of juveniles emerging from the nest stocked with the largest eggs had a visible yolk, compared with less than 1% for the nest stocked with the smallest eggs

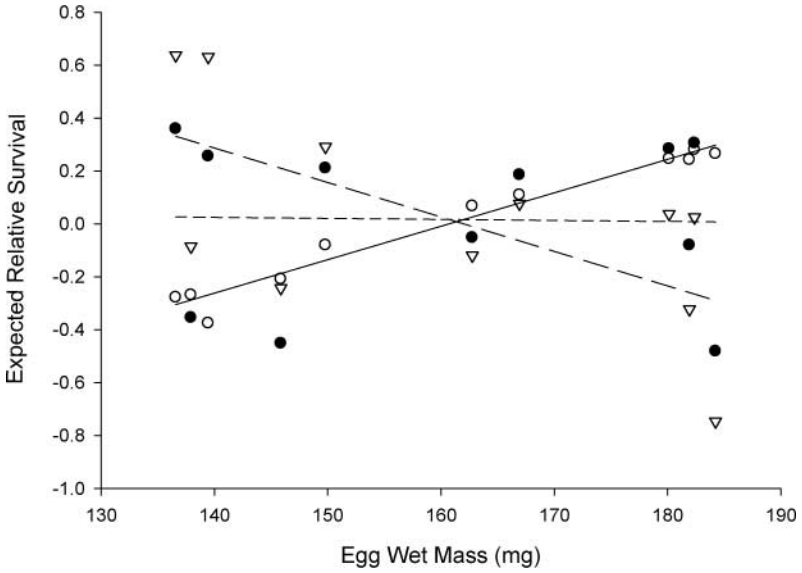

Fig. 2. Weighted averages of median day of emergence and mean dry mass were obtained for each paired replicate, then *z*-scores were calculated by subtracting each value from the overall mean and dividing it by the standard deviation. To calculate expected survival, *z*-scores were multiplied by 0.25 for dry mass data (solid line and open circles: $y = 0.0126(\text{EWM}) - 2.03$, $r^2 = 0.967$, $P < 0.001$) and by −0.39 for median day of emergence (long dash line and triangles: $y = 2.11 - 0.0130(\text{EWM})$, $r^2 = 0.393$, $P = 0.039$). The sum of these values, expected relative survival, did not correlate with egg wet mass (short dashed line and solid circles: $r^2 < 0.001$, $P = 0.95$).

(Fig. 1B). Larger juveniles often fare better than small juveniles after emergence (Hutchings, 1991; Einum and Fleming, 1999, 2000a, 2000b), but, to our knowledge, only two studies have evaluated the effect of residual yolk on juvenile survival in the wild. Letcher and Terrick (2001) found no evidence that yolk-sac juveniles fared worse in the wild than fully developed juveniles, although the type and abundance of predators was not studied. In contrast, Fresh and Schroder (1987) performed several large-scale releases spanning 2 years. Juvenile survival after 24 h was consistently inversely related to estimates of percent yolk, whereas the effect of mass and length on survival was inconsistent and variable in direction. Electrofishing surveys coupled with an analysis of stomach contents suggested that large rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) were abundant and were preferentially selecting yolk-sac juveniles.

There is indeed reason to suspect that yolk-sac juveniles are more susceptible to predation than juveniles that emerge just at the point when the yolk is completely absorbed. Many species of salmonids react most quickly to disturbance at 100% yolk absorption, but performance decreases quickly on either side of this optimum (Hale, 1996, 1999). Maximum swim velocity and distance travelled after disturbance tend to increase linearly or asymptotically with juvenile length, regardless of developmental stage (Hale, 1996, 1999), although developmental stage also tends to correlate positively with juvenile length (Killeen *et al.*, 1999). Given this disparity in performance, it is puzzling that yolk-sac juveniles will emerge at all from artificial and natural redds, apparently even when incubation conditions appear adequate, rather than delaying emergence (García de Leániz *et al.*, 2000; present study).

Although it seems that post-emergent juveniles from large eggs would be more likely to incur this disadvantage, the relative importance of size at emergence and developmental stage on predator avoidance is not understood.

Readers should note that by manipulating egg density and egg size, we also manipulated the biomass of eggs and, presumably, total nest respiration (Einum *et al.*, 2002; Rombough, 2006). We cannot, therefore, disentangle the effect of egg size from the effect of total egg biomass. For instance, if we multiply the number of eggs allocated to each treatment by the mean egg mass, we find that the biomass of eggs in each replicate varied between 2.9 g (EWM = 114.2 mg) and 4.6 g (EWM = 184.2 mg) for low densities and between 22.8 g and 36.8 g for high densities. Einum *et al.* (2002) provide a relationship between egg wet mass of Atlantic salmon and oxygen consumption (g O_2 per egg per hour) as $0.0014(\text{EWM, g})^{0.4434}$. Applying this equation to our smallest and our largest eggs (114.2 mg and 184.2 mg, respectively), we find that differences in oxygen consumption among density treatments were less pronounced for the smallest eggs (HD total O_2 consumption – LD total O_2 consumption = 0.0935 g O_2 per hour) than for the largest eggs (HD – LD = 0.116 g O_2 per hour). In other words, differences in O_2 consumption between high- and low-density treatments became increasingly pronounced as egg size increased. Also note that the range of clutch biomass (22.9–36.9 g) used in the present study was at the lower end of what would be expected in the field. Biomass per egg pocket increases in terms of female size according to the function 13.17 + 0.01(female body mass, g) (I.A. Fleming, unpublished data, cited in Einum *et al.*, 2002). Given that the range of female sizes used in the present study was 1240–3310 g (pre-oviposition), a clutch biomass of roughly 25.6–46.3 g per egg pocket may have more closely emulated wild conditions.

Finally, our calculations indicate that we had sufficient power to detect even a 0.5-mg difference in juvenile mass between density treatments with $n = 12$ pairs [power $(1 - \beta) = 0.83$; $\rho = 0.996$; $d = 0.826$; S.D. = 6.6; $\delta = 2.86$; analysis following Howell (2002, pp. 235–237)]. It is not clear, however, whether this lack of difference would persist in other nest environments. For example, although we attempted to mimic the natural rate of water flow through gravel, our design required that oxygenated water flow upwards through the gravel. In the wild, water would likely move laterally or downward through the gravel, and it may or may not be well oxygenated. Under low oxygen conditions, salmon larvae at high densities may be more prone to oxygen stress, and hence mutual physical disturbance (Bams, 1969), than larvae at lower densities, leading to a disparity in juvenile mass-at-emergence. In summary, we did not test for density-related effects over the full range of conditions experienced in the wild, and our findings should be interpreted with this in mind.

The present study fails to provide evidence that sub-gravel egg or larval density affects the phenotypes of emergent juveniles. Accordingly, we do not provide any evidence that the positive correlation between egg size and female body size is related to sub-gravel density *per se*. Our results do, however, reveal new reasons why large eggs may be disadvantageous in some environments. Future studies should investigate further the relationships between percent yolk at emergence, emergence time, and fitness of juveniles to evaluate the relative benefits associated with different maternal provisioning strategies. As the function relating maternal fitness to egg size is environment-dependent (Hutchings, 1991, 1997; Einum and Fleming, 1999), optimality would be best understood by testing these factors across a range of conditions, but within the context in which the eggs were originally provisioned (Marshall *et al.*, 2010).

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