

# Why does egg size of salmonids increase with the mean size of population spawning gravels?

Njal Rollinson and Jeffrey A. Hutchings

**Abstract:** Population mean egg size of salmonids increases with the mean size of gravels in which a population spawns. A long-standing hypothesis is that large larvae cannot navigate small-gravel interstices, so mothers must decrease per-offspring investment when spawning gravels are small. We manipulated the size of incubation gravels and egg size of Atlantic salmon (*Salmo salar*) to test whether size-related entombment of larvae occurs. We find little evidence of size-related entombment, but we find evidence that gravel size does not affect all larval sizes equally. Larger larvae emerge from small gravels before development is complete and with a visible yolk sac, possibly due to oxygen limitation in small gravels. Smaller larvae always complete development in the gravel and emerge without a yolk sac. Although growth and survival may increase with juvenile size following yolk-sac absorption, juveniles with yolk sacs may fare worse when depredation rates are high. The egg size – gravel size correlation may therefore reflect increased postemergence mortality among larger offspring in small-gravel environments. Alternatively, compaction stress in fine gravels coupled with size asymmetries in larval strength may have caused the patterns that we observed, in which case our data may not help explain the egg size – gravel size correlation.

**Résumé :** La taille moyenne des oeufs d'une population de salmonidés augmente en fonction de la taille moyenne du gravier dans lequel cette population fraie. Une hypothèse de longue date veut que les larves de grande taille ne peuvent pas se déplacer dans les interstices de gravier fin et que les mères doivent réduire leur investissement par rejeton lorsque le gravier de fraie est fin. Nous avons manipulé la taille du gravier d'incubation et la taille des œufs chez des saumons atlantiques (*Salmo salar*) afin de tester s'il se produit un ensevelissement des larves en fonction de la taille. Nous trouvons peu d'indications d'un ensevelissement relié à la taille, mais il y a des preuves que la taille du gravier n'affecte pas toutes les tailles de larves de même manière. Les larves plus grandes émergent des graviers fins avant la fin de leur développement et avec un sac vitellin bien visible, peut-être à cause de la limitation de l'oxygène dans les graviers fins. Les larves plus petites complètent toujours leur développement dans le gravier et émergent sans sac vitellin. Bien que la croissance et la survie puissent augmenter en fonction de la taille des jeunes après la absorption du sac vitellin, les jeunes avec sac vitellin peuvent réussir moins bien lorsque les taux de prédation sont élevés. La corrélation entre la taille des œufs et la taille du gravier peut donc refléter une mortalité accrue après l'émergence chez les rejetons de plus grande taille dans les environnements de gravier fin. Par ailleurs, le stress de compaction dans les graviers fins combiné aux asymétries reliées à la taille dans les forces des larves peuvent avoir causé les patrons que nous avons observés; dans ce cas, nos données peuvent ne pas aider à expliquer la corrélation entre la taille des œufs et la taille du gravier.

[Traduit par la Rédaction]

## Introduction

As mothers are expected to allocate the energy available for reproduction into offspring of an optimal size (Smith and Fretwell 1974), variation in offspring size among populations is often assumed to reflect a matching of maternal provisioning strategies to a particular environment (e.g., Gregersen et al. 2008). Indeed, interspecific variation in egg size may reflect broad patterns of canalization or adaptive plasticity in response to local selection pressure on eggs, embryos, and offspring (Berven and Gill 1983; Fox et al. 1997; Bashey 2006). Although a consistent difference among populations in the strength or direction of selection on offspring size can theoretically lead to adaptive divergence in per-offspring in-

vestment (Smith and Fretwell 1974; Allen et al. 2008; Marshall and Keough 2008), opposing patterns of selection at different stages of offspring development may also provide a framework in which adaptive interpopulation variation in offspring size can be understood (Hendry et al. 2001). For example, when offspring fitness is negatively related to size during the egg stage and positively related to size during the juvenile stage (e.g., Congdon and Gibbons 1987; Sinervo and Licht 1991), optimal offspring size becomes a function of the relative strength of selection on offspring size in the different life stages (Hendry et al. 2001). Although this mechanism can likely account for population divergence in egg size (e.g., Parciak 2002), few studies have explicitly examined its role in maintaining interspecific egg-size variation.

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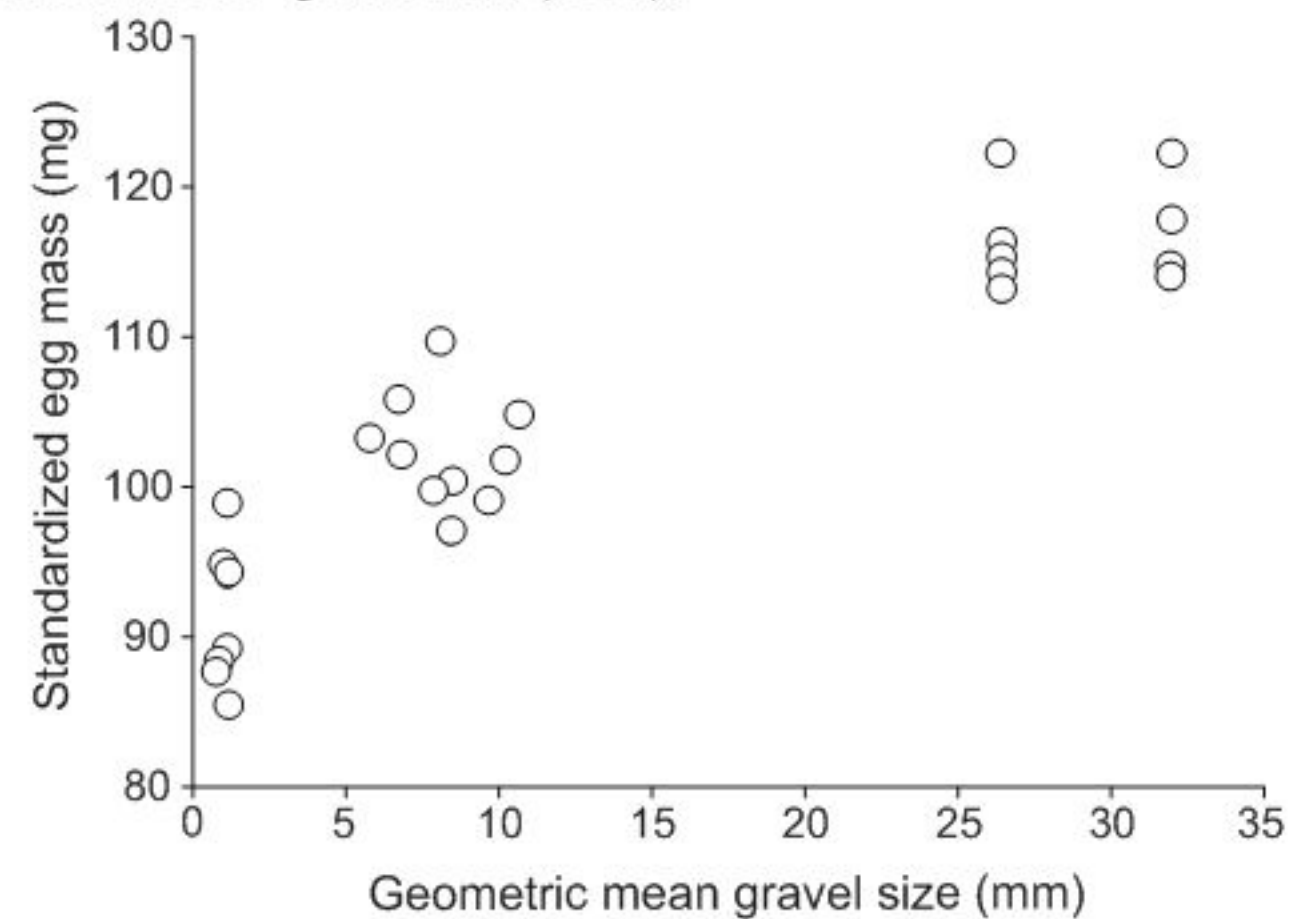
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Salmonids are largely autumnal spawners that deposit their eggs into riverine or lacustrine gravels. Embryos develop over winter and hatch into yolk-sac larvae (or “alevins”) in the following spring. Once yolk-sac absorption is complete, individuals emerge from the gravel as juveniles (or “fry”), and in some species, they establish feeding territories near their natal area (e.g., Bachman 1984). Size asymmetries at the juvenile stage (Hutchings 1991; Einum and Fleming 2000a, 2000b) and at the embryo or larval stage (Einum et al. 2002) can affect maternal and offspring fitness in salmonids such that this group has stimulated a wealth of research on offspring size–number strategies (reviewed in Einum et al. 2004).

Famously, a clear, positive correlation between population-specific spawning gravel size and population mean egg size has been observed among 21 Alaskan populations of sockeye salmon (*Oncorhynchus nerka*) (Quinn et al. 1995; Quinn 2005; Fig. 1). This pattern may occur because small incubation gravels represent (or are correlated with) a relatively benign selective environment in which variation in offspring size is not correlated with variation in offspring fitness. Under these conditions, maternal fitness is indeed maximized by producing many, poorly provisioned offspring (Smith and Fretwell 1974). It seems unlikely, however, that small gravels represent a benign selective environment, as small incubation gravels are generally associated with low egg-to-juvenile survival, which in turn has been attributed to low oxygen availability and small-gravel interstices (Phillips et al. 1975; Witzel and MacCrimmon 1981; Olsson and Persson 1986). The egg size–gravel size correlation may instead result from a decrease in offspring fitness with increasing egg size in small gravels. Only small larvae may be able to navigate the small pore spaces in finer gravels, whereas larger larvae may become entombed in the nest cavity. However, as the size of incubation gravels increases, there may be no size-related advantage during the embryo or larval stage, and given that larger juveniles generally exhibit greater fitness in the wild (Einum and Fleming 2000a, 2000b), a larger egg size may maximize maternal fitness when gravel sizes are large. Although this widely discussed hypothesis has never been explicitly tested (Witzel and MacCrimmon 1983; Quinn et al. 1995; Quinn 2005), it suggests that opposing patterns of selection at different life stages has led to a divergence in optimal offspring sizes among environments (Hendry et al. 2001).

The maximum difference between the smallest and largest population mean egg size in the original sockeye salmon populations was 25.6% (Quinn et al. 1995). The magnitude of this difference is indeed sufficient to warrant an investigation of adaptive hypotheses, as limited data suggest that a difference in egg size of 14%–30% is typical when reasonable evidence of adaptive egg-size plasticity exists (summarized by Fischer et al. 2011). On the other hand, although variation in egg size can be related to proximate factors such as variation in the offspring selective environment (e.g., Fox et al. 1997), it can also be traced to ultimate factors such as the rate at which a mother can provision primary oocytes (Sakai and Harada 2001). The point is that variation in mean egg size among environments does not necessarily mean variation in optimal offspring size. Manipulative experiments are nec-

**Fig. 1.** The relationship between population mean egg size of sockeye salmon (*Oncorhynchus nerka*) and geometric mean size of spawning gravels. Multiple points for the same gravel size are samples from the same population in different years. Egg mass is adjusted to a common maternal body size of 450 mm fork length. Modified from Quinn et al. (1995).



essary to test whether interpopulation egg-size variation reflects an adaptive response to local environments.

Here, we manipulate incubation environments of larval Atlantic salmon (*Salmo salar*) to test whether large embryos and larvae exhibit poor survival in small gravels because of size-related larval entombment. Assuming that size-related entombment is a function of larval size per se, we expect that larvae hatching from relatively large embryos will experience lower survival than larvae hatching from smaller embryos in fine gravels, but not in larger gravels. If large larvae have difficulty navigating the small interstices of fine gravels, then we also expect that they will use more energy during emergence from these gravels. Thus, we predict further that in smaller gravels, large juveniles surviving to emergence will have a lower specific energy content (in joules per gram of tissue) compared with smaller juveniles, but no difference in specific energy will be observed between large and small individuals that have emerged from a large-gravel substrate.

## Materials and methods

Atlantic salmon were reared to maturity at the Coldbrook Biodiversity Facility in Coldbrook, Nova Scotia, Canada. As part of a larger study on local adaptation in Atlantic salmon, multigenerational crosses were generated from 49 females and 54 males on 31 October 2008 and 4 November 2008; between four and eight males were crossed with a given female (for details, see Houde et al. 2011). Embryos were incubated at ambient temperatures in incubation trays within a single incubation trough at Coldbrook until the eyed stage. Embryos were then transferred to the Aquatron Facility at Dalhousie University where they were kept in plastic containers (Lee's Kritter Keepers, Lee's Aquarium & Pet Products, San Marcos, California) until the beginning of the experiment. Each container measured 27.6 × 17.0 × 20.3 cm but was modified to accommodate two families in 13.8 × 17.0 × 20.3 cm sections, and each container was perforated to allow water flow. Up to three containers (up to six families) occupied one 70 cm circular flow tank, and 60 flow tanks were used in to-

tal. Water levels were maintained so that embryos were submerged under 7 cm of water, and water volume was roughly 26.9 L·tank<sup>-1</sup>. Flow into each tank was 5–10 mL·s<sup>-1</sup>, and aeration originated from the middle of each tank, which promoted homogeneity in water quality. Ambient temperatures were maintained throughout incubation (mean ± standard deviation (SD): 3.1 ± 2.3 °C at Coldbrook; 5.2 ± 1.6 °C in the Aquatron). Percentage development was estimated from the sum of daily development, where daily development is estimated by Kane (1988) as

$$(1) \quad \ln(y) = 6.003 e^{(-0.0307 \times T)}$$

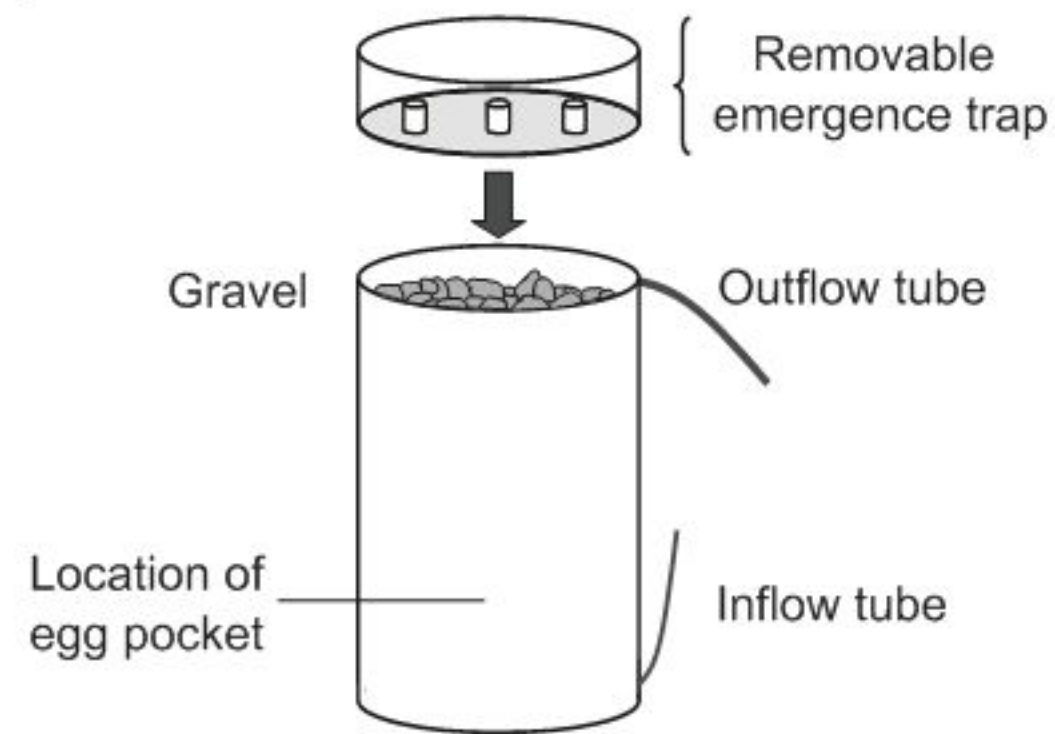
where “e” is the base of the natural logarithm, and  $T$  is the mean daily incubation temperature in degrees Celsius. Each daily value ( $y$ ) was divided into 100 and then summed across the incubation period to obtain percentage development (juveniles are expected to emerge from the gravel at 100% development).

We constructed water-tight containers designed to simulate natural egg pockets within salmon redds (Fig. 2). Containers were constructed from 15.2 cm diameter polyvinyl chloride (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 base of each container. Water flowed upward through the container and then out the top of the container through a 0.75 cm tube fixed to the lip. Water flow through each container was maintained at 26.1 ± 3.1 mL·s<sup>-1</sup>. Emergence traps were created by cutting 1 cm diameter tubing into 0.75 cm sections and then fixing those sections into similar-sized perforations in transparent, circular, 15 × 5 cm plastic trays. The trays were then secured in the top of each container.

The experiment was a 3 × 3 factorial design with three levels of egg size and three levels of gravel size, with three replicates per treatment (3 egg sizes × 3 gravel sizes × 3 replicates = 27 replicates in total). Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia) and sieved into three size classes. Mean ± SD diameter of 75 randomly selected pebbles (measured along the median or “B” axis) was 28.2 ± 3.4 mm (large gravel), 11.7 ± 1.6 mm (medium gravel), and 8.9 ± 1.6 mm (small gravel). The mean, median, and geometric mean gravel size was very similar for each size class. The range of geometric mean gravel sizes identified by Quinn et al. (1995) was 1.6–77.8 mm (pebble count method) and 0.9–32.1 mm (bulk samples).

Initial mean egg mass for each of the 49 females was estimated from a sample of 12–20 unfertilized eggs collected in Coldbrook. Based on these estimates, we identified six females that produced “small” eggs, six that produced “medium” eggs, and five that produced “large” eggs; the females that contributed eggs to each egg size class came from a mix of three different populations and hybrids thereof (Table 1). One female in each of these size classes was spawned on 4 November 2008, and the rest were spawned on 31 October 2008. On 10 and 11 March 2009, embryos were selected from relevant families (Table 1) and allocated evenly to the appropriate egg size class treatment. Allocation proceeded as in the following example: 19 large embryos may have been collected from each of four families (76 embryos in total)

**Fig. 2.** Artificial salmon egg pocket (nest replicate) used in the present study.



that share a common mother. In this case, two embryos per family (eight embryos in total) would then have been allocated to each of the nine large-egg replicates, with four of the 76 embryos remaining unused. One or two of the unused embryos from each family were retained and weighed to the nearest 0.1 mg to obtain embryo mass (Table 1), the others were discarded. Estimated mean embryo mass (± standard error, SE) allocated to each small-, medium-, and large-egg replicate was 69.5 ± 1.1 mg, 103.6 ± 1.5 mg, and 138.0 ± 2.6 mg, respectively, thus exceeding the range of population mean egg sizes identified by Quinn et al. (1995), which were between 84.5 ± 2.7 mg and 113.5 ± 1.2 mg. The small- and large-egg replicates each contained 44 embryos, medium-egg replicates contained 45 embryos, and all embryos were buried under 23 cm of gravel.

A natural photoperiod was initiated on 31 March 2009. Juvenile emergence occurred between 13 May and 18 June 2009. When juveniles were detected in an emergence trap, they were captured with a turkey baster, overanaesthetized in 0.1 mg·L<sup>-1</sup> tricaine methanesulphonate, rinsed in freshwater, blotted dry, weighed to the nearest 0.1 mg, and measured to the nearest millimetre (standard length) using calipers. We also noted the presence or absence of a yolk sac, as the mean percentage yolk content of juveniles emerging from a replicate is highly correlated with the percentage of individuals emerging with a visible yolk sac ( $r^2 = 0.971$ ,  $n = 12$  replicates,  $P < 0.001$ ; N. Rollinson and J.A. Hutchings, unpublished data). Juveniles were then frozen in individual 3 mL plastic tubes at -4 °C. All replicates were excavated on 24 June 2009, which is six days after the last juvenile had emerged, and the remaining live and dead alevins were counted.

Between 12 and 15 July 2010, we combusted a subsample of juveniles from each replicate to estimate their energetic content. First, the median day of emergence was calculated separately for each replicate. Then, three ( $n = 4$  replicates) or four ( $n = 23$  replicates) juveniles that emerged on the median day of emergence, or as close as possible to the median day of emergence, were vacuum dried for 18 h using a Labconco 4.5 L freeze-dryer ( $n = 104$  juveniles dried in total). Dry mass was then measured to the nearest 0.1 mg. To estimate energetic content of tissue, the subsample was homogenized and then combusted in a Parr 1266 semimicro oxygen bomb calorimeter. Each subsample was compressed to a 3 mm diameter pellet, and pellets were combusted using 1 of 2 Parr 1107 micro-bombs, as described by Michaud and Taggart (2007).

**Table 1.** Summary of embryo allocation to the experiment.

♀ ID	Egg size	Maternal cross	Embryo mass (mg)	SE	No. of embryos	No. of families
1	Small	Stewiacke	66.7	2.8	12	4
2	Small	Stewiacke	73.9	1.5	12	4
3	Small	Stewiacke	70.1	2.6	4	4
4	Small	Great Village	70.3	1.6	5	5
5	Small	Great Village	67.6	2.9	6	4
6	Small	Stewiacke	61.5	1.0	5	5
7	Medium	Stewiacke	107.7	0.9	9	4
8	Medium	Economy	100.1	4.3	8	5
9	Medium	Great Village	103.5	1.2	6	5
10	Medium	Economy	103.6	3.9	7	3
11	Medium	Eco × Stew	108.2	1.9	8	2
12	Medium	Grv × Stew	98.5	3.4	7	2
13	Large	Great Village	138.5	2.3	4	4
14	Large	Eco × Stew	137.4	1.0	8	2
15	Large	Eco × Stew	150.9	0.7	10	2
16	Large	Great Village	149.4	1.2	11	6
17	Large	Stewiacke	130.0	1.9	11	6

**Note:** “Maternal cross” reflects the genetic background of the female (Stewiacke River (Stew); Great Village River (Grv); Economy River (Eco)). “No. of embryos” is the number allocated to each relevant replicate from a given female. “No. of families” is the number of half-sib families (of embryos) allocated to the experiment from a given female. SE, standard error.

Factorial analyses of variance (ANOVAs) and Tukey post hoc tests were performed in Minitab v. 15.1.3 (Minitab Inc., State College, Pennsylvania). Weighted fits were used when cell means were computed from unequal sample sizes, and proportional data were  $\text{logit}(x + 0.025)$ -transformed before analysis.

## Results

We found a tight relationship between wet and dry mass of the 104 dried juveniles (dry mass, mg =  $0.188(\text{wet mass, mg}) - 3.23$ ,  $r^2 = 0.983$ ,  $P < 0.001$ ), so all values of juvenile mass reported below are dry mass values predicted from wet mass. Over the course of the experiment, 10 juveniles escaped from their replicates and were found in the overflow sink. They could not be assigned back to their original container, but they varied in size (range, 11.5–52.6 mg; mean  $\pm$  SD,  $21.3 \pm 4.2$  mg), which indicates that they did not come from a single replicate.

Emergence began on 13 May 2009 at 102.8% development and finished on 18 June 2009 at 164.8% development. We detected significant effects of egg size ( $F_{[2,18]} = 5.8$ ,  $P = 0.011$ ), gravel size ( $F_{[2,18]} = 142.9$ ,  $P < 0.001$ ), and their interaction ( $F_{[4,18]} = 3.3$ ,  $P = 0.032$ ) on percentage development at emergence. Similarly, we detected significant effects of egg size ( $F_{[2,18]} = 6.4$ ,  $P = 0.008$ ) and gravel size ( $F_{[2,18]} = 185.5$ ,  $P < 0.001$ ) on median day of emergence, as well as a gravel size – egg size interaction ( $F_{[4,18]} = 4.3$ ,  $P = 0.013$ ). Both estimates of emergence timing indicated that individuals from small embryos emerged relatively late in finer gravels, but there was no difference among egg size treatments in emergence time in large gravel (Fig. 3a).

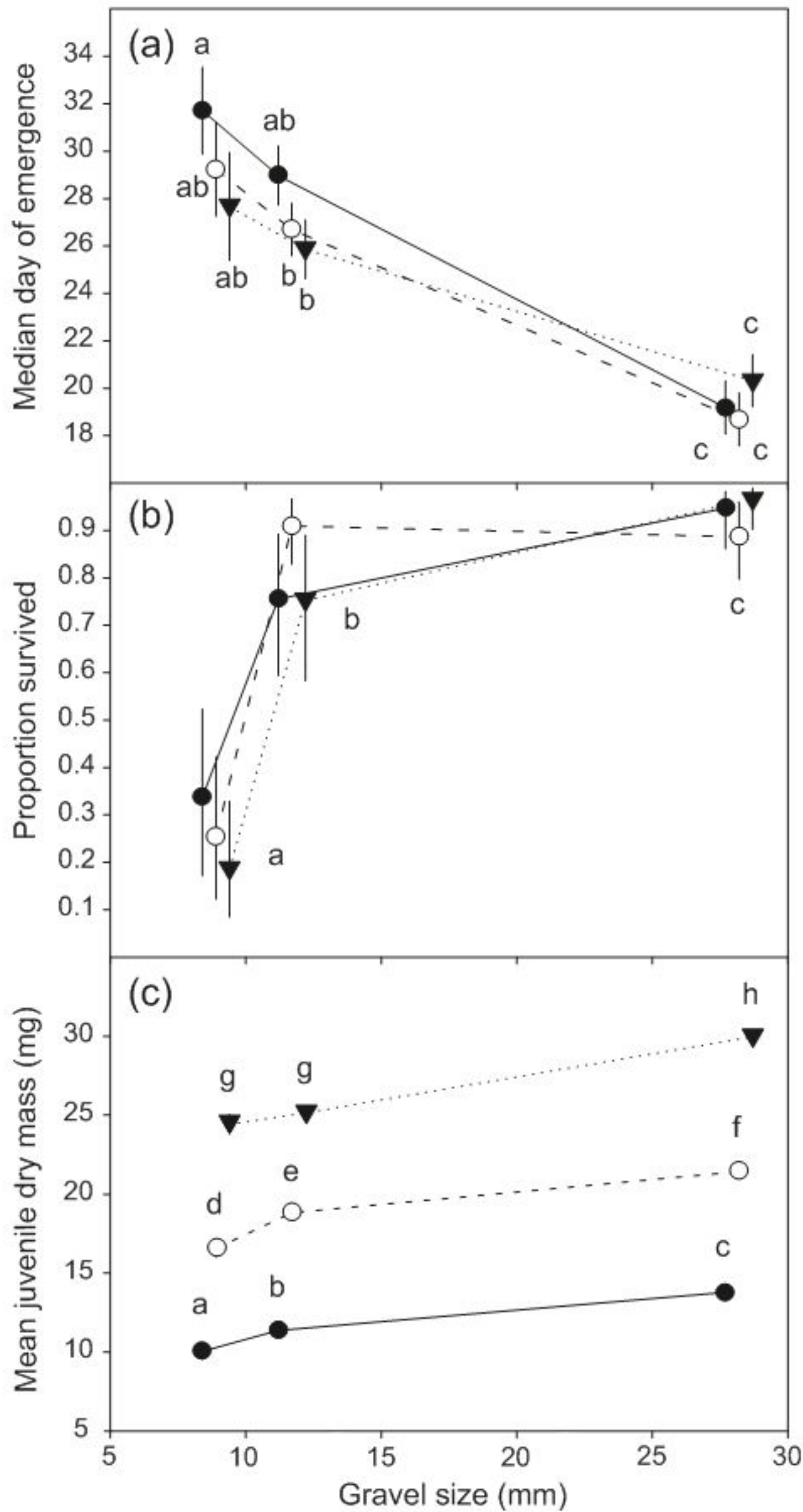
Survival increased with increasing gravel size ( $F_{[2,18]} = 66.5$ ,  $P < 0.001$ ), but survival was not related to egg size ( $F_{[2,18]} = 0.52$ ,  $P = 0.60$ ), and the egg size – gravel size interaction was not significant ( $F_{[4,18]} = 2.6$ ,  $P = 0.070$ ;

Fig. 3b). Dry mass at emergence was significantly affected by egg size ( $F_{[2,18]} = 3696.6$ ,  $P < 0.001$ ) and gravel size ( $F_{[2,18]} = 487.6$ ,  $P < 0.001$ ), and the egg size – gravel size interaction was significant ( $F_{[4,18]} = 19.5$ ,  $P < 0.001$ ). For large-egg treatments, juvenile mass did not differ between medium-gravel and small-gravel treatments, apparently because juveniles were smaller than expected in the large-egg – medium-gravel treatment. Differences in mass were apparent for medium- and small-egg treatments at each level of gravel size (Fig. 3c).

Specific energy of juveniles averaged  $21.86 \pm 0.12$  kJ·g<sup>-1</sup> across all treatments. We detected a significant effect of egg size ( $F_{[2,18]} = 4.8$ ,  $P = 0.021$ ) and an egg size – gravel interaction ( $F_{[4,18]} = 3.4$ ,  $P = 0.031$ ). Specific energy was significantly greater in the large-egg – small-gravel treatment compared with the small-egg – small-gravel and small-egg – medium-gravel treatments, but there was no difference among treatments in large gravel (Fig. 4a). To obtain total energy of juveniles (in joules), we multiplied the specific energy value obtained from each replicate by the mean dry mass of juveniles emerging from that replicate. In the subsequent ANOVA, we observed significant effects of egg size ( $F_{[2,18]} = 1166.5$ ,  $P < 0.001$ ), and gravel size ( $F_{[2,18]} = 145.4$ ,  $P < 0.001$ ), and the egg size – gravel size term was significant ( $F_{[4,18]} = 4.1$ ,  $P = 0.015$ ). Total energy increased with increasing gravel size for the medium-egg treatments, but total energy was not different among the two smaller gravel sizes for both small and large eggs. Within egg-size treatments, total energy was always greatest in large gravel (Fig. 4b).

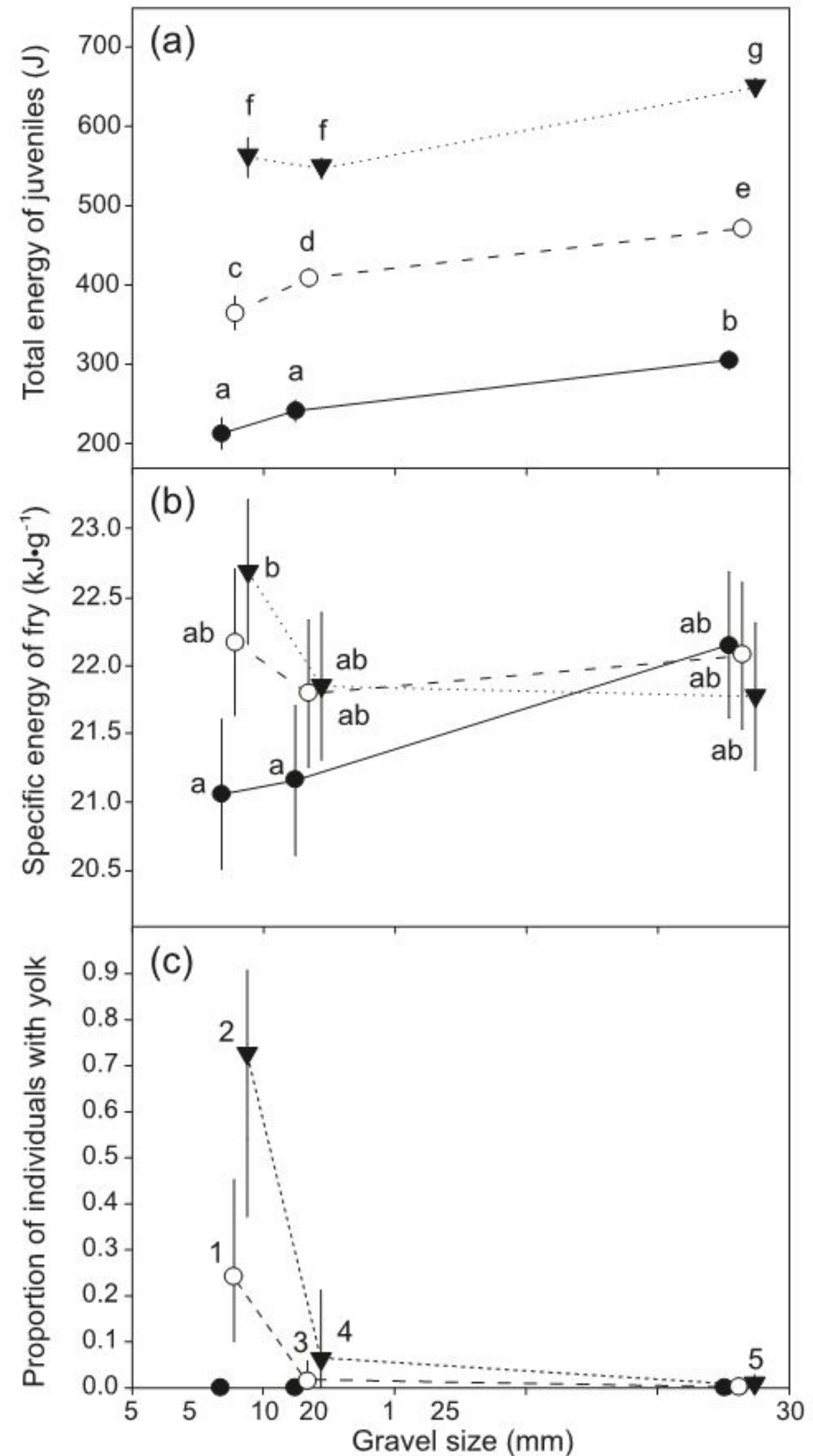
Individuals from small embryos never emerged with a yolk sac at any level of gravel size, whereas many individuals from medium and large embryos emerged from small gravels with a yolk sac (Fig. 4c). Accordingly, the proportion of fry emerging with a yolk sac (“proportion with yolk”) was zero-heavy and not normally distributed (Kolmogorov–Smirnov test statistic = 0.352,  $P < 0.001$ ). These data pose a statisti-

**Fig. 3.** (a) Median day of juvenile emergence for each level of egg size and gravel. Note that post-hoc tests show an identical pattern of significance when emergence time is expressed as percentage development. (b) Embryo-to-juvenile survival as a function of egg size and gravel size. Proportions are back-transformed from logits. Note that only the main effect of gravel was significant, and that the single letter at each level of gravel size denotes differences for the main effect of gravel size. (c) Mean dry mass of juveniles. Solid circles, small eggs; open circles, medium eggs; solid triangles, large eggs. Letters denote significant differences after a Tukey's honestly significant difference (HSD) test; means are least-squares means and error bars are 95% confidence intervals.



cal problem, as they indicate an invariance of “proportion with yolk” in the small-egg level of the egg-size factor. Most statistical tests assume that data are drawn from populations with the same distribution of data, even if the distribution is non-normal. In our case, the proportion of individuals emerging with a yolk sac from small-egg treatments was always zero, and hence distribution-free, so a more appropriate approach is to calculate the probability of drawing a zero from a population with a mean and standard deviation equal to

**Fig. 4.** (a) Least squares mean total energy of juveniles. (b) Least squares mean specific energy of juveniles. (c) Weighted mean proportion of individuals that emerged from the gravel with a visible yolk sac as a function of gravel size and egg size. Data are back-transformed from logits. Values identify one-tailed  $z$  tests ( $\alpha = 0.01$ ) performed on logit-transformed data, which evaluated whether non-zero means were significantly greater than zero (1,  $z = -2.72$ ,  $P = 0.003$ ; 2,  $z = -3.11$ ,  $P = 0.0009$ ; 3,  $z = -0.57$ ,  $P = 0.28$ ; 4,  $z = -1.09$ ,  $P = 0.14$ ; 5,  $z = -0.56$ ,  $P = 0.28$ ). Solid circles, small eggs; open circles, medium eggs; solid triangles, large eggs. Letters denote significant differences after a Tukey's honestly significant difference (HSD) test; error bars are 95% confidence intervals.



those observed in the other treatments. To evaluate whether treatments with a nonzero mean were significantly greater than zero, we used one-tailed  $z$  tests,

$$(2) \quad z = (x_i - \bar{x}) \times \sigma^{-1}$$

where  $x_i$  is 0,  $\bar{x}$  is the weighted mean of a given treatment, and  $\sigma$  is the weighted standard deviation (Zar 1984, p. 84). We also applied a Bonferroni correction to account for multiple paired comparisons (five tests were performed, so  $\alpha =$

0.01). Weighted mean proportion of individuals emerging with a yolk sac was greater than zero for the large-egg–small-gravel treatment ( $\text{logit}(\bar{x} + 0.025) = 1.20$ ,  $\text{logit}(\sigma + 0.025) = 1.56$ ,  $\text{logit}(x_i + 0.025) = -3.66$ ,  $z = -3.11$ ,  $\alpha = 0.01$ ,  $P = 0.0009$ ) and for the medium-egg–small-gravel treatment ( $\bar{x} = -0.933$ ,  $\sigma = 1.00$ ,  $x_i = -3.66$ ,  $z = -2.72$ ,  $\alpha = 0.01$ ,  $P = 0.003$ ), whereas other treatments were not greater than zero (Fig. 4c). This suggests an egg size – gravel size interaction, as there is no difference among levels of egg size in larger gravels, but “proportion with yolk” increases in juveniles from medium and large eggs when gravel size is small (Fig. 4c). Finally, “proportion with yolk” correlated positively with specific energy (Spearman’s  $\rho = 0.516$ ,  $n = 27$ ,  $P = 0.006$ ), indicating that juveniles emerging with yolk sacs had a higher specific energy.

Six days after the last juvenile emerged from the gravel (24 June 2009), we assumed that all larvae surviving within the replicates were unable to escape (entombed) and excavated all replicates. We analyzed these data to provide insight into the mechanism(s) of mortality within the gravel (e.g., oxygen limitation versus entombment). All live individuals detected were emaciated, suggesting entombment. Only four live individuals were found in the nine medium-gravel replicates, all four of which were in two large-egg replicates. No live individuals were found in any of the large-gravel replicates. Hence, we analyzed entombment data only within small gravels using a one-way ANOVA, where we tested the effect of egg size. The percentage of live individuals entombed in small-, medium-, and large-egg treatments was 11.1%, 12.3%, and 23.7%, respectively, and these values are inversely related to survival to emergence in small gravels (Fig. 3b). The one-way ANOVA was not significant ( $F_{[2,6]} = 2.5$ ,  $P = 0.16$ ), however, which indicates that survival after entombment was not related to egg size in small gravel.

Dead individuals were too decomposed to determine stage-at-death (i.e., yolk-sac vs. non-yolk-sac larvae). Not surprisingly, the percentage of dead individuals found after excavation was higher in small gravel ( $F_{[1,12]} = 53.4$ ,  $P < 0.001$ ) but unaffected by egg size ( $F_{[2,12]} = 2.2$ ,  $P = 0.15$ ) or their interaction ( $F_{[2,12]} = 1.4$ ,  $P = 0.29$ ). Note that no dead individuals were detected in the large-gravel replicates, so the large-gravel level of the gravel size factor was not included in the preceding analysis.

## Discussion

In the present study, we test whether interpopulation variation in egg size could be maintained by size-specific survival of embryos or larvae in particular incubation substrates. We observed that the effect of egg size on offspring phenotypes differed markedly among incubation substrates: the egg size – gravel size interaction term was significant in four of five ANOVAs. Although our survival data suggest that individuals from small embryos fared similarly in both small and medium gravel (which cannot be said of individuals from medium and large embryos), the relevant interaction term for this effect was not significant ( $P = 0.07$ ). Our data do not, therefore, support our focal hypothesis, but they instead intimate the possibility that a novel mechanism may effect divergent selection on egg size among populations of salmonids. Below, we re-evaluate traditional hypotheses for the egg

size – gravel size correlation and examine whether our data can help explain this correlation.

First, we observed interactions among egg size and specific energy, day of emergence, and percentage of juveniles emerging with a yolk sac. These interactions are not independent, as both specific energy and emergence time may correlate with percentage yolk (developmental stage) within gravel size fractions. These interactions broadly reflect a positive correlation between initial egg size and the proportion of juveniles emerging with yolk in smaller gravels and a lack of this correlation in larger gravels. Moreover, small larvae emerged relatively late in small gravel, but not in larger gravels, and this is reflected in the lower specific energy (or percentage yolk) of small larvae in small gravels. Overall, these patterns suggest that relatively large larvae emerge prematurely in small incubation gravels, whereas small larvae delay emergence until yolk absorption is complete. A common observation is indeed that premature emergence occurs when incubation gravels are small (Phillips et al. 1975; Witzel and MacCrimmon 1983; Olsson and Persson 1986) and that larger larvae take longer to absorb their larger yolk sacs (Killeen et al. 1999). Our findings are novel, however, in that premature emergence in stressful environments is much more common among larger larvae.

Flow in artificial incubators can be largely confined to the smooth incubator walls when gravel size is small (Rose and Rizk 1949; Witzel and MacCrimmon 1983), and given that larval mobility is restricted in smaller gravels (Dill and Northcote 1970), oxygen stress may have occurred in the present study. Assuming zero mortality in our replicates, estimated total oxygen consumption for small-, medium-, and large-egg replicates was 0.0853, 0.141, and 0.191  $\text{mg}\cdot\text{h}^{-1}$ , respectively, at the eyed embryo stage, and 0.0985, 0.261, and 0.479  $\text{mg}\cdot\text{h}^{-1}$ , respectively, just after hatching (i.e., the beginning of the larval stage) (calculations following Rombough 2007). For large-egg replicates, this suggests an increase of 60.1% in oxygen consumption from the embryo stage to the larva stage compared with a 13.4% increase for small-egg replicates. Of course, we did not detect a size-related difference in survival to emergence or in the number of larvae surviving while entombed in small gravels at the end of the experiment, so if a size-related oxygen effect occurred, it was likely sublethal.

Yet it is unlikely that size-related patterns of emergence reflect different hypoxic tolerances of small and large embryos. Small embryos have indeed been long expected to fare better in low-oxygen environments because of larger ratios of surface area to volume, which was presumed to translate into more favourable rates of gas exchange (Krogh 1959); however, this supposition has been discredited. Although geometric constraints dictate that egg and embryo surface areas increase at a rate of  $(\text{embryo mass})^{0.67}$ , the rate of metabolic expansion is much slower ( $b = 0.44$  (Einum et al. 2002);  $b = 0.30$  (Rombough 2007)) such that larger embryos actually have a greater surface area for gas exchange relative to metabolic oxygen demand. Despite this difference, embryo size has no effect on hypoxic tolerance during the embryo stage, so it is unlikely that embryo surface area is a major factor limiting oxygen uptake (Rombough 2007; but see Einum et al. 2002). Size-related differences in hypoxic tolerance during the larva stage are also unlikely to result in adaptive, interpo-

pulation variation in egg size. Although hypoxic tolerance is indeed lower for larvae from larger embryos, this effect is confined to the first half of the larval period when gills are underdeveloped and oxygen acquisition must be primarily cutaneous. Moreover, this effect of initial egg size on larval hypoxic tolerance is very small (Rombough 2007), so it is unlikely that hypoxic tolerance per se is responsible for the patterns observed in the present study.

On the other hand, metabolic rate increases relatively quickly with size in larvae, so a small larva requires less oxygen per unit of time than a larger larva, all else being equal (Rombough 2007). Assuming larval oxygen consumption scales at the rate of (embryo mass)<sup>0.62</sup> (Rombough 2007), we estimate that the per-capita oxygen consumption is roughly 16.7% higher in the sockeye population with the largest eggs compared with that with the smallest (Quinn et al. 1995). This difference may be important in small incubation gravels where flow rates are relatively low such that smaller larvae could persist in a given interstitial space for a longer period before running low on oxygen. We observed that total energy of juveniles increased with gravel size, which is indeed consistent with lower yolk-conversion efficiency in small-gravel environments (e.g., Olsson and Persson 1986) or at lower levels of dissolved oxygen per se (e.g., Mason 1969). We suggest that oxygen depletion rates by larvae, rather than hypoxic tolerance per se, may explain why large juveniles emerge prematurely from small gravels. In this scenario, however, larval biomass per unit area is likely an important factor that influences oxygen availability, but unfortunately, Quinn et al. (1995) do not report whether larval density (or fecundity) varies with gravel size among their focal populations.

If size-specific oxygen use by larvae is responsible for the patterns of emergence observed in the present study, then a novel hypothesis for the egg size–gravel size correlation arises. Although the relative importance of residual yolk and size-at-emergence on the probability of depredation is not understood, there is credible evidence to suggest that yolk-sac juveniles fare worse when depredation is high. For example, in a large-scale release programme spanning two years, juvenile survival was consistently and inversely related to a metric of residual yolk content, whereas selection on juvenile size was inconsistent and variable in direction (Fresh and Schroder 1987). An analysis of predator stomach contents in this study suggested that individuals with yolk sacs were being preferentially depredated (Fresh and Schroder 1987). Moreover, laboratory experiments have shown that yolk-sac larvae generally have a slower escape response than their fully developed counterparts (Hale 1996, 1999). If yolk-sac larvae are indeed more prone to depredation, then small individuals may have greater fitness when incubated in small gravels because they emerge as juveniles not as yolk-sac larvae. Although it is clear that yolk-sac juveniles also emerge under natural conditions (García De Leániz et al. 2000), our hypothesis depends critically on the benefits of increased size-at-emergence versus the propensity of these larger individuals with yolk sacs to be depredated, so further testing is certainly warranted.

Alternatively, the patterns observed in the present study may have nothing to do with the egg size–gravel size correlation observed by Quinn et al. (1995). First of all, it is not

clear that a propensity to deplete local oxygen reserves in gravel interstices will necessarily result in premature emergence for larger larvae, as hypothesized above. Compaction stress in fine gravels, on the other hand, may encourage larvae of all sizes to emerge prematurely (Witzel and MacCrimmon 1983), but it may concomitantly result in delayed emergence in small larvae if small larvae are weaker and have more difficulty navigating the small interstitial spaces. This mechanism can explain every pattern that we observed in the present study, and this explanation is perhaps more parsimonious than the oxygen-stress hypothesis described above. It seems less likely that adaptive interpopulation egg-size variation would be maintained by size asymmetries in larval strength, as this explanation suggests that small juveniles expend more energy emerging from small gravels, which may have subsequent effects on growth and survival (e.g., Dias and Marshall 2010). Clearly, more research is needed to understand both the mechanism responsible for the pattern of emergence observed in the present study and to understand the egg size–gravel size correlation.

Another long-standing hypothesis for the egg size–gravel size correlation is that egg and embryo predators such as sculpins (*Cottus* sp.) and gobies (*Neogobius* sp.) are better able to penetrate larger gravels, such that large eggs might be favoured in large gravels if predators are gape-limited (Quinn et al. 1995; Einum et al. 2002). We think that this hypothesis is unlikely. Although egg and embryo predators may be an important source of mortality (Foote and Brown 1998), there is little evidence that gobies and sculpins can differentially penetrate the gravel sizes described in the 21 populations of sockeye salmon (Biga et al. 1998; Chotkowski and Marsden 1999; Palm et al. 2009). Moreover, assuming that egg diameter ( $d$ ; in mm) scales to egg wet mass according to the equation given by Einum and Fleming (2002),

$$(3) \quad d = \left(6 \times v \times (1/\pi)\right)^{1/3}$$

where  $v$  is egg volume, and given that the range of egg masses described by Quinn et al. (1995) was 84.5–113.5 mg, this hypothesis requires that the predators be gape-limited to the extent that an estimated 0.56 mm difference in egg or embryo diameter decreases the likelihood of depredation in larger gravels.

A recent study also supports the finding that size-related survival of embryos and larvae may not occur in incubation gravels of different sizes (Rollinson and Hutchings 2011), though this study was limited in scope. The present study is a more thorough test of the focal hypothesis, but we note that it has three important drawbacks, any of which may explain why we observed no pattern of size-related survival. First, we cannot exclude the possibility that the original sockeye salmon populations (Quinn et al. 1995) exhibit traits at the embryo, larval, or maternal stage that act in concert with variation in egg size to effect the positive egg size–gravel size correlation. For example, the depth at which mothers bury their embryos may vary with spawning substrate (e.g., Kon-dolf and Wolman 1993), but we buried embryos at a depth of 23 cm at all levels of gravel size, which is the average burial depth of Atlantic salmon embryos (Devries 1997). Burial depth may correlate negatively with dissolved oxygen levels (e.g., Ingendahl 2001; Malcolm et al. 2004; but see Peterson

and Quinn 1996) and given that large larvae have a higher per-capita oxygen consumption (Rombough 2007), mothers may do well to spawn small eggs in shallow nests when gravel size is small and flow is limited. We note that survival in our smallest gravel was very low at all levels of egg size, which underlines the possibility that the present study did not effectively emulate conditions in the wild. Second, the gravel sizes used in the present study were homogenous, and this is unlikely to occur in nature. A silt, sand, and cobble mixture, for example, may have the same geometric mean gravel size a homogeneous pebble mixture, but the two mixtures may not have the same effects on offspring survival or phenotypes (Tappel and Bjornn 1983). Our results may have differed if we had more closely emulated gravel mixtures found in the wild rather than using homogenous gravel mixtures. Third, and with the other caveats in mind, it is difficult to overlook the possibility that we committed a type II error with respect to size-related survival in different gravel size fractions. The relevant interaction term approached significance ( $P = 0.07$ ), and small embryos appeared to fare similarly in both small and medium gravels, which would provide at least some support to the focal hypothesis.

In summary, our data do not support the hypothesis that size-related entombment of salmonid larvae occurs (Quinn et al. 1995; Quinn 2005), though caveats particular to the present experiment may have precluded a proper test of the focal hypothesis. We identify the possibility that interpopulation variation in egg size is maintained by size-related oxygen demand of larvae coupled with a propensity for larger larvae to deplete oxygen reserves more quickly in fine gravels, which ultimately results in premature emergence of large larvae from small gravels. It is also possible that large larvae emerge sooner from small gravel because they are simply stronger than larvae from small embryos, in which case it seems less likely that the pattern that we observed in the present study can explain the egg size–gravel size correlation. The present study shows that the mechanism(s) involved in effecting the positive correlation between egg size and gravel size, if any, may be more complex than originally anticipated.

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