

1 **Offspring size effects vary over fine spatio-temporal scales in Atlantic**

2 **salmon (*Salmo salar*)**

4 **Grethe Robertsen, Helge Skoglund and Sigurd Einum**

6 **G. Robertsen** (corresponding author, e-mail: grethe.robertsen@nina.no, phone number: +47
7 98464786). Centre for Conservation Biology, Department of Biology, Norwegian University
8 of Science and Technology, Realfagbygget, NO-7491 Trondheim, Norway; present address:
9 Norwegian Institute for Nature Research, Tungasletta 2, NO-7047 Trondheim, Norway.

11 **H. Skoglund** (e-mail: helge.skoglund@uni.no). Laboratory of Freshwater Ecology and Inland
12 Fisheries, Uni Research, Thormøhlensgt 49, NO-5006, Bergen, Norway.

14 **S. Einum** (e-mail: sigurd.einum@bio.ntnu.no). Centre for Conservation Biology, Department
15 of Biology, Norwegian University of Science and Technology, Realfagbygget, NO-7491
16 Trondheim, Norway.

23 Running title: Offspring size effects vary over fine spatio-temporal scales

Abstract: Classic offspring-size theory predicts that a single level of investment per offspring maximizes parental reproductive success in a given environment. Yet, substantial variation in offspring size is often observed among females within populations. Variation at this scale may occur because spatio-temporal variation in stabilizing selection prevents erosion of genetic variation. We tested if patterns of size-specific offspring survival of Atlantic salmon (*Salmo salar*) varies across location and season within a short stretch of a natural stream by manipulating the emergence timing of juveniles from 12 families with different mean egg sizes and assessing their performance at two locations. The relationship between egg size and juvenile survival varied temporally and spatially: large eggs were advantageous for early emergers in one location, whereas egg size had no effect in the other. Furthermore, the performance of later emerging juveniles did not depend on egg size in either location, possibly because the early emergers had grown or established territories. Thus, selection on offspring size can be complex and vary across short periods of time and small geographic distances, thereby preventing the erosion of genetic variation expected under consistent stabilising selection.

Key words: breeding timing, density-dependent selection, maternal effects, natural selection

Introduction

Parental fecundity and investment per offspring tend to be positively correlated with parental fitness. However, these traits trade off, such that parents experience stabilizing selection on investment per offspring. Smith and Fretwell (1974) proposed that, in a given environment, one level of per offspring investment will optimize the trade-off between size and number of offspring, thereby maximizing parental reproductive success. Yet, despite this prediction, a common observation is that offspring size varies among parents in a population. Identifying mechanisms responsible for maintaining this variation has received much attention in evolutionary biology. Theoretical models predicting such variation are primarily based on the assumption that different mothers provide different environmental conditions for their offspring, and that this influences size optima (Parker and Begon 1986; McGinley et al. 1987; Hendry et al. 2001). Indeed, large offspring size has been found to be particularly advantageous under adverse conditions (e.g. Fox 2000; Wilson et al. 2009; Monro et al. 2010) or under high population densities (Coltman et al. 1999; Marshall et al. 2006; Svanbäck and Persson 2009). Thus, spatial heterogeneity in environmental conditions within populations is a good candidate mechanism (Levene 1953; Bulmer 1971; Gillespie and Turelli 1989). Temporal variation in environmental conditions is also expected to have the potential to conserve within-population variation in fitness-related traits (Ellner and Hairston 1994; Ellner and Sasaki 1996). Even though there is empirical support that both temporal and spatial variation in selection pressures can contribute to maintain genetic variation in fitness-related traits (Kittelson and Maron 2001; Siepielski et al. 2009) few studies address how these can work in concert to shape selection for offspring size. This is particularly relevant for organisms that live in seasonal environments where important environmental characteristics can vary across small spatial scales as well as within one year. Under such circumstances the

location and timing of reproduction could be expected to interact and shape the pattern of offspring size selection.

Stream-rearing salmonid fishes are one group of organisms that are well suited for studying spatial and temporal variation in selection on offspring size. First, abiotic and biotic environmental conditions in nursery streams can vary considerably across space within populations (Arnekleiv et al. 2006; Finstad et al. 2009). This includes variation in juvenile densities and competitive intensity over small spatial scales (Einum et al. 2011) caused by patchy egg distribution (Finstad et al. 2010) and limited juvenile movements (Einum et al. 2008a; Foldvik et al. 2010). Second, the breeding season typically stretches over a long period of time (up to 10 weeks within populations of Atlantic salmon (*Salmo salar* L., Fleming 1996), which results in considerable variation in timing of emergence from nests (Garcia de Leaniz et al. 2007). Depending on their timing of emergence the environmental conditions experienced by the juveniles can vary over the season (Brännäs 1995). Juvenile density is, for instance, expected to increase across the season as more juveniles hatch and enter the population. Finally, even though the evolution of offspring size has been the subject of much interest in stream-rearing salmonid fishes (e.g. Hutchings 1991; Kinnison et al. 2001; Rollinson and Hutchings 2010), interactive effects of egg size, distribution of breeding and timing of emergence remain unknown.

In a recent field study the performance of Atlantic salmon juveniles from groups varying in emergence timing (experimentally manipulated) differed between locations within the same stream (Skoglund et al. 2011a). Spatial variation in intraspecific competition was identified as the most important factor responsible for this pattern (Skoglund et al. 2011a). The present study is partly based on the same data as Skoglund et al. (2011a), but utilising additional information on the juvenile's family origin (based on microsatellite genotyping), and hence egg size. The combined use of egg size data and experimental manipulation of both

spatial distribution of eggs in the river and emergence timing allowed us to study how distribution and timing of breeding together shape the patterns of selection on offspring size.

Methods

Experimental fish

Twenty-four hatchery reared Atlantic salmon from the Imsa population (12 females and 12 males) with a mean weight of 2400 g were used to create 12 full-sib family groups at the Norwegian Institute for Nature Research (NINA) Research Station, Ims, Norway on 22 November 2007. At the same time, samples of the parents' adipose fins were taken and stored in ethanol for later genetic analyses (see electronic supplementary material, Genotyping and parental allocation). Fifteen unfertilized eggs were sampled from each family and fixed in a 4% formalin buffer and weighed to the nearest 0.1 mg. Egg mass differed significantly among the families (ANOVA: $F_{12,168} = 5917.8$, $P < 0.005$, range in mean values: 0.12 – 0.17 g). To create offspring with normal, early and late emergence relative to the wild River Imsa population, the fertilized eggs were divided into three groups, consisting of the same number of eggs from each family, which were incubated at different temperatures. The group with normal emergence timing was incubated in ambient temperature water (average incubation temperature 4.4 °C) drawn from Lake Liavatn (source of the River Imsa), whereas the early and the late emerging groups were incubated in heated (7 °C) or cooled water (3.7 °C), respectively. According to predictions based on a development model (Crisp 1981, 1988) and daily incubation temperatures, this resulted in median dates of emergence for the early, normal and late groups at 4th April, 7th May and 29th May, respectively. The development model has previously been shown to accurately predict dates at emergence across a wide range of temperature regimes for this population (Skoglund et al. 2011b).

At the eyed stage the resulting embryos had their otoliths marked with a fluorescent dye by immersion of the eggs for 8 h in a solution of 175 mg L⁻¹ alizarin red S (Baer and Rösch 2008). During the immersion procedure a water pump and two air pumps ensured that the water was adequately mixed and aerated. Depending on emergence timing group the alevins received one, two or three fluorescent marks (by repeating the procedure at intervals of 16-23 days). This marking procedure is commonly used to mark large numbers of fish (Wright et al. 2002) and is not known to influence embryonic survival or growth (Baer and Rösch 2008).

Study site and experimental design

The experiment was carried out in the Stream Osalandsbekken (Fig. 1a), a tributary to the River Imsa (the origin of the fish used in this study). This stream has no natural salmon population due to a migration barrier, but has a natural population of resident brown trout (*S. trutta*, see Einum et al. 2006 for details). Alevins from all timing groups were outplanted across two stream reaches (locations) in well aerated artificial nests (Vibert®, Federation of Fly Fishermen, Bozeman, MT, USA) at different times (early 13 March; normal 22 April; late 21 May) but at similar developmental stages. At outplanting the alevins had undergone 90.7 % (early), 84.1 % (normal) and 87.6 % (late) of the development from fertilization to emergence (based on development model of Crisp 1981, 1988). To ensure variation in environmental conditions between these two locations in addition to the potential effects of non-measured variables (e.g. food availability, habitat suitability) we stocked out 3600 alevins in either of two spatial configurations known to have different effects on competitive regimes, food availability and mortality rates (see Einum & Nislow 2005; Einum et al. 2008a). First, we stocked out 72 individuals (2 individuals × 12 families × 3 timing groups) in 25 nests that were spaced 12.5 m apart (Fig 1b). The even distribution of alevins across this relatively long

stream reach should lead to overall low juvenile densities, good growth conditions and high survival. Approximately 750 m downstream from the lowermost nest in the upper location we planted out 1800 alevins (50 individuals \times 12 families \times 3 timing groups) in a single nest (Fig. 1b). Due to limited dispersal of salmon fry the high number of alevins located at this location should result in depleted food resources and elevated mortality rates (e.g. Einum et al. 2008a). In the following these two reaches will be referred to as the low density and the high density location, respectively.

Sampling and lab analyses

During 7-8 July 2008 the stream was divided in 26 sections of about 50 m which were electrofished one to four times depending on the number of salmon caught. Salmon juveniles captured were killed by an overdose of anaesthetics (Clove Oil Extract, $>150 \text{ mg L}^{-1}$) and frozen for later processing, whereas brown trout were released back into the stream. In the lab, the salmon juveniles were weighed ($\pm 0.1 \text{ mg}$), sampled for subsequent genetic analyses (see electronic supplementary material, Genotyping and parental allocation), and identified to emergence timing groups. The timing group identity of most fish from the late timing group ($<1.1 \text{ g}$) and the largest fish from the early group ($>3.9 \text{ g}$) was obvious from the size distributions. Fish in the size range where there was overlap between the timing groups (549 individuals) were identified by otolith analysis. This was done by first extracting otoliths from the fish and mounting them onto slide glasses with a transparent adhesive (Crystalbond, Buehler, Lake Bluff, IL, USA). They were then polished with grit paper and analysed for dye marks with an epifluorescent microscope (Wright et al. 2002).

Statistics

Apparent survival was calculated as the number of captured individuals of each family from each timing group in each of the two experimental locations. Because specific growth rate depends on body size we used the standardized mass-specific growth rate (Ω %) (Ostrovsky 1995):

$$\Omega = \frac{M_t^b - M_0^b}{b \times t} \times 100,$$

where M_t is final weight, M_0 is the estimated initial weight at emergence for each family (see below), b is the allometric mass exponent for the relationship between specific growth rate and body mass (estimated to be 0.31 for Atlantic salmon juveniles, Elliott and Hurley 1997) and t is the time between date of emergence (estimated using Crisp 1981, 1988) and sampling. To calculate mean fry weight at emergence for each family we used mean family egg size (adjusted for 16.4 % increase in mass due to water absorption, Einum and Fleming 2000) and the relationship between egg mass and mass at emergence (mass at emergence [g] = 0.880 + 0.881 \times ln egg mass, Einum 2003).

All statistical analyses were conducted in R, v. 2.11.1. (R Development Core Team 2010).

The effect of mean family egg mass on three performance proxies (apparent survival, final mass and growth) was tested in separate models.

To test for an effect of mean family egg mass on survival we used a generalized linear mixed model (GLMM) with a poisson error structure (using the function *lmer* from the *lme4* package, Bates and Maechler 2010) and family as a random factor (intercept). The initial fixed factors included the main effects of mean family egg mass, timing group, location and all interaction effects. To test the effect of mean family egg mass on final mass we used a linear mixed effects model (using the function *lme* from the *nlme* package, Pinheiro et al.

2009) that included family as a random factor (intercept). The fixed factors in the initial model were the main effects of mean family egg mass, timing group, location and their interactions. Both final mass and egg mass were ln-transformed prior to analysis. Variation in growth rate was analysed using a similar model, but where none of the variables were transformed. To account for heterogeneity of residuals among the timing groups in the latter model we applied the function *varIdent* from the *nlme* package. This led to a decrease in AIC ($\Delta AIC = 179.0$, comparison done with models fitted using Restricted Maximum Likelihood).

For the three initial models, the fixed effects were assessed using a backwards selection procedure (Zuur et al. 2009). Thus, starting with a global model (fitted with Maximum Likelihood), fixed factors were sequentially removed and the resulting simpler models were compared to preceding models using likelihood ratio tests. This removal of fixed factors was done consecutively until the removal of any further terms resulted in a significant ($p < 0.05$) decrease in log-likelihoods.

To obtain a general measure of the upper limits of the selective forces in each location (i.e. not depending on our choice of phenotypic character, egg size), we calculated the opportunity for selection (I , Arnold and Wade 1984) as:

$$I = \frac{SD^2}{\bar{X}^2},$$

where SD^2 is the variance in apparent offspring survival (i.e. number of juveniles recaptured) among families under the different treatments (location and emergence timing) and \bar{X}^2 is the squared mean family survival within each treatment group. Confidence intervals (CI , 95%) for the values of opportunity for selection were obtained by using the 2.5% and 97.5%

quantiles from 1000 resamplings of the distribution of family-specific apparent survivals using the function *bootstrap* (from the package *bootstrap*, Leich 2007).

The statistical significance of variation in the relationship between egg mass and offspring survival among groups is best obtained through the GLMM approach described above. However, to complement these analyses we also present estimates for directional (unstandardized, β , and standardized, β') and non-linear selection gradients (unstandardized, γ , and standardized, γ') relating relative offspring survival to egg size in the different locations and timing groups based on regression coefficients (Lande and Arnold 1983).

Results

Of the 715 juvenile Atlantic salmon recaptured, 475 came from the low density and 240 from the high density location ($\chi^2 = 85.03$, $p < 0.0001$). In the low density location a migration barrier prevented upstream migration and all juveniles were captured within 100 m downstream of the lowermost nest site. In the high density location, 74 % of the captured juveniles were found within 150 m above and below the nest site. None were caught further up than 160 m above, or further down than 270 m below, the nest site. It is therefore very unlikely that individuals had moved between the localities. In addition 2435 young of the year and 839 older brown trout were caught. Of the salmon juveniles captured 666 (440 from the low density location and 226 from the high density location) were successfully genotyped and allocated to their respective family (electronic supplementary material, Genotyping and parental allocation). The remaining 7 % of the juveniles that were not allocated had insufficient marker amplification due to low quality DNA in their samples. All seven loci were in Hardy-Weinberg equilibrium.

Apparent survival

We found no significant correlation between mean family egg mass and apparent survival overall (Pearsons rank order correlation, $r = 0.28$, $T = 0.91$, d.f. = 10, $p = 0.39$). Furthermore, the GLMM that best described variation in apparent survival included only the main effect of timing group and location (comparisons of preceding models $p > 0.05$, removal of further terms $p < 0.001$, Table 1). According to this model, apparent survival depended only on locality and timing group. Removal of the three-way interaction (between egg mass, location and timing group) from the initial model, however, lead to an almost significant decrease in log-likelihood ($p = 0.051$), suggesting that survival effects of egg mass varied between the locations and among the timing groups. Furthermore, low numbers of fish were retrieved from both the normal and late timing groups, causing high uncertainty in survival estimates when individuals were divided among their original families. Since the normal and late timing groups both experienced competition with early emerging conspecifics, we merged them into a single group ('normal/late timing', termed NLT). We then performed a second model selection with an initial model identical to the first one but with the ln-transformed number of eggs per family at different locations included as an offset variable (accounting for the number of eggs in the NLT group being twice that in the early timing group). The resulting best model included all main effects together with the three-way interaction (decrease in log-likelihood for excluding any terms, $p = 0.02$). Thus, the relationship between egg mass and apparent survival depended on location and emergence timing group. According to this model, the slopes of the relationship between egg mass and apparent survival (Fig. 2) did not differ significantly from zero in either the early or the normal/late emergers at the low density location (slope values $\pm SE$ for the different timing groups [early, ET and normal/late, NLT] given on the log scale: ET = -0.22 ± 4.65 , $p = 0.96$; NLT = 4.29 ± 3.64 , $p = 0.24$). At the high density location, the relationship between egg mass and apparent survival was positive in the early timing group, and not significantly different from zero in the normal/late group (ET =

12.14 \pm 6.0, $p = 0.04$; NLT = -6.28 \pm 5.44, $p = 0.25$). Also according to this model, the slopes did not differ significantly between the low density (LD) and high density (HD) location within either the early or normal/late timing group (differences between slopes given on the log scale: HD relative to LD for ET = 12.35 \pm 7.58, $p = 0.10$; HD relative to LD for NLT = -10.57 \pm 6.55, $p = 0.11$). Neither did they differ significantly between the early and normal/late timing group in the low density location (NLT relative to ET = 4.51 \pm 5.90, $p = 0.44$). However, in the high density location, the effect of egg mass was significantly more positive in the early compared to in the normal/late timing group (NLT relative to ET = -18.41 \pm 8.09, $p = 0.02$).

Final mass and growth

Variation in final body mass was best explained by a linear mixed model including all main effects (egg mass, location and timing of emergence) together with the two-way interaction between location and timing of emergence (comparison of log-likelihoods of preceding models, $p \geq 0.1$, removal of further terms, $p \leq 0.045$). According to this model there was an overall positive effect of larger egg mass and early emergence on final mass (Table 2). In addition, the relative final mass of juveniles in the low density and high density location varied slightly depending on timing group (Table 2).

The linear mixed model that best explained variation in growth included only the main effect of emergence group (comparisons of log-likelihoods of preceding models, $p \geq 0.06$; exclusion of further terms, $p < 0.0001$). Thus, the model selection did not reveal any correlation between growth and egg mass or differences in growth between the locations (Parameter estimates \pm SE for the fixed effects were: Intercept [early timing] = 3.12 \pm 0.05, $p < 0.001$; normal relative to early timing = 1.43, \pm 0.05, $p < 0.001$; late relative to early timing = 2.08 \pm 0.08, $p < 0.001$).

Opportunity for selection

Overall, the opportunity for selection (I) was substantially lower in the low density (0.09, 95 % CI = 0.049, 0.126) than in the high density (0.26, 95 % CI = 0.138, 0.375) location. Calculations of separate values of I for the different timing groups show that this difference was particularly pronounced for the early emergers. Whereas I tended to be higher for the normal and late emergers than for the early ones in the low density location, the opposite was true for the high density location (Fig. 3).

Selection gradients

Directional selection gradients relating relative offspring survival to egg size in the different locations and timing groups were highest in the early timing group at the high density locality (Table 3). This is consistent with the patterns revealed in the GLMM where there was a significant positive effect of large egg size in the early timing group at the high density location. The estimates for non-linear selection gradients were of a positive sign for all groups (Table 3). This suggests disruptive rather than stabilizing selection, which seems unlikely to reflect real biological processes.

Discussion

By manipulating timing of emergence within family groups and assessing family-specific performance at locations within the same stream we were able to reveal spatio-temporal complexity in the selection on offspring size (egg mass) in Atlantic salmon. In a previous analysis we showed how the effect of timing of emergence on the success of the offspring varied among locations (Skoglund et al. 2011a). The results presented here provide additional support for the hypothesis that selection pressures can vary over small spatial scales (100s of

meters), as well as throughout the season. More specifically, large egg size led to survival advantages for early emergers in one location, whereas no such effect was found in the other location. Furthermore, performance of juveniles that emerged later in the season did not depend on egg size.

Even though the underlying causative agents shaping this selection pattern remain unknown in the present study, variation in competitive intensity and differences in body sizes among the different emergence timing groups represent potential candidates. Two findings in this study provide support for this statement. First, the spatial configurations of nests employed in this study have previously been shown to generate differences in competitive regimes and lead to different survival patterns (Einum and Nislow 2005; Skoglund et al. 2011a). Indeed, juveniles in the location with a spatial configuration of nests expected to result in high levels of competition (high density) had significantly lower survival than those in the locality where levels of competition were expected to be low (low density). Thus, the advantage of large egg size for early emergers in the high density locality could be due to higher levels of competition, and the lack of an effect in the low density locality due to low levels of competition. This observation is consistent with density-dependent selection theory (reviewed in e.g. Reznick et al. 2002; Einum et al. 2008b) and also with lab experiments on salmonids, showing that the competitive advantages provided by large egg size (Hutchings 1991) are only important in the presence of competition (Einum and Fleming 1999). Second, the lack of egg size effects among later emergers in the high density location can be attributed to asymmetric competition, where earlier emerging juveniles systematically won contests over later emerging ones irrespective of the relative body size of these later ones. This could be either due to the relatively large body size of early emergers achieved through growth, their prior residency (e.g. Cutts et al. 1999; Kvingedal and Einum 2011) or a combination. A similar effect of older cohorts on offspring size-performance has been found

in the side-blotched lizards *Uta stansburiana* (Svensson and Sinervo 2000). Our results also show how the opportunity for selection can vary over space and time. Consistent with the findings for egg size, the opportunity for selection was highest for the early timing group and at the high density location.

Even though there is considerable residual variation (e.g. Fig. 2) and restricted sample sizes (12 families) in this study, we find it unlikely that the pattern revealed is caused by low statistical power for two reasons. First, the finding that selection is strong at high density in the absence of older competitors, is consistent with previous studies (see above). Second, the lack of significant relationships between egg mass and survival in the other treatments (LD, ET; LD, NLT; HD, NLT) are convincing based on visual inspection of the variation, which does not suggest selection patterns to be obscured by outliers (Fig. 2).

According to previous experiments performed in Stream Osalandsbekken, the low apparent survival in the high density location relative to that in the low density location was unlikely to have been caused by habitat differences between the sites. In a previous experiment (Einum and Kvingedal 2011), equal numbers of juveniles (two size classes, mean body mass 0.35 and 2.51 g) were released at nine different sites situated 150 m apart along the stream within the stretch used in the current study. One of these sites was identical to our high-density location, and four others were within the low-density location. The estimated survival rate for the smallest size class, which is most relevant for the present study, at the location corresponding to our high density location was 46 %. At the four sites situated in our low density location the mean \pm *SD* survival rate was 29 ± 10 %. Thus, there was no indication that the high density location in the present study had a habitat providing lower survival rate than in the low density location. If anything, the trend was in the opposite direction, suggesting that the habitat in the high density site was better than that in the low density site.

Our study is likely to be highly relevant for wild salmonid populations since timing of juvenile emergence varies considerably within populations (Garcia de Leaniz et al. 2007). Furthermore, if the difference in apparent survival between the locations was induced by differences in juvenile densities, among-year variation in adult population size (e.g. Myers et al. 1998; Krkosek et al. 2011) could be predicted to shape selection patterns on a coarser temporal scale. Finally, the females distribute their eggs in patches (Geist and Dauble 1998; Moir et al. 1998) which affects the distribution of the resulting juveniles (Foldvik et al. 2010; Teichert et al. 2011). This may result in heterogeneous densities over small spatial scales (e.g. Einum et al. 2008a). Accordingly, it seems likely that egg size selection patterns can vary both temporally and spatially in natural salmon populations, even within one season. Such variation in selection patterns may, together with other processes such as variation in pre-emergence selection (Hendry and Day 2003) and straying among populations, contribute to maintenance of the observed additive genetic variation in salmonid egg size ($h^2 = 0.3 - 0.6$, Gall and Huang 1988; Su et al. 1997).

Spatio-temporal variation in selection pressures caused by differences in local environmental conditions and presence of older juveniles is presumably to a large extent unpredictable. Under such conditions, bet-hedging (reducing temporal variance in fitness) may be an adaptive offspring provisioning strategy. This may involve the production of fewer and larger offspring (conservative), or variable offspring sizes within clutches (diversified) (Philippi and Seger 1989), with the optimal alternative depending on the shape of the egg size-offspring fitness curve (McGinley et al. 1987). In the most extreme case, where egg size-offspring fitness curves are dome shaped, diversified bet-hedging may increase maternal fitness in a variable environment (Marshall et al. 2008). However, a previous study on Atlantic salmon found the relationship between offspring size and fitness to be positive and asymptotic, not dome shaped (Einum and Fleming 2000). Simulations that utilized this

positive, asymptotic fitness surface suggest that conservative bet hedging almost always yields the greatest maternal fitness, and that diversifying bet-hedging will be advantageous only in extremely variable environments (Einum and Fleming 2004). In the present study, the linear relationships between offspring size and fitness were either positive or non-existent (e.g. Fig 2). Our estimates of non-linear selection (γ') were of a positive sign, suggesting diversifying rather than stabilizing selection on offspring size, which is a pattern that seems highly unlikely to reflect real biological processes. Thus, given current estimates of non-linearity in selection gradients it is becoming increasingly clear that diversified bet-hedging (i.e. intra-clutch variation in egg size) is unlikely to represent an adaptive strategy for salmonids. If the variation in selection patterns is to some extent predictable and in accordance with the findings in the present study, fish breeding early in the season could be expected to benefit from having larger eggs than the ones breeding later in the season. To our knowledge it is not known whether egg size and breeding time are phenotypically and/or genetically correlated within salmonid populations, and future studies assessing this may yield insights into the possible role of correlational selection on these two traits.

We found an overall positive relationship between mean family egg mass and final mass across the emergence timing groups and locations, implying that the size advantages of hatching from a large egg is maintained for at least 78 days after hatching for the early, 46 days for the normal and 22 days for the late emerging juveniles. Thus, even though large egg size only provided survival advantages in the early group at one location, it may still have positive fitness effects among later emergers due to survival benefits of a large body size at later stages (e.g. increased over-winter survival, Schultz et al. 1998; Biro et al. 2004, but see Hendry et al. 2003). Growth rate was, however, not influenced by egg size but only by emergence timing.

In conclusion, we show that the relationship between egg size and juvenile survival in Atlantic salmon may depend strongly on the joint effect of two other maternal traits: the spatial distribution of eggs and the timing of breeding. Accordingly, the optimal solution to the trade-off between egg size and number may vary spatially over small scales and temporally within one season as well as among seasons. These results highlight the complexities shaping selection in general and selection on offspring size in particular. Such complexities will have pronounced impacts on the evolutionary dynamics of this trait, and may be important in maintaining genetic variation within populations.

Acknowledgements

We thank the land owners for cooperation, the staff at NINA Research Station Ims for technical assistance, Maxim Teichert and Ola Sandven for assistance during field work, Frode Fossøy and Sarah E. Yeates for advice regarding genotyping, Ivar Herfindal for statistical advice, and two anonymous reviewers for helpful comments. Financial support was provided by the Norwegian Research Council and the Norwegian University of Science and Technology. This study was conducted in accordance with national animal care guidelines under licence number 051 granted by the Norwegian Animal Research Authority to the NINA Research Station, Ims.

References

- Arnekleiv, J.V., Finstad, A.G., and Rønning, L. 2006. Temporal and spatial variation in growth of juvenile Atlantic salmon. *J. Fish. Biol.* **68**: 1062-1076.
- Arnold, S.J., and Wade, M.J. 1984. On the measurement of natural and sexual selection: Theory. *Evolution* **38**: 709-719.

439 Baer, J., and Rösch, R. 2008. Mass-marking of brown trout (*Salmo trutta* L.) larvae by
 440 alizarin: method and evaluation of stocking. J. Appl. Ich. **24**: 44-49.

441 Bates, D., and Maechler, M. 2010. lme4: Linear mixed-effects models using S4 classes. R
 442 package version 0.999375-35. <http://CRAN.R-project.org/package=lme4>

443 Biro, P.A., Morton, A.E., Post, J.R., and Parkinson, E.A. 2004. Over-winter lipid depletion
 444 and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **61**:
 445 1513-1519.

446 Brännäs, E. 1995. First access to territorial space and exposure to strong predation pressure- a
 447 conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. Evol. Ecol. **9**: 411-420.

448 Bulmer, M.G. 1971. Stable equilibria under the two-island model. Heredity **27**: 321-330.

449 Coltman, D.W., Smith, J.A., Bancroft, D.R., Pilkington, J., MacColl, A.D.C., and Clutton-
 450 Brock, T.H. 1999. Density-dependent variation in lifetime breeding success and natural
 451 and sexual selection in Soay rams. Am. Nat. **154**: 730-746.

452 Crisp, D.T. 1981. A desk study of the relationship between temperature and hatching time for
 453 the eggs of 5 species of salmonid fishes. Freshwater Biol. **11**: 361-368.

454 Crisp, D.T. 1988. Prediction, from temperature, of eyeing, hatching and swim-up times for
 455 salmonid embryos. Freshwater Biol. **19**: 41-48.

456 Cutts, C.J., Metcalfe, N.B., and Taylor, A.C. 1999. Competitive asymmetries in territorial
 457 juvenile Atlantic salmon, *Salmo salar*. Oikos **86**: 479-486.

458 Einum, S., and Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*):
 459 norms of reaction to environmental quality. P. R. Soc. Lond. B **266**: 2095-2100.

460 Einum, S., and Fleming, I.A. 2000. Highly fecund mothers sacrifice offspring survival to
 461 maximize fitness. Nature **405**: 565-567.

462 Einum, S. 2003. Atlantic salmon growth in strongly food-limited environments: Effects of
 463 egg size and paternal phenotype? Environ. Biol. Fish. **67**: 263-268.

Einum, S., and Fleming, I.A. 2004. Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evol. Ecol. Res.* **6**: 443-455.

Einum, S., and Nislow, K.H. 2005. Local-scale density-dependent survival of mobile organisms in continuous habitats: an experimental test using Atlantic salmon. *Oecologia* **143**: 203-210.

Einum, S., Sundt-Hansen, L., and Nislow, K.H. 2006. The partitioning of density-dependent dispersal, growth and survival throughout ontogeny in a highly fecund organism. *Oikos* **113**: 489-496.

Einum, S., Nislow, K.H., McKelvey, S., and Armstrong, J. D. 2008a. Nest distribution shaping within-stream variation in Atlantic salmon juvenile abundance and competition over small spatial scales. *J. Anim. Ecol.* **77**: 167-172.

Einum, S., Robertsen, G., and Fleming, I.A. 2008b. Adaptive landscapes and density-dependent selection in declining salmonid populations: going beyond numerical responses to human disturbance. *Evol. Appl.* **1**: 239-251.

Einum, S., and Kvingedal, E. 2011. Relative importance of size-based competitive ability and degree of niche overlap in inter-cohort competition of Atlantic salmon (*Salmo salar*) juveniles. *Can. J. Fish. Aquat. Sci.* **68**: 969-976.

Einum, S., Robertsen, G., Nislow, K.H., McKelvey, S., and Armstrong, J.D. 2011. The spatial scale of density-dependent growth and implications for dispersal from nests in juvenile Atlantic salmon. *Oecologia* **165**: 959-969.

Elliott, J.M., and Hurley, M.A. 1997. A functional model for maximum growth of Atlantic salmon parr, *Salmo salar*, from two populations in northwest England. *Funct. Ecol.* **11**: 592-603.

Ellner, S., and Hairston, N.G. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* **143**: 403-417.

489 Ellner, S., and Sasaki, A. 1996. Patterns of genetic polymorphism maintained by fluctuating
 490 selection with overlapping generations. *Theor. Popul. Biol.* **50**: 31-65.

491 Finstad, A.G., Einum, S., Ugedal, O., and Forseth, T. 2009. Spatial distribution of limited
 492 resources and local density regulation in juvenile Atlantic salmon. *J. Anim. Ecol.* **78**: 226-
 493 235.

494 Finstad, A.G., Einum, S., Sættem, L.M., and Hellen, B.A. 2010. Spatial distribution of
 495 Atlantic salmon (*Salmo salar*) breeders: among- and within-river variation and predicted
 496 consequences for offspring habitat availability. *Can. J. Fish. Aquat. Sci.* **67**:1993-2001.

497 Fleming, I.A. 1996. Reproductive strategies of Atlantic salmon: Ecology and evolution. *Rev.*
 498 *Fish. Biol. Fisher.* **6**: 379-416.

499 Foldvik, A., Finstad, A.G., and Einum, S. 2010. Relating juvenile spatial distribution to
 500 breeding patterns in anadromous salmonid populations. *J. Anim. Ecol.* **79**: 501-509.

501 Fox, C.W. 2000. Natural selection on seed-beetle egg size in nature and the laboratory:
 502 Variation among environments. *Ecology* **81**: 3029-3035.

503 Gall, G.A.E., and Huang, N. 1988. Heritability and selection schemes for rainbow trout –
 504 female reproductive performance. *Aquaculture* **73**: 57-66.

505 Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S.,
 506 Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F., Webb, J.H., Vøllestad, L.A.,
 507 Villanueva, B., Ferguson, A., and Quinn, T.P. 2007. A critical review of adaptive genetic
 508 variation in Atlantic salmon: implications for conservation. *Biol. Rev.* **82**: 173-211.

509 Geist, D.R., and Dauble, D.D. 1998. Redd site selection and spawning habitat use by fall
 510 chinook salmon: The importance of geomorphic features in large rivers. *Environ. Manage.*
 511 **22**: 655-669.

512 Gillespie, J.H., and Turelli, M. 1989. Genotype-environment interactions and the maintenance
 513 of polygenic variation. *Genetics* **121**: 129-138.

514 Hendry, A.P., Day, T., and Cooper, A.B. 2001. Optimal size and number of propagules:
 515 allowance for discrete stages and effects of maternal size on reproductive output and
 516 offspring fitness. *Am. Nat.* **157**: 387-407.
 517 Hendry, A.P., and Day, T. 2003. Revisiting the positive correlation between female size
 518 and egg size. *Evol. Ecol. Res.* **5**: 421-429.
 519 Hendry, A.P., Letcher, B.H., and Gries, G. 2003. Estimating natural selection acting on
 520 stream-dwelling Atlantic salmon: Implications for the restoration of extirpated
 521 populations. *Conserv. Biol.* **17**: 795-805.
 522 Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in
 523 brook trout *Salvelinus fontinalis*. *Evolution* **45**: 1162-1168.
 524 Kinnison, M.T., Unwin, M.J., Hendry, A.P., and Quinn, T.P. 2001. Migratory costs and the
 525 evolution of egg size and number in introduced and indigenous salmon populations.
 526 *Evolution* **55**: 1656-1667.
 527 Kittelson, P.M., and Maron, J.L. 2001. Fine-scale genetically based differentiation of life-
 528 history traits in the perennial shrub *Lupinus arboreus*. *Evolution* **55**: 2429-2438.
 529 Krkosek, M., Hilborn, R., Peterman, R.M., and Quinn, T.P. 2011. Cycles, stochasticity and
 530 density dependence in pink salmon population dynamics. *P. R. Soc. Lond. B* **278**: 2060-
 531 2068.
 532 Kvingedal, E., and Einum, S. 2011. Prior residency advantage for Atlantic salmon in the wild:
 533 effects of habitat quality. *Behav. Ecol. Sociobiol.* **65**: 1295-1303.
 534 Lande, R., and Arnold, S.J. 1983. The measurement of selection on correlated characters.
 535 *Evolution* **37**: 1210-1226.
 536 Leich, F. 2007. bootstrap: Functions for the Book 'An Introduction to the Bootstrap'. S
 537 original, from StatLib, by Tibshirani, R. R package version 1.0-22. [http://CRAN.R-](http://CRAN.R-project.org/package=bootstrap)
 538 [project.org/package=bootstrap](http://CRAN.R-project.org/package=bootstrap)

539 Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am.
540 Nat. **87**: 331-333.

541 Marshall, D.J., Cook, C.N., and Emlet, R.B. 2006. Offspring size effects mediate competitive
542 interactions in a colonial marine invertebrate. Ecology **87**: 214-225.

543 Marshall, D.J., Bonduriansky, R. and Bussière, L.F. 2008. Offspring size variation within
544 broods as a bet-hedging strategy in unpredictable environments. Ecology **89**:2506-2517.

545 McGinley, M.A., Temme, D.H., and Geber, M.A. 1987. Parental investment in offspring in
546 variable environments – theoretical and empirical considerations. Am. Nat. **130**: 370-398.

547 Moir, H.J., Soulsby, C., and Youngson, A. 1998. Hydraulic and sedimentary characteristics of
548 habitat utilized by Atlantic salmon for spawning in the Girnock Burn, Scotland. Fisheries
549 Manag. Ecol. **5**: 241-254.

550 Monro, K., Sinclair-Taylor T., and Marshall, D.J. 2010. Selection on offspring size among
551 environments: the roles of environmental quality and variability. Funct. Ecol. **24**: 676-684.

552 Myers, R.A., Mertz, G., Bridson, J.M., and Bradford, M.J. 1998. Simple dynamics underlie
553 sockeye salmon (*Oncorhynchus nerka*) cycles. Can. J. Fish. Aquat. Sci. **55**: 2355-2364.

554 Ostrovsky, I. 1995. The parabolic pattern of animal growth-determination of equation
555 parameters and their temperature dependencies. Freshwater Biol. **33**: 357-371.

556 Parker, G.A., and Begon, M. 1986. Optimal egg size and clutch size - effects of
557 environmental and maternal phenotype. Am. Nat. **128**: 573-592.

558 Philippi, T., and Seger, J. 1989. Hedging ones evolutionary bets, revisited. Trends Ecol. Evol.
559 **4**: 41-44.

560 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and the R Core team. 2009. nlme: Linear and
561 nonlinear mixed effects models. R package version 3.1-96.

562 R Development Core Team. 2010. R: A language and environment for statistical computing.
 563 R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available
 564 from <http://www.R-project.org>.
 565 Reznick, D., Bryant, M.J., and Bashey, F. 2002. *r*- and *K*-selection revisited: The role of
 566 population regulation in life-history evolution. *Ecology* **83**: 1509-1520.
 567 Rollinson, N., and Hutchings, J.A. 2010. Why does egg size increase with maternal size?
 568 Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo*
 569 *salar*). *Evol. Ecol. Res.* **12**: 949-960.
 570 Schultz, E.T., Conover, D.O., and Ehtisham, A. 1998. The dead of winter: size dependent
 571 variation and genetic differences in seasonal mortality among Atlantic silverside
 572 (Atherinidae: *Menidia menidia*) from different latitudes. *Can. J. Fish. Aquat. Sci.* **55**:
 573 1149-1157.
 574 Siepielski, A.M., DiBattista, J.D., and Carlson, S.M. 2009. It's about time: the temporal
 575 dynamics of phenotypic selection in the wild. *Ecol. Lett.* **12**: 1261-1276.
 576 Skoglund, H., Einum, S., and Robertsen, G. 2011*a*. Competitive interactions shape offspring
 577 performance in relation to seasonal timing of emergence in Atlantic salmon. *J. Anim. Ecol.*
 578 **80**: 365-374.
 579 Skoglund, H., Einum, S., Forseth, T. and Barlaup, B.T. 2011*b*. Phenotypic plasticity in
 580 physiological status at emergence from nests as a response to temperature in Atlantic
 581 salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **68**:1470-1479.
 582 Smith, C.C., and Fretwell, S.D. 1974. Optimal balance between size and number of offspring.
 583 *Am. Nat.* **108**: 499-506.
 584 Su, G.S., Liljedahl, L.E., and Gall, G.A.E. 1997. Genetic and environmental variation of
 585 female reproductive traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **154**: 115-
 586 124.

587 Svanbäck, R., and Persson, L. 2009. Population density fluctuations change the selection
588 gradient in Eurasian perch. *Am. Nat.* **173**: 507-516.

589 Svensson, E.I., and Sinervo, B. 2000. Experimental excursions on adaptive landscapes:
590 density-dependent selection on egg size. *Evolution* **54**: 1396-1403.

591 Teichert, M.A.K., Foldvik, A., Forseth, T., Ugedal, O., Einum, S., Finstad, A.G., Hedger,
592 R.D., and Bellier, E. 2011. Effects of spawning distribution on juvenile Atlantic salmon
593 (*Salmo salar*) density and growth. *Can. J. Fish. Aquat. Sci.* **68**: 43-50.

594 Wilson, A.J., Pemberton, J.M., Pilkington, J.G., Clutton-Brock, T.H., and Kruuk, L.E.B. 2009.
595 Trading offspring size for number in a variable environment: selection on reproductive
596 investment in female Soay sheep. *J. Anim. Ecol.* **78**: 354-364.

597 Wright, P.J., Panfili, J., Folkvord, A., Mosegaard, H., and Meunier, F.J. 2002. Direct
598 validation. *In* Manual of Fish Sclerochronology, Ifremer-IRD coedition, Brest, France.
599 *Edited by* J. Panfili, H. de Pontual, H. Troadec, and P.J. Wright. pp. 114-127.

600 Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G.M. 2009. Mixed effects
601 models and extensions in ecology with R. Springer, New York.

Tables

Table 1. Parameter estimates from the generalized linear mixed model (with family as a random intercept) best explaining variation in apparent survival of Atlantic salmon juveniles given at the log scale and as treatment contrasts*.

	Estimate \pm SE	Z	p
Intercept (LD, ET)	2.69 \pm 0.68	39.43	< 0.001
HD	-0.67 \pm 0.08	-8.14	< 0.001
NT	-0.2 \pm 0.09	-2.17	0.03
LT†	-0.39 \pm 0.09	-4.09	< 0.001

*Abbreviations are given for all parameters: Low density location (LD), High density location (HD), early timing (ET), normal timing (NT) and late timing (LT).

†LT relative to NT: estimated value = -0.2 ± 0.1 , $Z = -1.95$, $p = 0.051$.

Table 2. Parameter estimates from the general linear mixed model with family as a random effect (intercept) that best explain variation in final body mass (ln-transformed) of Atlantic salmon juveniles given as treatment contrasts*.

	Estimate \pm SE	<i>T</i>	<i>P</i>
Intercept (Egg mass, LD, ET)	2.43 \pm 0.35	6.9	< 0.0001
HD	-0.05 \pm 0.03	-1.85	0.07
Slope (Egg mass)	0.75 \pm 0.19	3.95	0.003
NT	-0.29 \pm 0.02	-12.07	< 0.0001
LT	-1.13 \pm 0.03	-42.31	< 0.0001
LL:NT	0.1 \pm 0.04	2.5	0.01
LL:LT	0.04 \pm 0.04	1.0	0.31

* LD is the low density location, HD is the high density location, ET is early timing, NT is normal timing, LT is late timing and egg mass is the ln-transformed mean family egg mass (g).

Table 3. Selection gradients relating the relative proportions of juveniles recaptured to family mean egg mass (g) under the different locations (Low density, LD; High density, HD) and emergence timing groups (Early timing, ET; normal/late timing, NLT). Directional selection gradients (unstandardized, β ; standardized, β') are estimated from linear regression coefficients, and non-linear (stabilizing) selection gradients (unstandardized, γ ; standardized, γ') are estimated from regression coefficients of squared deviations from the mean. Standard errors are given in parentheses.

	β	β'	γ	γ'
LD, ET	-0.06 (0.97)	-0.004 (0.06)	11.53 (58.27)	0.01 (0.06)
LD, NLT	0.98 (0.76)	0.07 (0.06)	46.73 (42.91)	0.06 (0.06)
HD, ET	2.02 (1.35)	0.22 (0.14)	114.96 (71.42)	0.21 (0.13)
HD, NLT	-0.66 (0.55)	-0.11 (0.09)	25.26 (32.04)	0.07 (0.09)

Figure legends

Fig. 1. (a) Map of the Stream Osalandsbekken and its location within Norway. Open and filled circles represent experimental Atlantic salmon nests in the low and high density reach, respectively. Arrows indicate the direction of water flow. (b) Experimental design giving the number of alevins from 12 Atlantic salmon families and three emergence timing groups that were distributed among 25 nests in the low density reach and one nest in the high density reach of Stream Osalandsbekken.

Fig. 2. The observed proportions of recaptured Atlantic salmon juveniles originating from families with different mean sized eggs that were outplanted in the (a) low density and (b) high density location in Stream Osalandsbekken. Filled and open circle(s) indicate the early and normal/late timing groups respectively. Solid and dashed lines are the estimated relationships (from a poisson generalized linear mixed model where differences in initial numbers are taken into account using *offset*) between juvenile recaptures and mean family egg mass within the early and normal/late timing groups, respectively.

Fig. 3. The opportunity for selection (SD^2 / \bar{X}^2) and corresponding 95 % confidence intervals (CI) for Atlantic salmon juveniles (12 families) from the early, normal and late timing group recaptured at the low density (open circles) and high density (closed circles) location in Stream Osalandsbekken.

Fig. 1.

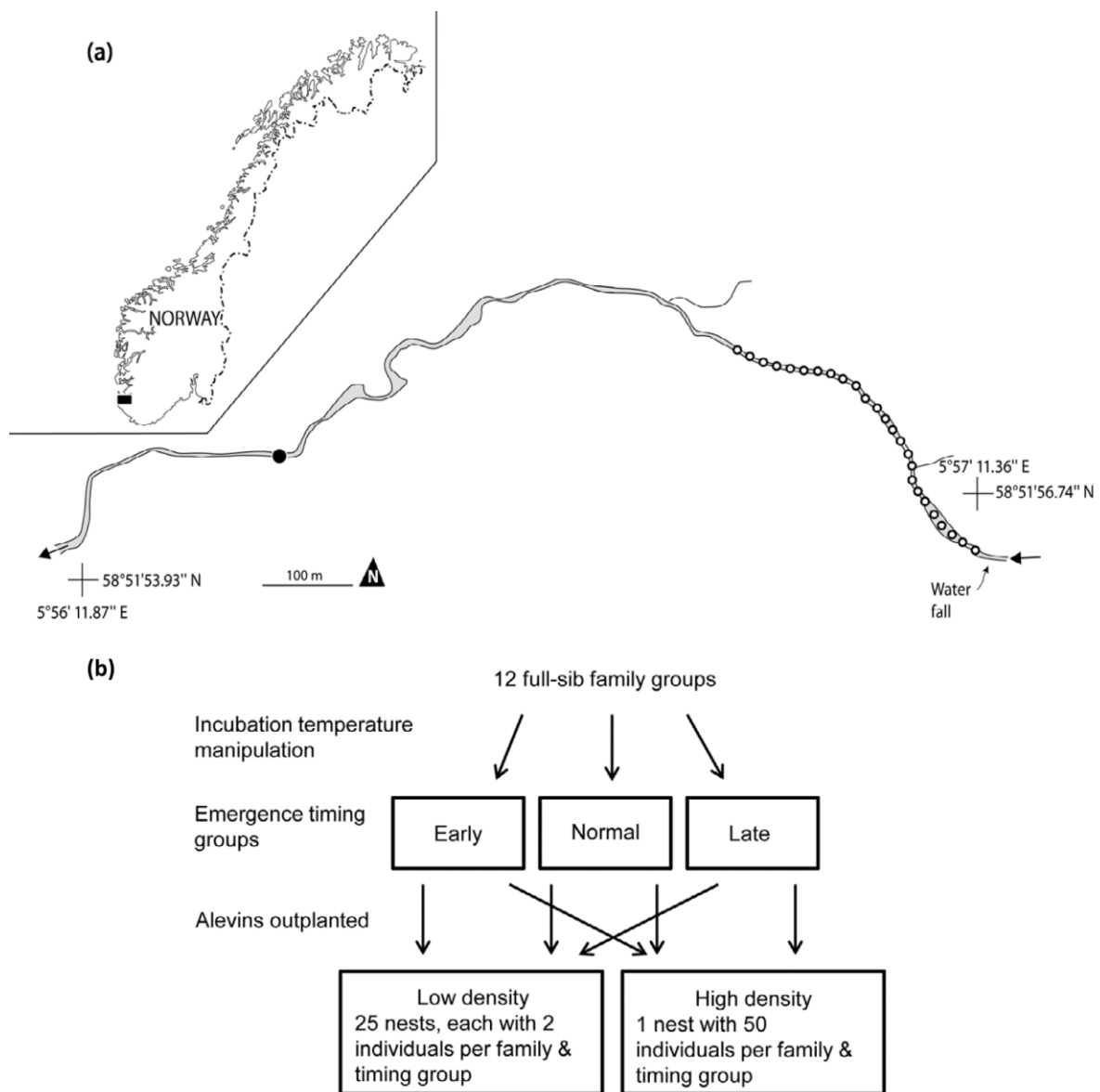


Fig. 2.

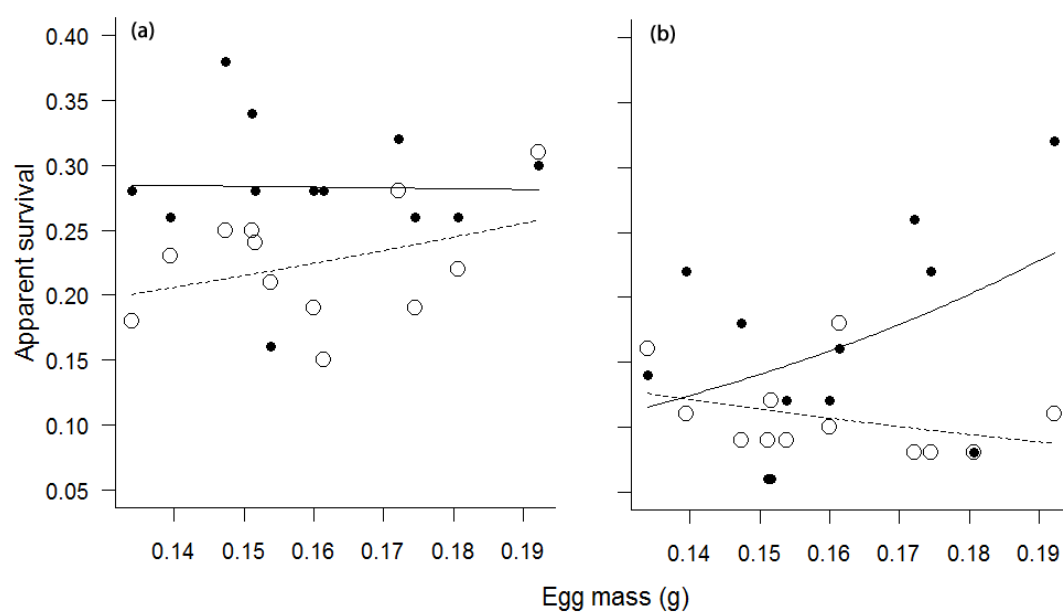


Fig. 3.

