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RESEARCH ARTICLE

Testing the effects of genetic crossing distance on embryo survival within a metapopulation of brown trout (*Salmo trutta*)

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Abstract Predicting progeny performance from parental genetic divergence can potentially enhance the efficiency of supportive breeding programmes and facilitate risk assessment. Yet, experimental testing of the effects of breeding distance on offspring performance remains rare, especially in wild populations of vertebrates. Recent studies have demonstrated that embryos of salmonid fish are sensitive indicators of additive genetic variance for viability traits. We therefore used gametes of wild brown trout (*Salmo trutta*) from five genetically distinct populations of a river catchment in Switzerland, and used a full factorial design to produce over 2,000 embryos in 100 different crosses with varying genetic distances (F_{ST} range 0.005–0.035). Customized egg capsules allowed recording the survival of individual embryos until hatching under natural field conditions. Our breeding design enabled us to evaluate the role of the environment, of genetic and non-genetic parental contributions, and of interactions between these factors, on embryo viability. We found that embryo survival was strongly affected by maternal environmental (i.e. non-genetic) effects and by the microenvironment, i.e. by the location within the gravel. However, embryo survival was not predicted by population divergence, parental allelic dissimilarity, or heterozygosity, neither in the field nor under laboratory conditions. Our findings suggest that the genetic effects of inter-population hybridization within a genetically differentiated meta-population can be minor in comparison to environmental effects.

Keywords Genetic distance · Inbreeding · Maternal effects · Outbreeding · Optimal outcrossing distance · Additive genetic variance · *Salmo trutta* · Salmonidae

Introduction

The effect of parental genetic distance on offspring fitness is of fundamental interest in population biology, and identification of the genetic distance producing maximally fit offspring can be useful for population management. Anthropogenic impacts increasingly affect the genetic composition and fitness of natural populations (reviewed in Hendry et al. 2008; Smith and Bernatchez 2008). Artificial migration barriers, for instance, may sub-structure populations and cause inbreeding depression (Wang et al. 2001; Epps et al. 2005). Genetic introgression from non-native gene pools (e.g. from introduced domestic stock or from mixing populations in supportive breeding programmes) on the other hand can cause outbreeding depression (Goldberg et al. 2005; Muhlfeld et al. 2009). Although supportive breeding programmes are widely used in an attempt to halt population declines and local extinction (Keller and Waller 2002; Wang et al. 2002), little is known about their long-term fitness consequences (Araki et al. 2007; Fraser 2008). Systematic comparison of the fitness of crosses with different genetic distances could provide important insight into the optimisation of such programmes, and help assess the risk of introducing non-native stock. Yet, experimental

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testing of the fitness consequences of breeding distance remains rare, especially in wild populations.

Theoretically, a dome-shaped relationship could be expected between fitness and parental genetic distance (Price and Waser 1979; Campbell and Waser 1987; Schierup and Christiansen 1996). At small distances, e.g. between closely related individuals but also between individuals from populations with low genetic diversity, inbreeding depression may occur because increased levels of homozygosity can unmask deleterious alleles (Charlesworth and Willis 2009). At large distances, e.g. between individuals from divergent populations and heterospecifics, offspring fitness can decrease due to outbreeding depression, i.e. genetic incompatibilities, negative epistasis, and disruption of beneficial gene complexes (Lynch 1991; Edmands 2002). The fitness peak may thus be expected to reside in the area of crosses between moderately diverged populations within species (Neff 2004), where effects of inbreeding and outbreeding depression are minimal. Effects of heterosis (dominance and overdominance) and positive epistasis (Willi et al. 2007) may additionally enhance fitness.

Despite these theoretical predictions, there is only scant evidence for stabilizing selection on the genomic divergence of breeders in wild populations, especially in vertebrates (Marshall and Spalton 2000; Neff 2004). Results reported in the literature on the relationship between parental genetic distance and offspring performance are generally mixed. While some studies found support for maximized performance at intermediate genetic crossing distances (e.g. Moll et al. 1965; Willi and Van Buskirk 2005), others found performance to increase with distance (e.g. Moran et al. 1995; Xiao et al. 1996; Amos et al. 2001; Gonzalez et al. 2007; Jagosz 2011), decrease with distance (e.g. McClelland and Naish 2007; Pekkala et al. 2012), or no effect of genetic distance was observed at all (e.g. Edmands 1999; Stokes et al. 2007; Robinson et al. 2009; Hung et al. 2012). Overall, the genetic distance at which fitness peaks in natural animal and plant systems seems hard to predict, and results seem strongly dependent on the phenotypic traits used as proxies for fitness, the genetic markers used, and the range of parental distances considered.

Salmonids such as brown trout (*Salmo trutta*) represent powerful vertebrate models for experimental studies in ecology and evolution. They can be easily crossed in vitro and reared in large numbers under controlled conditions. Recent studies have demonstrated that an embryo's survival to hatching can be significantly affected by its genotype (e.g. Pitcher and Neff 2007; Wedekind et al. 2008b; von Siebenthal et al. 2009; Jacob et al. 2010; Pompini et al. 2013). Salmonid fish are also of considerable cultural and economical importance, and they are typically keystone species in their respective habitat. There is an urgency to better understand their biology since salmonid populations are declining in many parts of the world (ICES 2006; Krkosek et al. 2007; Ford and Myers 2008). Severe population declines have also been observed in many Swiss rivers where catches of brown trout have dropped by more than 50 % during the last three decades—a pattern that is well documented but relatively poorly understood (Fischnetz 2004; Borsuk et al. 2006; Burkhardt-Holm 2008). Supportive breeding programmes are currently in operation whereby artificially fertilised eggs are raised in hatcheries and fry are released back into the wild to supplement the natural populations.

There typically is considerable genetic differentiation on neutral markers between neighbouring populations of brown trout (e.g. Keller et al. 2011). A recent study from a river network in Switzerland found migration barriers to be associated with increased genetic distance between populations (Stelkens et al. 2012a). Even on a microgeographic scale, populations differed substantially in genotype (F_{ST}) and phenotype (body shape, especially locomotory and trophic morphology), though the same populations tested negative for local adaptation (Stelkens et al. 2012b). Here, we used five brown trout populations from the same river network (a subset of those geno- and phenotyped in Stelkens et al. 2012a) and generated full-factorial intra- and inter-population crosses to test whether we would find a genetic crossing distance that is optimal with respect to embryo viability under natural conditions. The crosses yielded over 2,000 embryos in 100 different half-sib families, covering a range of genetic distances (F_{ST} 0.005–0.035) that is typical for natural stream-dwelling metapopulations of brown trout (see literature cited in Stelkens et al. 2012a). The current stocking regime in the study area stipulates that populations can only be stocked with hatchery-bred offspring of spawners from the same population. We wanted to see if, within a typical metapopulation of salmonids, a particular breeding strategy can enhance offspring performance. Embryos were either buried in incubation capsules in a natural streambed or, as a control, raised under benign conditions in the laboratory. Our breeding design also enabled us to evaluate the role of the environment, of genetic and non-genetic parental contributions, and of interactions between these factors, on embryo viability.

Materials and methods

Sampling of genetic and phenotypic data of adults

Stelkens et al. (2012a) collected tissue samples of 563 brown trout from 21 locations in the Aare river system during

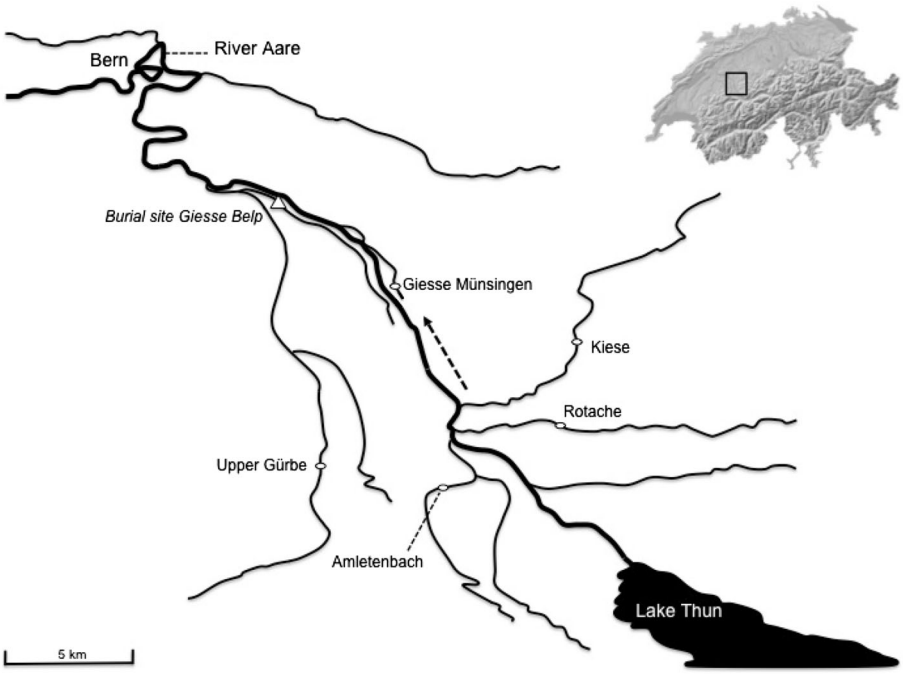

Fig. 1 Map of the River Aare catchment between Thun and Bern (Switzerland), indicating the five sampling sites (modified from Stelkens et al. 2012a). The *triangle* indicates the site where eggs were reared in the streambed. The *dashed arrow* indicates direction of water flow. The *box* in the *upper-right inset* indicates the location of the catchment in Switzerland

summer and autumn of 2009. Further reproductive-age adults were collected shortly before the breeding season of the same year by electro-fishing from five of these locations (Fig. 1; GPS data in Table 1 of "Appendix") and transported to the cantonal hatchery facility at Reutigen, Bern canton, where they were held for about 4 weeks. At 1 day around the peak of the breeding season, 20 adult males and 20 gravid females (i.e. four of each sex from each of the five populations) were haphazardly selected from among the captive fish for use in our crossing experiment. Fish were anaesthetized with clove oil and processed as follows: Photos and tissue samples were taken for these fish to be included in the analyses of Stelkens et al. (2012a). Then, the fish were pressed along the length of the abdomen to expel their gametes, which were collected in sterile Petri dishes. Next, tissue was collected from the pectoral fin for the estimation of genetic parameters. Body length (tip of snout to end of caudal fin) was recorded of each breeder and total egg mass for each female.

Fertilization protocol and treatment of fertilized embryos

We employed a full-factorial breeding design with respect to the five populations, in that females from every population were crossed with males from every population, with each population-by-population combination replicated four times by individual crosses (see Fig. 2 in Clark et al. 2013a). Overall, the design yielded 100 different crosses (full-sib groups), with a total of 2,115 fertilized eggs (mean number of embryos per cross 21 ± 1.1 SD, range 15–22) that were used in the present study. Further embryos resulting from these crosses were used in a parallel study on parental effects on pathogen resistance (Clark et al. 2013a).

Fertilizations were carried out at the Reutigen hatchery at 6.5 °C. Fertilization was induced in 90 mm Petri dishes by adding ca. 20 µl of milt to ca. 80 eggs per dish (this amount of sperm over-saturates the number of eggs yielding maximum success of fertilization in every dish). Fifteen ml of standardized water (sterilized and aerated, chemically defined water prepared according to OECD guidelines (OECD 1992) were added to activate the sperm, and dishes were gently agitated to mix gametes. After 5 min, 50 ml of standardized water were added and eggs were left undisturbed for 2 h of egg hardening. A subsample of eggs from each female was then photographed. From digital analyses of these photographs (ImageJ; http://rsbweb.nih.gov/ij/), we estimated mean egg size and egg redness measured as the R/G colour ratio relative to a standard yellow reference (Gladbach et al. 2010).

Fertilized eggs were transferred to a cold chamber at the University of Lausanne, (6.22 ± 0.14 °C) where they were rinsed under running tap water for 30 s (flow rate: 4 l/min) and then distributed individually to wells of 24-well cell culture plates (Falcon, Becton–Dickinson), which had been pre-filled with 2 ml per well of standardized water. Each plate received one embryo from each of the 20 crosses involving the four females sampled from a given population and five males from five different populations. For the following 27 days, embryos remained in the cold chamber with a daily light/dark cycle of 12 h/12 h (in order to have a repeatable light regime while allowing for monitoring embryo development). At the end of this period, all

embryos were carefully examined on a light table (Hama professional, LP 555) to determine survival with a stereo zoom microscope (Olympus SZX9).

"Stream" and "Control" rearing treatments

On day 27 after fertilization, i.e. at around 170° days (accumulated daily mean temperatures) when embryos were at the early eyed stage, they were allocated to either 'Stream' or 'Control' treatments. Stream treatment eggs were distributed individually to the compartments of custom-designed egg capsules (Fig. 2). Each capsule comprised a vertical stack of ten compartments enclosed with a fine stainless steel wire mesh tube, which allowed good through-flow of water while keeping embryos separate and thus individually identifiable. Because capsules were to be buried upright in the streambed, where upper and lower compartments could experience different physicochemical conditions, eggs were distributed into capsules with some compartments left empty so that each individual cross was represented at each capsule position, yielding a total of 128 capsules.

On day 28 after fertilization, capsules were transported in chilled standardized water to Giesse Belp (Fig. 1), where they were randomly allocated to one of two sites (46.907352N, 7.513543E and 46.906327N, 7.516094E) recognizable by their appearance as natural brown trout redds. The redds were briefly turned with a hoe to loosen the gravel and to reduce the sediment load. Capsules were inserted into the streambed one by one (Supplementary Video S1) after displacing the gravel with a steel spike and sleeve as per the methods of Dumas and Marty (2006; p 289). Although the Giesse Belp stream is part of the Aare catchment, none of the fish used in our crosses were collected from this stream. The burial sites thus represent a novel environment to all populations in this experiment (in order to avoid effects of local adaptation in our sample even if we had not found such effects in Stelkens et al. 2012b). At the first burial site, streambed water temperature, recorded at 15 min intervals with an Escort iLog data-logger (http://www.escortcoldchain.com/), ranged from 3.22 to 7.91 °C during the burial period (mean 5.55 ± 1.03 °C). At the second burial site temperature ranged from 3.16 to 7.76 °C (mean 5.45 ± 1.02 °C).

Embryos remained in the streambed until their retrieval at 460° days (at a time when hatching has usually started) when they were dug up and transported back to the laboratory. Upon arrival, embryos were removed from their capsules, redistributed to individual wells of 24-well plates, and examined using a stereomicroscope. Embryos were scored as alive or dead, depending on whether or not the heart was visibly beating. Mortality was typically associated with infection of typical saprophytes such as *Saprolegnia* sp.

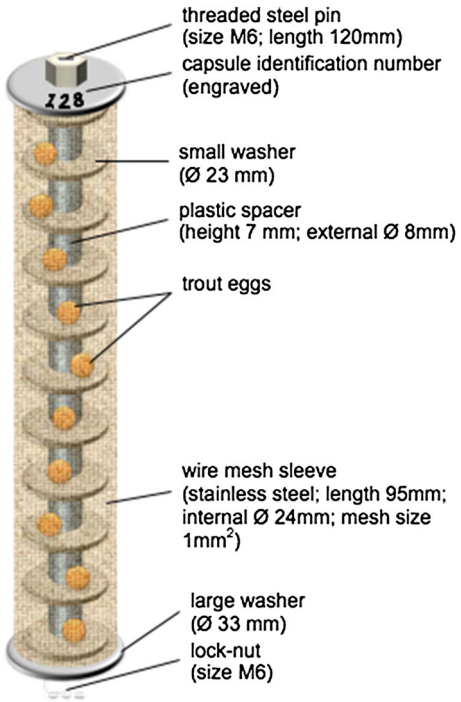

Fig. 2 Egg capsule design

Control group embryos ($n = 12$ per cross) were examined regularly from 170 degree days on using a stereomicroscope. For comparison with Stream group embryos, we determined survivorship at a comparable point in development, i.e. 460° days. These embryos also served as control group of another experiment that studied timing of hatching and larval growth in response to pathogen infection (Clark et al. 2013a). Embryos of the present study that survived to the end of the monitoring period were returned to the Reutigen hatchery to supplement an ongoing supportive breeding program.

Estimation of genetic differentiation of populations and breeders

Stelkens et al. (2012a) estimated pairwise genetic distances between 21 populations, of which five are represented in our study, based on allele frequencies at 11 microsatellite markers using FSTAT 2.9.4 (Goudet 2002). Because the 40 breeders used in our study were included in their data, we extracted pairwise F_{ST}-distances for the five populations our breeders originated from (see Table 2 in "Appendix"), and other variables characterizing the populations' genetic variability (see Table 1 in "Appendix"). Table 1 in "Appendix" also shows how many individuals per population (including our breeders) entered these calculations. Note that sampling sites are called 'populations' here for simplicity even though they may not represent biological populations.

To describe the genetic constitution of individual breeders, we estimated their heterozygosity (H, the proportion of heterozygous loci among all loci examined), which is expected to negatively correlate with the degree of inbreeding in the individual's recent ancestry. Because we employed breeders drawn from a wild population without known pedigrees, we also used genetic information to estimate the 'relatedness' between individual breeders crossed in our experiment. Specifically, we calculated W, a coefficient describing the genetic dissimilarity of two individuals, taking into account the allele frequencies of their respective populations of origin (Wang et al. 2002). W has been shown to be robust to small sample sizes and highly polymorphic loci. We calculated W using the software MER3 (http://www.zsl.org/science/research/software/mer,1152,AR.html). Increasing values of W indicate increasing genetic dissimilarity.

Finally, we also calculated projected heterozygosity ($H_{\text{projected}}$), the mean level of heterozygosity expected for offspring from each individual cross. Since the genotypes of all parents were known, we could estimate, for each of the 11 microsatellite loci, the probability that offspring from a particular parental combination would be homozygous or heterozygous at a particular locus. $H_{\text{projected}}$ was calculated as the average of these 11 probabilities.

As reported in Stelkens et al. (2012a), pairwise population differentiation (F_{ST}), after sequential Bonferroni correction, was significant between all populations ($p < 0.05$; see Table 2 in "Appendix"; see Stelkens et al. 2012a for more information). Global genetic population differentiation was in the range expected for a network of brown trout populations within the same catchment (global $F_{ST} = 0.021$, 95 % CI 0.014–0.027; comparable estimates were found in Carlsson and Nilsson 2000; Heggenes and Roed 2006; Griffiths et al. 2009; Hansen et al. 2010). In none of the populations, F_{IS} values (Wright's inbreeding coefficient) differed significantly from zero, suggesting there was no heterozygote deficit, i.e. inbreeding was not evident (see Table 1 in "Appendix"). As expected, genetic dissimilarity of breeders from different populations (W) was significantly positively correlated with the genetic differentiation of populations (F_{ST}: $r_s = -0.17$, $p < 0.001$). W was also negatively associated with the expected offspring heterozygosity ($H_{\text{projected}}$: $r_s = -0.68$, $p < 0.001$).

Statistical analyses

Hypothesis testing was performed using R (R Core Team 2013). Except where stated otherwise, we analyzed our data with a series of generalized linear mixed effect models (GLMMs, lme4 package; Bates et al. 2013), in each case using a binomial fit for the binary response variable (i.e. survival at 460° days). Variables grouping embryos by their extent of common heritage were treated as random factors. These included *sire* and *dam* identity (corresponding to half-sib groups), interaction between *dam* × *sire* (corresponding to full-sib groups), and the variable *population cross* (representing more distant genetic links, but common heritage nonetheless). The identity of the capsule in which an embryo was reared, as well as the position within this capsule, were treated as random factors. F_{ST}, W, $H_{\text{projected}}$ as well as burial *site* (because only two levels) were treated as fixed factors. To evaluate the explanatory importance of a factor, alternative models with or without this factor were compared using log-likelihood ratio tests (LRT) with restricted maximum likelihood (REML) for random factors and maximum likelihood (ML) for fixed effects (Zuur et al. 2009).

Stream ($n = 932$) and control ($n = 1{,}183$) reared embryos were analyzed separately. We constructed a first GLMM that included key environmental factors (*site*, *capsule* and *position*, only available for stream-reared embryos), various random factors defining the amount of shared heritage among groups of embryos, but none of the measured attributes of particular dams or sires. Our base model thus had the following structure: *survival* ~ *burial site* + (1 | *capsule*) + (1 | *position within capsule*) + (1 | *dam*) + (1 | *sire*) + (1 | *dam*:*sire*) + (1 | *population cross*). From this point on, all candidate predictors of survivorship were evaluated individually by testing changes in the likelihood of models with or without the factor of interest. Correlations were calculated using Spearman's rank correlations r_s.

Results

Effects of genetic crossing distance on offspring survival

Mean survival across different population crosses varied between 61.1 and 80.0 % for the stream-reared embryos, and between 83.3 and 100 % for the control embryos. Variation in survival of stream-reared embryos was not related to the divergence of parental populations (F_{ST}: linear fit (LRT): $\chi^2_1 = 0.02$, $p = 0.90$; quadratic fit: $\chi^2_2 = 0.60$, $p = 0.74$; Fig. 3a). The same applies to the control environment (linear: $\chi^2_1 = 1.09$, $p = 0.30$; quadratic: $\chi^2_2 = 5.01$, $p = 0.08$; Fig. 3a).

Across individual crosses, survival ranged from 20 to 100 % in stream-reared embryos, and from 45.5 to 100 % in control embryos. No relationship between embryo survival and the genetic dissimilarity of breeders (W) was evident in the stream (linear: $\chi^2_1 = 0.02$, $p = 0.88$; quadratic: $\chi^2_2 = 0.87$, $p = 0.65$; Fig. 3b). In the laboratory, however, W had a nearly significant linear effect on

offspring survival (linear: $\chi^2_1 = 3.79$, $p = 0.051$; quadratic: $\chi^2_2 = 4.43$, $p = 0.11$; Fig. 3b).

The expected heterozygosity ($H_{\text{projected}}$) of stream-reared embryos had no significant linear ($\chi^2_1 = 0.05$, $p = 0.83$) but showed a convex quadratic relationship with survival ($\chi^2_2 = 7.04$, $p = 0.03$; Fig. 3c). Under control conditions, $H_{\text{projected}}$ had no significant effect on embryo survival (linear: $\chi^2_1 = 1.13$, $p = 0.29$; quadratic: $\chi^2_2 = 4.84$, $p = 0.09$; Fig. 3c).

Parental effects on offspring survival

We found a significant positive correlation between female body length and embryo survival in the stream ($r_s = 0.53$, $p = 0.02$; Fig. 4) but not between sire body length and embryo survival ($r_s = 0.12$, $p = 0.62$). There was no significant effect of reproductive investment on offspring survival (absolute brood mass: $r_s = 0.30$, $p = 0.19$; mean egg volume: $r_s = 0.25$, $p = 0.28$; egg redness: $r_s = 0.02$, $p = 0.93$). Survival was not predicted by the within-individual genetic diversity of dams (H: $r_s = 0.24$, $p = 0.31$) or sires (H: $r_s = -0.29$, $p = 0.22$).

By virtue of our experimental breeding design, offspring variously shared the same dams, sires or populations of origin, allowing us to disentangle these parental effects. In our base model, *dam* identity explained a significant part of the variation (exclusion from base model: stream: $\chi^2_1 = 6.24$, $p = 0.01$; control: $\chi^2_1 = 11.40$, $p < 0.001$), while *sire* identity had no significant effects on embryo survival (LRT: stream: $\chi^2_1 = 0.01$, $p = 0.92$; control: $\chi^2_1 = 0.00$, $p = 1.00$). Non-additive genetic effects (*dam* × *sire* interaction) were negligible for the stream environment (LRT: $\chi^2_1 = 0.00$, $p = 1.00$) but close to significance in the control environment (LRT: $\chi^2_1 = 3.70$, $p = 0.055$). The factor *population cross identity* in our models did not explain any variation in mortality, neither in the stream-reared (LRT: $\chi^2_1 < 0.01$, $p = 1.00$) nor in the control-reared embryos (LRT: $\chi^2_1 = 0.16$, $p = 0.69$).

Environmental influences on offspring survival

Rearing environment had a strong effect on embryo survival. Stream-reared eggs had an overall survival rate of 70.5 % from fertilisation to 460° days, while survival in the control group through the same period was 93.2 % (proportion test with continuity correction: $\chi^2_1 = 191.4$, $p < 0.001$).

Within the stream environment, there was a strong association effect of capsule identity on embryo survival ($\chi^2_1 = 19.35$, $p < 0.001$). Mean survival was not different at the two burial sites (Site 1: 67.5 % [95 % CI 63.4–71.4 %]; Site 2: 74.3 % [95 % CI 69.9–78.3 %]; *site* effect $\chi^2_1 = 32.69$, $p = 0.10$), and there was no significant overall effect of position within capsule (LRT; $\chi^2_1 = 2.14$, $p = 0.14$).

Discussion

We generated intra- and inter-population crosses between brown trout sampled within a metapopulation that has previously been shown to be genetically and phenotypically diverse (Stelkens et al. 2012a). We tested for effects of parental genetic distance (measured on both the individual breeder level and on the population level) on embryo survival under natural and laboratory conditions. Our breeding design also enabled us to measure the role of the environment on embryo viability (laboratory vs. stream environment, egg position in the gravel), of genetic and

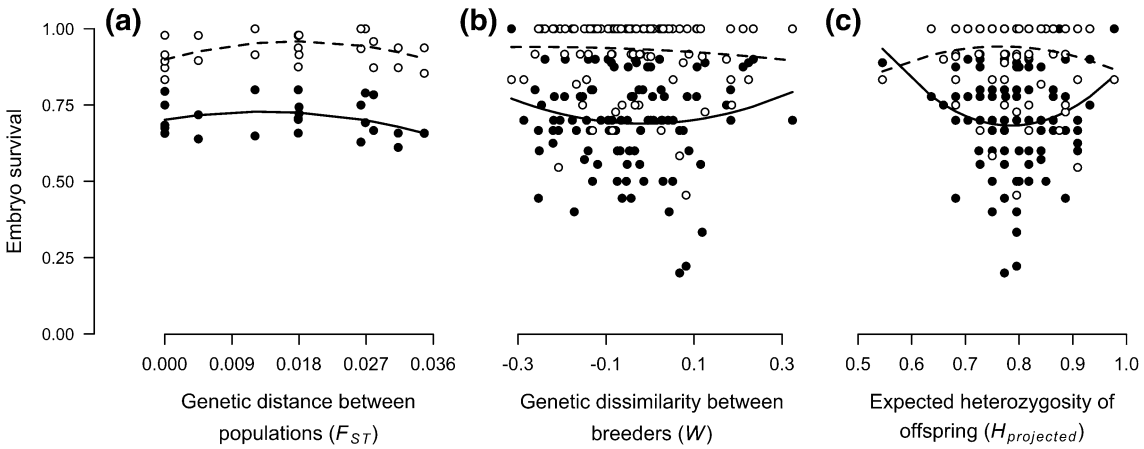

Fig. 3 Mean survival of offspring resulting from crosses of varying genetic distances. *Open symbols* (and *dashed line*) denote means for groups of laboratory-reared embryos, while *closed symbols* (and *solid line*) denote groups reared within natural streambeds. See text for relevant statistics

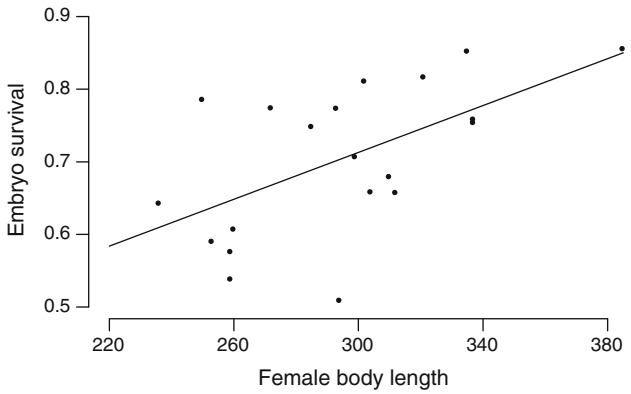

Fig. 4 Mean offspring survival versus dam body total length (mm). The *line* gives the regression. See text for statistics

non-genetic parental contributions, and of interactions between these factors.

Only one predictor of genetic distance between individual breeders, parental genetic dissimilarity (*W*), showed a nearly significant positive correlation to embryo survival, and this was only evident in the laboratory treatment where environmental noise was kept minimal (Fig. 3b). The other predictor, parental heterozygosity (*H*), had no effect. It should, however, be cautioned that 11 microsatellite markers may not be sufficient to adequately reflect individual heterozygosity at genome-wide level (Grueber et al. 2011).

Interestingly, the projected heterozygosity ($H_{\text{projected}}$) of stream-reared embryos predicted individuals with either low or high degrees of heterozygosity to survive better in the stream than individuals with intermediate levels of heterozygosity (Fig. 3c). Although speculative, high survival rates at the two ends of the heterozygosity continuum may be caused by the preservation of beneficial parental allelic combinations and/or positive epistatic effects in the least heterozygous offspring (underdominance), and by heterozygote advantage (overdominance; Lynch 1991) or recombinant hybrid vigor (epistasis or complementation; Rieseberg et al. 1999) in the most heterozygous offspring.

Embryo survival was not predicted by population divergence (F_{ST}), neither in the field nor under laboratory conditions (Fig. 3a). We consider three non-mutually exclusive possible explanations for this result.

Firstly, although the populations we used were significantly structured, with subpopulations genetically distinct from one another (see Table 1 in "Appendix"; Stelkens et al. 2012a), the overall range of genetic distances our crosses yielded may not have provided sufficient breadth to reveal inbreeding or outbreeding depression. Regarding outbreeding depression, it is difficult to predict a priori at what genetic distance we should expect to see effects. Although direct comparison between species of the genetic distances of crosses is impaired by the variability of genetic markers used, in a study of largemouth bass (*Micropterus salmoides*), crosses with rather small distance ($G_{ST} = 0.05$) resulted in up to 58 % reductions in viral resistance among F2 individuals compared to ancestral individuals (Goldberg et al. 2005), yet no reduction in F1 embryonic survival was observed in Atlantic salmon crossed over substantial genetic distances (Nei's $D > 0.43$; Fraser et al. 2010). A meta-analysis comprising 670 pairwise comparisons of fish populations cautioned that few general predictions could be made about the size or direction of outbreeding effects, and observed that genetic distance explained little of the variation in effect size across studies (McClelland and Naish 2007).

Secondly, it is possible that inbreeding or outbreeding effects are not influential enough (i) to cause mortality at benign laboratory conditions and (ii) to overrule the effects of typical environmental variation at the early ontogenetic life-stages on which we focused. Perhaps, inbreeding or outbreeding effects are more pronounced later in life for traits such as survival to maturity, attractiveness to mates, fecundity, reproductive success, and longevity (Stearns 1992; Szulkin et al. 2007; Grueber et al. 2010). For example, Gharrett et al. (1999) found no effect of genetic distance on salmon fertilization rates but the rate of return of adults to the spawning grounds was reduced for more outcrossed fish. Life stage-specific effects of inbreeding depression, outbreeding depression, and heterosis have been observed in other animal and plant species (Husband and Schemske 1996; Koelewijn et al. 1999; Escobar et al. 2008). Moreover, outbreeding depression in particular may only become evident in later hybrid generations, i.e. in or after the F2 (Edmands 2007).

Thirdly, the specific evolutionary history of a population can potentially mitigate the effects of inbreeding and outbreeding. The severity of inbreeding depression, for example, depends on the genetic load carried in a population, but inbreeding during severe or frequent population bottlenecks in the past can purge detrimental alleles and reduce the costs of inbreeding (Bijlsma et al. 1999; Glemin 2003). Meanwhile, the effects of outbreeding depression, which involves the disruption of locally built up coadaptations, can be diminished by pre-existing gene flow (Lynch 1991). Extrinsic effects can also influence the shape of the genetic distance-fitness function, such as the mode of selection (e.g. directional versus balancing selection; Frankham 2009) and the type of environment (Armbruster and Reed 2005). Thus, it is conceivable that historical factors influence the genetic composition of our sampled populations in a way that reduces the likelihood of inbreeding or outbreeding depression in the present time.

Besides genetic crossing distance, we also investigated how other factors affected offspring survival. We were able

to estimate the relative impact of the environment, of genetic and non-genetic parental contributions (for the latter we assumed that variation in maternally inherited mitochondrial genes has no significant effects on embryo performance), and of interactions between these factors. We found that embryo survival was strongly affected by maternal environmental effects (i.e. non-genetic, environmental conditions faced by mothers before egg laying) and by the microenvironment, i.e. by the location within the gravel. Rearing conditions strongly affected offspring phenotype, with stream-reared embryos showing reduced survival compared to embryos reared in the laboratory. The specific causes of this elevated mortality in the stream could not be identified. They may have included pathogens, micro-predators, or physicochemical stresses. The intensity of these environmental stresses will vary through time and space, and accordingly, we found that an embryo's position within the streambed had a strongly significant influence on its survival confirming previous findings at other locations (Stelkens et al. 2012b).

We found significant dam effects on offspring fitness, but no paternal effects, which suggests that most of the maternal effect was not due to additive genetic effects (i.e. mediated through the maternally-provided environment or caused by epigenetic effects). As such, our results add to a growing body of evidence for the evolutionary significance of maternal effects (Mosseau and Fox 1998). Given that the survival of a dam's offspring was not significantly related to the mean size of her eggs, or their redness (a proxy for carotenoid content that may partly reflect maternal investment; L. G. E. Wilkins and C. Wedekind, unpublished results), it is likely that many of these maternal effects were mediated by qualitative—rather than quantitative—provisioning of nutrients, protective structures, or immune-active substances within the egg. Alternatively, egg size may be unrelated to offspring viability if fitness is maximised at optimal (i.e. environment-dependent), rather than maximal, egg sizes (Smith and Fretwell 1974).

Dam × sire interaction effects were negligible for survival in the stream but nearly significant in the control environment. This suggests that non-additive genetic effects (e.g. dominance interactions) may play a role but that their importance is mitigated by environmental variation.

In contrast to maternal environmental effects, sires did not have much influence on the survival of their offspring in our experiment. This result is consistent with other salmonid studies employing various group-rearing conditions (e.g. Beacham 1988; Nagler et al. 2000; Urbach et al. 2008; Janhunen et al. 2010). Janhunen et al. (2010) and others suggested that detecting paternal effects at embryonic stages depends on the kind and amount of environmental variance allowed for in the experiment, a prediction verified in recent studies on brown trout (Jacob et al. 2010) and whitefish *Coregonus palaea* (e.g. von Siebenthal et al. 2009). Our findings confirm the significance of environmental variation in affecting early embryo survival within the gravel of a natural red. It seems that additive genetic effects during embryogenesis are best observed under controlled laboratory conditions. For instance, significant sire effects could be found on the timing of hatching after sub-lethal infections of embryos with *Pseudomonas fluorescens*, indicating additive genetic variation in infection tolerance in brown trout (Clark et al. 2013a) and in the whitefish *C. palaea* (Clark et al. 2014). On a side note, Clark et al. (2013a) found no significant role of genetic crossing distance on infection tolerance, analogous to our findings.

While paternal effects are sometimes small at very early developmental stages (Wedekind et al. 2001, 2008a), studies on late embryo viability often found considerable additive genetic effects (e.g. Wedekind et al. 2001, 2007, 2008b; Jacob et al. 2007; Pitcher and Neff 2007; Evans et al. 2010, Clark et al. 2014), of which some were linked to allelic variation on major histocompatibility complex (MHC) loci in a quantitative genetic breeding experiment (Pitcher and Neff 2006), and in a selection experiment within full-sib families (Wedekind et al. 2004). Other examples for paternal effects on traits expressed later in life include MHC expression shortly before hatching (Clark et al. 2013b), resistance to pathogens after hatching (Evans and Neff 2009), hatchling size (Eilertsen et al. 2009), growth after hatching (Vandeputte et al. 2002), and territorial behaviour (Petersson and Jarvi 2007).

Conclusions

We found no evidence that the genetic distance between populations affected offspring survival under the conditions of this study, i.e. when embryo survival was recorded during incubation in a natural redd (i.e. under potentially stressful conditions) or in the laboratory under benign conditions. We conclude that, at the embryonic life-stage, the fitness consequences of inter-population hybridization within this metapopulation (such as occurs during supportive breeding programmes; Edmands 2007) can be minor in comparison to other factors affecting embryo viability such as the incubation microhabitat or maternal environmental effects. This does not exclude the possibility that the genetic distance between parents may be important over different genetic distance scales, for different traits or life-stages, or when applied to a different population network.

Brown trout are known to have complex population structure within river catchments, often with substantial genetic differentiation, vast phenotypic diversity, and large variation in life history strategies (e.g. Nielsen et al. 2003;

Hermida et al. 2009). Although supportive breeding programmes are widely used to avoid population declines (Keller and Waller 2002; Wang et al. 2002) stocking with non-native individuals is a controversial practice because it can lead to the loss of local adaptation and lower long-term fitness due to outbreeding depression (Araki et al. 2007; Fraser 2008; Eldridge et al. 2009; Muhlfeld et al. 2009). While the divergence observed between populations in this study is representative of that within brown trout metapopulations, our results are not conclusive with regard to fitness effects that would result from hybridization between much more divergent populations, e.g. between the members of separate metapopulations. As such, our results cannot refute the potential risks of cross-population stocking in general. Future systematic comparison of the fitness of crosses with larger genetic distances could help assess the risk of introducing non-native stock.

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Conflict of interest The authors declare that they have no competing interests.

Appendix

See Tables 1 and 2.

Table 1 Location, GPS coordinates, *n* of individuals sampled, and neutral genetic variation parameters of the study populations as determined from eleven microsatellite loci

Sampling site	Coordinates	*n* individuals	*n* alleles	*k*	H_E	H_O	F_{IS}
Giesse Münsingen	7°32′44,00″	40	13.18	9.90	0.77	0.69	0.105
	46°53′5,33″		(4–22)				
Upper Gürbe	7°30′56,03″	63	15.82	10.6	0.8	0.77	0.027
	46°47′19,25″		(5–26)				
Kiese	7°37′11,27″	45	13.64	9.78	0.78	0.77	0.001
	46°50′55,85″		(4–22)				
Rotache	7°36′52,71″	35	12.45	9.51	0.78	0.74	0.040
	46°48′29,31″		(4–22)				
Amletenbach	7°34′04,73″	57	11.54	8.61	0.76	0.72	0.054
	46°47′05,95″		(4–19)				

These data are a subset of those presented in Stelkens et al. (2012a)

N alleles mean number of alleles across loci (range across loci in parentheses), *k* allelic richness (corrected for variation in sample size), H_E gene diversity, H_O observed heterozygosity, F_{IS} Wright's inbreeding coefficient

Table 2 Pairwise population comparisons of genetic differentiation (F_{ST}) at eleven microsatellite loci

	Giesse Münsingen	Upper Gürbe	Kiese	Rotache	Amletenbach
Giesse Münsingen	–	0.018	0.027	0.028	0.031
Upper Gürbe		–	0.005	0.012	0.018
Kiese			–	0.018	0.026
Rotache				–	0.035
Amletenbach					–

All comparisons are significant after Bonferroni correction ($p < 0.005$). These data are a subset of those presented in Stelkens et al. (2012a)

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