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1 **Testing the effects of genetic crossing distance on embryo survival within a**
2 **metapopulation of brown trout (*Salmo trutta*)**
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4 Rike B. Stelkens[1,2], Manuel Pompini[1], Claus Wedekind[1*]
5
6 [1] Department of Ecology and Evolution, Biophore, University of Lausanne, CH-1015
7 Lausanne, Switzerland
8 [2] Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK
9
10 [*] Correspondence: Tel. +41 21 692 42 50; Fax: +41 21 692 42 65; claus.wedekind@unil.ch
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17

18 ## Abstract
19 Predicting progeny performance from parental genetic divergence can potentially enhance the
20 efficiency of supportive breeding programmes and facilitate risk assessment. Yet,
21 experimental testing of the effects of breeding distance on offspring performance remains
22 rare, especially in wild populations of vertebrates. Recent studies have demonstrated that
23 embryos of salmonid fish are sensitive indicators of additive genetic variance for viability
24 traits. We therefore used gametes of wild brown trout (*Salmo trutta*) from five genetically
25 distinct populations of a river catchment in Switzerland, and used a full factorial design to
26 produce over 2,000 embryos in 100 different crosses with varying genetic distances (F_{ST}
27 range: 0.005-0.035). Customized egg capsules allowed recording the survival of individual
28 embryos until hatching under natural field conditions. Our breeding design enabled us to
29 evaluate the role of the environment, of genetic and non-genetic parental contributions, and
30 of interactions between these factors, on embryo viability. We found that embryo survival
31 was strongly affected by maternal environmental (i.e. non-genetic) effects and by the
32 microenvironment, i.e. by the location within the gravel. However, embryo survival was not
33 predicted by population divergence, parental allelic dissimilarity, or heterozygosity, neither in
34 the field nor under laboratory conditions. Our findings suggest that the genetic effects of
35 inter-population hybridization within a genetically differentiated meta-population can be
36 minor in comparison to environmental effects.
37 **Keywords:** genetic distance; inbreeding; maternal effects; outbreeding; optimal outcrossing
38 distance; additive genetic variance; *Salmo trutta;* Salmonidae
39

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 & 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 & 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 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 & Waser 1979; Campbell & Waser 1987; Schierup & 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 & 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 & 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 & 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 & 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 & 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

90 (ICES 2006; Krkosek et al. 2007; Ford & Myers 2008). Severe population declines have also
91 been observed in many Swiss rivers where catches of brown trout have dropped by more than
92 50% during the last three decades - a pattern that is well documented but relatively poorly
93 understood (Fischnetz 2004; Borsuk et al. 2006; Burkhardt-Holm 2008). Supportive breeding
94 programmes are currently in operation whereby artificially fertilised eggs are raised in
95 hatcheries and fry are released back into the wild to supplement the natural populations.
96   There typically is considerable genetic differentiation on neutral markers between
97 neighbouring populations of brown trout (e.g. Keller et al. 2011). A recent study from a river
98 network in Switzerland found migration barriers to be associated with increased genetic
99 distance between populations (Stelkens et al. 2012a). Even on a microgeographic scale,
100 populations differed substantially in genotype (F_{ST}) and phenotype (body shape, especially
101 locomotory and trophic morphology), though the same populations tested negative for local
102 adaptation (Stelkens et al. 2012b). Here, we used five brown trout populations from the same
103 river network (a subset of those geno- and phenotyped in Stelkens et al. 2012a) and generated
104 full-factorial intra- and inter-population crosses to test whether we would find a genetic
105 crossing distance that is optimal with respect to embryo viability under natural conditions.
106 The crosses yielded over 2,000 embryos in 100 different half-sib families, covering a range
107 of genetic distances (F_{ST} 0.005-0.035) that is typical for natural stream-dwelling
108 metapopulations of brown trout (see literature cited in Stelkens et al. 2012a). The current
109 stocking regime in the study area stipulates that populations can only be stocked with
110 hatchery-bred offspring of spawners from the same population. We wanted to see if, within a
111 typical metapopulation of salmonids, a particular breeding strategy can enhance offspring
112 performance. Embryos were either buried in incubation capsules in a natural streambed or, as
113 a control, raised under benign conditions in the laboratory. Our breeding design also enabled
114 us to evaluate the role of the environment, of genetic and non-genetic parental contributions,
115 and of interactions between these factors, on embryo viability.
116

Material and Methods

Sampling of genetic and phenotypic data of adults
119 Stelkens et al. (2012a) collected tissue samples of 563 brown trout from 21 locations in the
120 Aare river system during summer and autumn of 2009. Further reproductive-age adults were
121 collected shortly before the breeding season of the same year by electro-fishing from five of
122 these locations (Figure 1; GPS data in Appendix 1) and transported to the cantonal hatchery
123 facility at Reutigen, Bern canton, where they were held for about 4 weeks. At one day around
124 the peak of the breeding season, 20 adult males and 20 gravid females (i.e. four of each sex
125 from each of the five populations) were haphazardly selected from among the captive fish for
126 use in our crossing experiment. Fish were anaesthetized with clove oil and processed as
127 follows: Photos and tissue samples were taken for these fish to be included in the analyses of
128 Stelkens et al. (2012a). Then, the fish were pressed along the length of the abdomen to expel
129 their gametes, which were collected in sterile Petri dishes. Next, tissue was collected from the
130 pectoral fin for the estimation of genetic parameters. Body length (tip of snout to end of
131 caudal fin) was recorded of each breeder and total egg mass for each female.
132

Fertilization protocol and treatment of fertilized embryos
134 We employed a full-factorial breeding design with respect to the five populations, in that
135 females from every population were crossed with males from every population, with each
136 population-by-population combination replicated four times by individual crosses (see Figure
137 2 in Clark et al. 2013a). Overall, the design yielded 100 different crosses (full-sib groups),
138 with a total of 2,115 fertilized eggs (mean number of embryos per cross 21±1.1 SD, range 15
139 to 22) that were used in the present study. Further embryos resulting from these crosses were

140 used in a parallel study on parental effects on pathogen resistance (Clark et al. 2013a).

141 Fertilizations were carried out at the Reutigen hatchery at 6.5 °C. Fertilization was
142 induced in 90 mm Petri dishes by adding ca. 20 µl of milt to ca. 80 eggs per dish (this amount
143 of sperm over-saturates the number of eggs yielding maximum success of fertilization in
144 every dish). Fifteen ml of standardized water (sterilized and aerated, chemically defined
145 water prepared according to OECD guidelines (OECD 1992) were added to activate the
146 sperm, and dishes were gently agitated to mix gametes. After 5 minutes, 50 ml of
147 standardized water were added and eggs were left undisturbed for 2 h of egg hardening. A
148 sub-sample of eggs from each female was then photographed. From digital analyses of these
149 photographs (ImageJ; http://rsbweb.nih.gov/ij/), we estimated mean egg size and egg redness
150 measured as the R/G colour ratio relative to a standard yellow reference (Gladbach et al.
151 2010).

152 Fertilized eggs were transferred to a cold chamber at the University of Lausanne,
153 (6.22 ± 0.14 °C) where they were rinsed under running tap water for 30 seconds (flow rate: 4
154 L/minute) and then distributed individually to wells of 24-well cell culture plates (Falcon,
155 Becton-Dickinson), which had been pre-filled with 2 ml per well of standardized water. Each
156 plate received one embryo from each of the 20 crosses involving the four females sampled
157 from a given population and five males from five different populations. For the following 27
158 days, embryos remained in the cold chamber with a daily light/dark cycle of 12h/12h (in
159 order to have a repeatable light regime while allowing for monitoring embryo development).
160 At the end of this period, all embryos were carefully examined on a light table (Hama
161 professional, LP 555) to determine survival with a stereo zoom microscope (Olympus SZX9).

162

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

174 On day 28 after fertilization, capsules were transported in chilled standardized water
175 to Giesse Belp (Fig. 1), where they were randomly allocated to one of two sites (46.907352 N
176 7.513543 E and 46.906327 N, 7.516094 E) recognizable by their appearance as natural brown
177 trout redds. The redds were briefly turned with a hoe to loosen the gravel and to reduce the
178 sediment load. Capsules were inserted into the streambed one by one (Supplementary Video
179 S1) after displacing the gravel with a steel spike and sleeve as per the methods of Dumas and
180 Marty (p289; 2006). Although the Giesse Belp stream is part of the Aare catchment, none of
181 the fish used in our crosses were collected from this stream. The burial sites thus represent a
182 novel environment to all populations in this experiment (in order to avoid effects of local
183 adaptation in our sample even if we had not found such effects in Stelkens et al. 2012b). At
184 the first burial site, streambed water temperature, recorded at 15 min intervals with an Escort
185 iLog data-logger (http://www.escortcoldchain.com/), ranged from 3.22 to 7.91 °C during the
186 burial period (mean 5.55 ± 1.03 °C). At the second burial site temperature ranged from 3.16
187 to 7.76 °C (mean 5.45 ± 1.02 °C).

188 Embryos remained in the streambed until their retrieval at 460 degree days (at a time
189 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.

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 degree 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 (Appendix 2), and other variables characterizing the populations' genetic variability (Appendix 1). Appendix 1 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 ($H_{projected}$), 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. $H_{projected}$ 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 ($p < 0.05$; Appendix 2; 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 & Nilsson 2000; Heggenes & 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 (Appendix 1). 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 ($H_{projected}$: $r_s = -0.68$, p <0.001).

Statistical analyses
241 Hypothesis testing was performed using R (R Development Core Team 2004). Except where
242 stated otherwise, we analyzed our data with a series of generalized linear mixed effect models
243 (GLMMs, lme4 package; Bates & Maechler 2009), in each case using a binomial fit for the
244 binary response variable (i.e. survival at 460 degree days). Variables grouping embryos by
245 their extent of common heritage were treated as random factors. These included *sire* and *dam*
246 identity (corresponding to half-sib groups), interaction between *dam x sire* (corresponding to
247 full-sib groups), and the variable *population cross* (representing more distant genetic links,
248 but common heritage nonetheless). The identity of the capsule in which an embryo was
249 reared, as well as the position within this capsule, were treated as random factors. F_{ST}, W,
250 $H_{projected}$ as well as burial *site* (because only 2 levels) were treated as fixed factors. To
251 evaluate the explanatory importance of a factor, alternative models with or without this factor
252 were compared using log-likelihood ratio tests (LRT) with restricted maximum likelihood
253 (REML) for random factors and maximum likelihood (ML) for fixed effects (Zuur et al.
254 2009).
255 Stream (n = 932) and control (n = 1,183) reared embryos were analyzed separately.
256 We constructed a first GLMM that included key environmental factors (*site*, *capsule* and
257 *position*, only available for stream-reared embryos), various random factors defining the
258 amount of shared heritage among groups of embryos, but none of the measured attributes of
259 particular dams or sires. Our base model thus had the following structure: *survival ~ burial*
260 *site* + (1|*capsule*) +(1|*position within capsule*) + (1|*dam*) + (1|*sire*) + (1|*dam:sire*) +
261 (1|*population cross*). From this point on, all candidate predictors of survivorship were
262 evaluated individually by testing changes in the likelihood of models with or without the
263 factor of interest. Correlations were calculated using Spearman's rank correlations r_s.
264

Results
Effects of genetic crossing distance on offspring survival
267 Mean survival across different population crosses varied between 61.1% and 80.0 % for the
268 stream-reared embryos, and between 83.3% and 100% for the control embryos. Variation in
269 survival of stream-reared embryos was not related to the divergence of parental populations
270 (F_{ST}: linear fit (LRT): $\chi^2_1 = 0.02$, p = 0.90; quadratic fit: $\chi^2_2 = 0.60$, p = 0.74; Figure 3a). The
271 same applies to the control environment (linear: $\chi^2_1 = 1.09$, p = 0.30; quadratic: $\chi^2_2 = 5.01$, p
272 = 0.08; Figure 3a).
273 Across individual crosses, survival ranged from 20 % to 100 % in stream-reared
274 embryos, and from 45.5% to 100% in control embryos. No relationship between embryo
275 survival and the genetic dissimilarity of breeders (*W*) was evident in the stream (linear: $\chi^2_1 =$
276 0.02, p = 0.88; quadratic: $\chi^2_2 = 0.87$, p = 0.65; Figure 3b). In the laboratory, however, *W* had
277 a nearly significant linear effect on offspring survival (linear: $\chi^2_1 = 3.79$, p = 0.051; quadratic:
278 $\chi^2_2 = 4.43$, p = 0.11; Figure 3b).
279 The expected heterozygosity *($H_{projected}$)* of stream-reared embryos had no significant
280 linear ($\chi^2_1 = 0.05$, p = 0.83) but showed a convex quadratic relationship with survival ($\chi^2_2 =$
281 7.04, p = 0.03; Figure 3c). Under control conditions, $H_{projected}$ had no significant effect on
282 embryo survival (linear: $\chi^2_1 = 1.13$, p = 0.29; quadratic: $\chi^2_2 = 4.84$, p = 0.09; Figure 3c).
283

Parental effects on offspring survival
285 We found a significant positive correlation between female body length and embryo survival
286 in the stream ($r_s = 0.53$, p = 0.02; Figure 4) but not between sire body length and embryo
287 survival ($r_s = 0.12$, p = 0.62). There was no significant effect of reproductive investment on
288 offspring survival (absolute brood mass: $r_s = 0.30$, p = 0.19; mean egg volume: $r_s = 0.25$, p =

289   0.28; egg redness: $r_s = 0.02$, p = 0.93). Survival was not predicted by the within-individual
290   genetic diversity of dams (H: $r_s = 0.24$, p = 0.31) or sires (H: $r_s = -0.29$, p = 0.22).

291   By virtue of our experimental breeding design, offspring variously shared the same
292   dams, sires or populations of origin, allowing us to disentangle these parental effects. In our
293   base model, *dam* identity explained a significant part of the variation (exclusion from base
294   model: stream: $\chi^2_1 = 6.24$, p = 0.01; control: $\chi^2_1 = 11.40$, p < 0.001), while *sire* identity had
295   no significant effects on embryo survival (LRT: stream: $\chi^2_1 = 0.01$, p = 0.92; control: $\chi^2_1 = $
296   0.00, p = 1.00). Non-additive genetic effects (*dam* x *sire* interaction) were negligible for the
297   stream environment (LRT: $\chi^2_1 = 0.00$, p = 1.00) but close to significance in the control
298   environment (LRT: $\chi^2_1 = 3.70$, p = 0.055). The factor *population cross identity* in our models
299   did not explain any variation in mortality, neither in the stream-reared (LRT: $\chi^2_1 < 0.01$, p =
300   1.00) nor in the control-reared embryos (LRT: $\chi^2_1 = 0.16$, p = 0.69).

301

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

307   Within the stream environment, there was a strong association effect of capsule
308   identity on embryo survival ($\chi^2_1 = 19.35$, p < 0.001). Mean survival was not different at the
309   two burial sites (Site 1: 67.5 % [95% CI: 63.4 – 71.4 %]; Site 2: 74.3 % [95% CI: 69.9 –
310   78.3%]; *site* effect $\chi^2_1 = 32.69$, p = 0.10), and there was no significant overall effect of
311   position within capsule (LRT; $\chi^2_1 = 2.14$, p = 0.14).

312

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

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

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

336   Embryo survival was not predicted by population divergence (F_{ST}), neither in the field
337 nor under laboratory conditions (Figure 3a). We consider three non-mutually exclusive
338 possible explanations for this result.
339   Firstly, although the populations we used were significantly structured, with
340 subpopulations genetically distinct from one another (Appendix 1 and Stelkens et al. 2012a),
341 the overall range of genetic distances our crosses yielded may not have provided sufficient
342 breadth to reveal inbreeding or outbreeding depression. Regarding outbreeding depression, it
343 is difficult to predict *a priori* at what genetic distance we should expect to see effects.
344 Although direct comparison between species of the genetic distances of crosses is impaired
345 by the variability of genetic markers used, in a study of largemouth bass (*Micropterus*
346 *salmoides*), crosses with rather small distance ($G_{ST} = 0.05$) resulted in up to 58% reductions
347 in viral resistance among F2 individuals compared to ancestral individuals (Goldberg et al.
348 2005), yet no reduction in F1 embryonic survival was observed in Atlantic salmon crossed
349 over substantial genetic distances (Nei's D > 0.43; Fraser et al. 2010). A meta-analysis
350 comprising 670 pairwise comparisons of fish populations cautioned that few general
351 predictions could be made about the size or direction of outbreeding effects, and observed
352 that genetic distance explained little of the variation in effect size across studies (McClelland
353 & Naish 2007).
354   Secondly, it is possible that inbreeding or outbreeding effects are not influential
355 enough (i) to cause mortality at benign laboratory conditions and (ii) to overrule the effects of
356 typical environmental variation at the early ontogenetic life-stages on which we focused.
357 Perhaps, inbreeding or outbreeding effects are more pronounced later in life for traits such as
358 survival to maturity, attractiveness to mates, fecundity, reproductive success, and longevity
359 (Stearns 1992; Szulkin et al. 2007; Grueber et al. 2010). For example, Gharrett *et al.* (1999)
360 found no effect of genetic distance on salmon fertilization rates but the rate of return of adults
361 to the spawning grounds was reduced for more outcrossed fish. Life stage-specific effects of
362 inbreeding depression, outbreeding depression, and heterosis have been observed in other
363 animal and plant species (Husband & Schemske 1996; Koelewijn et al. 1999; Escobar et al.
364 2008). Moreover, outbreeding depression in particular may only become evident in later
365 hybrid generations, i.e. in or after the F2 (Edmands 2007).
366   Thirdly, the specific evolutionary history of a population can potentially mitigate the
367 effects of inbreeding and outbreeding. The severity of inbreeding depression, for example,
368 depends on the genetic load carried in a population, but inbreeding during severe or frequent
369 population bottlenecks in the past can purge detrimental alleles and reduce the costs of
370 inbreeding (Bijlsma et al. 1999; Glemin 2003). Meanwhile, the effects of outbreeding
371 depression, which involves the disruption of locally built up coadaptations, can be diminished
372 by pre-existing gene flow (Lynch 1991). Extrinsic effects can also influence the shape of the
373 genetic distance-fitness function, such as the mode of selection (e.g. directional versus
374 balancing selection; Frankham 2009) and the type of environment (Armbruster & Reed
375 2005). Thus, it is conceivable that historical factors influence the genetic composition of our
376 sampled populations in a way that reduces the likelihood of inbreeding or outbreeding
377 depression in the present time.
378   Besides genetic crossing distance, we also investigated how other factors affected
379 offspring survival. We were able to estimate the relative impact of the environment, of
380 genetic and non-genetic parental contributions (for the latter we assumed that variation in
381 maternally inherited mitochondrial genes has no significant effects on embryo performance),
382 and of interactions between these factors. We found that embryo survival was strongly
383 affected by maternal environmental effects (i.e. non-genetic, environmental conditions faced
384 by mothers before egg laying) and by the microenvironment, i.e. by the location within the
385 gravel. Rearing conditions strongly affected offspring phenotype, with stream-reared

386 embryos showing reduced survival compared to embryos reared in the laboratory. The
387 specific causes of this elevated mortality in the stream could not be identified. They may
388 have included pathogens, micro-predators, or physicochemical stresses. The intensity of these
389 environmental stresses will vary through time and space, and accordingly, we found that an
390 embryo's position within the streambed had a strongly significant influence on its survival
391 confirming previous findings at other locations (Stelkens et al. 2012b).
392   We found significant dam effects on offspring fitness, but no paternal effects, which
393 suggests that most of the maternal effect was not due to additive genetic effects (i.e. mediated
394 through the maternally-provided environment or caused by epigenetic effects). As such, our
395 results add to a growing body of evidence for the evolutionary significance of maternal
396 effects (Mosseau & Fox 1998). Given that the survival of a dam's offspring was not
397 significantly related to the mean size of her eggs, or their redness (a proxy for carotenoid
398 content that may partly reflect maternal investment; L.G.E. Wilkins and C. Wedekind,
399 unpublished results), it is likely that many of these maternal effects were mediated by
400 qualitative - rather than quantitative - provisioning of nutrients, protective structures, or
401 immune-active substances within the egg. Alternatively, egg size may be unrelated to
402 offspring viability if fitness is maximised at optimal (i.e. environment-dependent), rather than
403 maximal, egg sizes (Smith & Fretwell 1974).
404   Dam × sire interaction effects were negligible for survival in the stream but nearly
405 significant in the control environment. This suggests that non-additive genetic effects (e.g.
406 dominance interactions) may play a role but that their importance is mitigated by
407 environmental variation.
408   In contrast to maternal environmental effects, sires did not have much influence on
409 the survival of their offspring in our experiment. This result is consistent with other salmonid
410 studies employing various group-rearing conditions (e.g. Beacham 1988; Nagler et al. 2000;
411 Urbach et al. 2008; Janhunen et al. 2010). Janhunen et al. (2010) and others suggested that
412 detecting paternal effects at embryonic stages depends on the kind and amount of
413 environmental variance allowed for in the experiment, a prediction verified in recent studies
414 on brown trout (Jacob et al. 2010) and whitefish *Coregonus palaea* (e.g. von Siebenthal et al.
415 2009). Our findings confirm the significance of environmental variation in affecting early
416 embryo survival within the gravel of a natural red. It seems that additive genetic effects
417 during embryogenesis are best observed under controlled laboratory conditions. For instance,
418 significant sire effects could be found on the timing of hatching after sub-lethal infections of
419 embryos with *Pseudomonas fluorescens*, indicating additive genetic variation in infection
420 tolerance in brown trout (Clark et al. 2013a) and in the whitefish *C. palaea* (Clark et al.
421 2013b). On a side note, Clark et al. (2013a) found no significant role of genetic crossing
422 distance on infection tolerance, analogous to our findings.
423   While paternal effects are sometimes small at very early developmental stages
424 (Wedekind et al. 2001, 2008a), studies on late embryo viability often found considerable
425 additive genetic effects (e.g. Wedekind et al. 2001; Jacob et al. 2007; Pitcher et al. 2007;
426 Wedekind et al. 2007; Wedekind et al. 2008b; Evans et al. 2010, Clark et al. 2013b), of which
427 some were linked to allelic variation on major histocompatibility complex (MHC) loci in a
428 quantitative genetic breeding experiment (Pitcher & Neff 2006), and in a selection
429 experiment within full-sib families (Wedekind et al. 2004). Other examples for paternal
430 effects on traits expressed later in life include MHC expression shortly before hatching (Clark
431 et al. 2013c), resistance to pathogens after hatching (Evans & Neff 2009), hatchling size
432 (Eilertsen et al. 2009), growth after hatching (Vandeputte et al. 2002), and territorial
433 behaviour (Petersson & Jarvi 2007).
434
435 **Conclusions**

9

436 We found no evidence that the genetic distance between populations affected offspring
437 survival under the conditions of this study, i.e. when embryo survival was recorded during
438 incubation in a natural redd (i.e. under potentially stressful conditions) or in the laboratory
439 under benign conditions. We conclude that, at the embryonic life-stage, the fitness
440 consequences of inter-population hybridization within this metapopulation (such as occurs
441 during supportive breeding programmes; Edmands 2007) can be minor in comparison to
442 other factors affecting embryo viability such as the incubation microhabitat or maternal
443 environmental effects. This does not exclude the possibility that the genetic distance between
444 parents may be important over different genetic distance scales, for different traits or life-
445 stages, or when applied to a different population network.
446   Brown trout are known to have complex population structure within river catchments,
447 often with substantial genetic differentiation, vast phenotypic diversity, and large variation in
448 life history strategies (e.g. Nielsen et al. 2003; Hermida et al. 2009). Although supportive
449 breeding programmes are widely used to avoid population declines (Keller & Waller 2002;
450 Wang et al. 2002) stocking with non-native individuals is a controversial practice because it
451 can lead to the loss of local adaptation and lower long-term fitness due to outbreeding
452 depression (Araki et al. 2007; Fraser 2008; Eldridge et al. 2009; Muhlfeld et al. 2009). While
453 the divergence observed between populations in this study is representative of that within
454 brown trout metapopulations, our results are not conclusive with regard to fitness effects that
455 would result from hybridization between much more divergent populations, e.g. between the
456 members of separate metapopulations. As such, our results cannot refute the potential risks of
457 cross-population stocking in general. Future systematic comparison of the fitness of crosses
458 with larger genetic distances could help assess the risk of introducing non-native stock.
459

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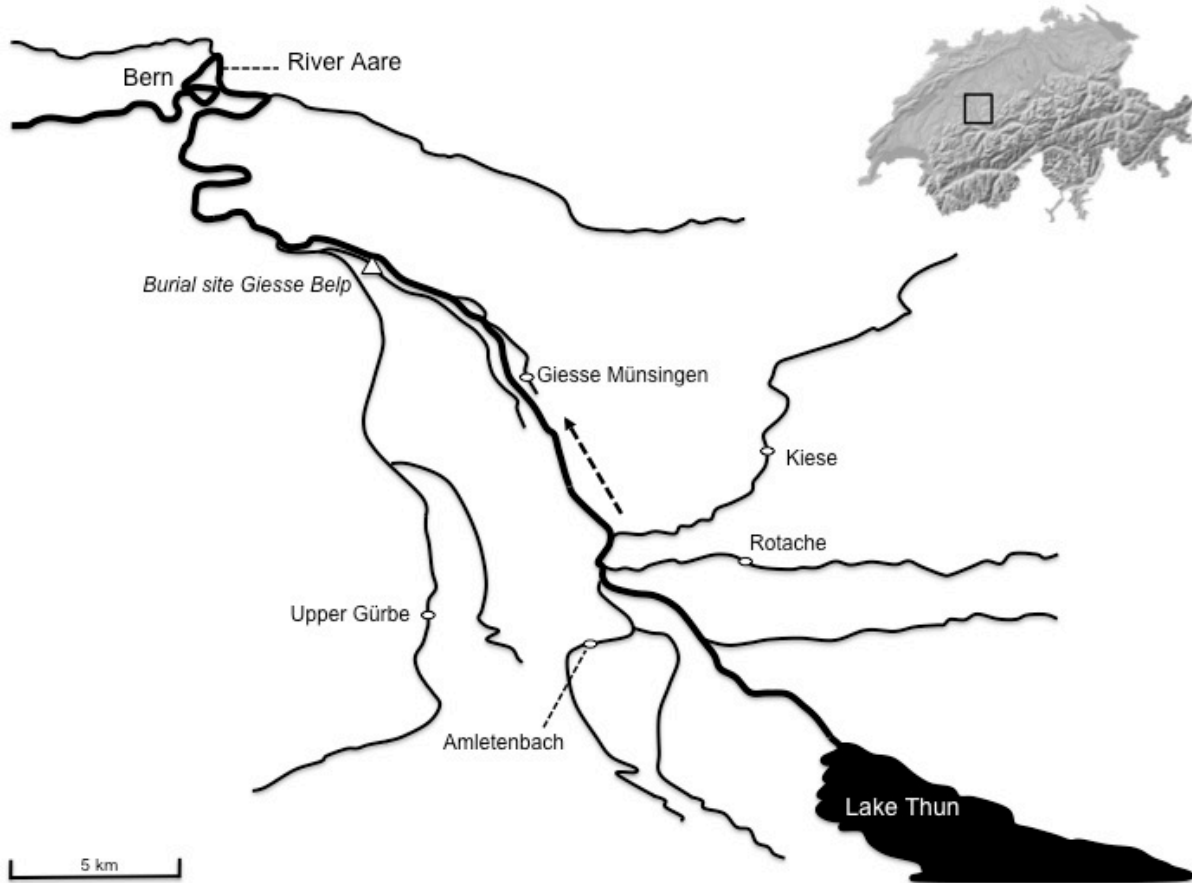703   2357-2364.
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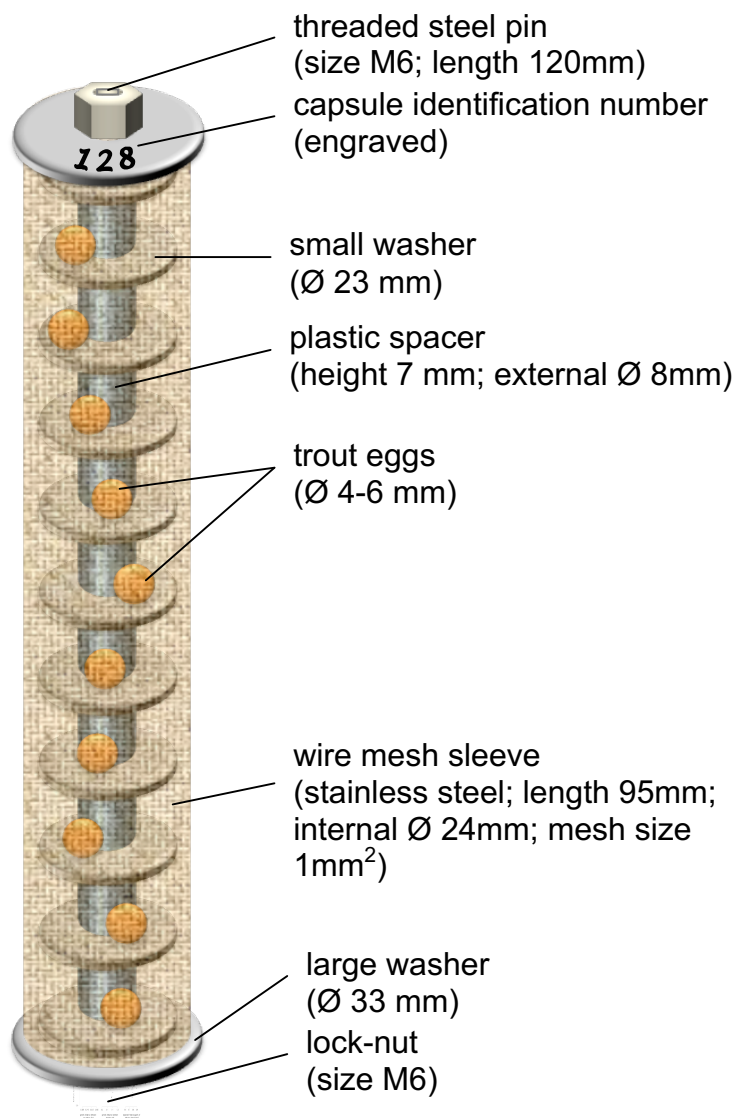708   andextensions in ecology with R. Springer, New York.
709
710

711 **Figure 1. Map of the River Aare catchment** between Thun and Bern (Switzerland),
712 indicating the five sampling sites (modified from Stelkens et al. 2012a). The triangle
713 indicates the site where eggs were reared in the streambed. The dashed arrow indicates
714 direction of water flow. The box in the upper-right insert indicates the location of the
715 catchment in Switzerland.
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717
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721   **Figure 2. Egg capsule design.**
722

threaded steel pin
(size M6; length 120mm)

capsule identification number
(engraved)

small washer
(Ø 23 mm)

plastic spacer
(height 7 mm; external Ø 8mm)

trout eggs
(Ø 4-6 mm)

wire mesh sleeve
(stainless steel; length 95mm;
internal Ø 24mm; mesh size
$1mm^2$)

large washer
(Ø 33 mm)

lock-nut
(size M6)

723
724

725 **Figure 3. Mean survival of offspring resulting from crosses of varying genetic distances.**
726 Open symbols (and dashed line) denote means for groups of laboratory-reared embryos,
727 while closed symbols (and solid line) denote groups reared within natural streambeds. See
728 text for relevant statistics.
729
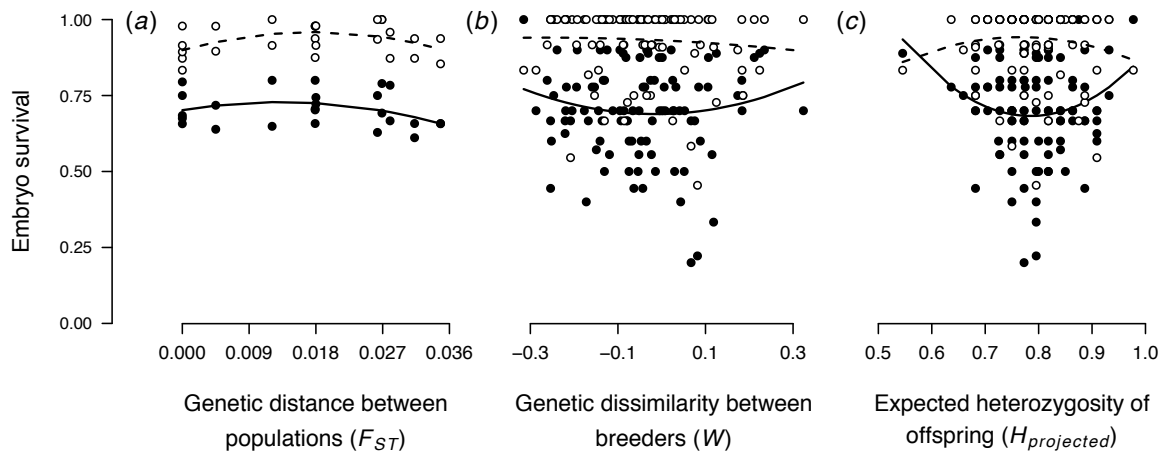
730

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

733
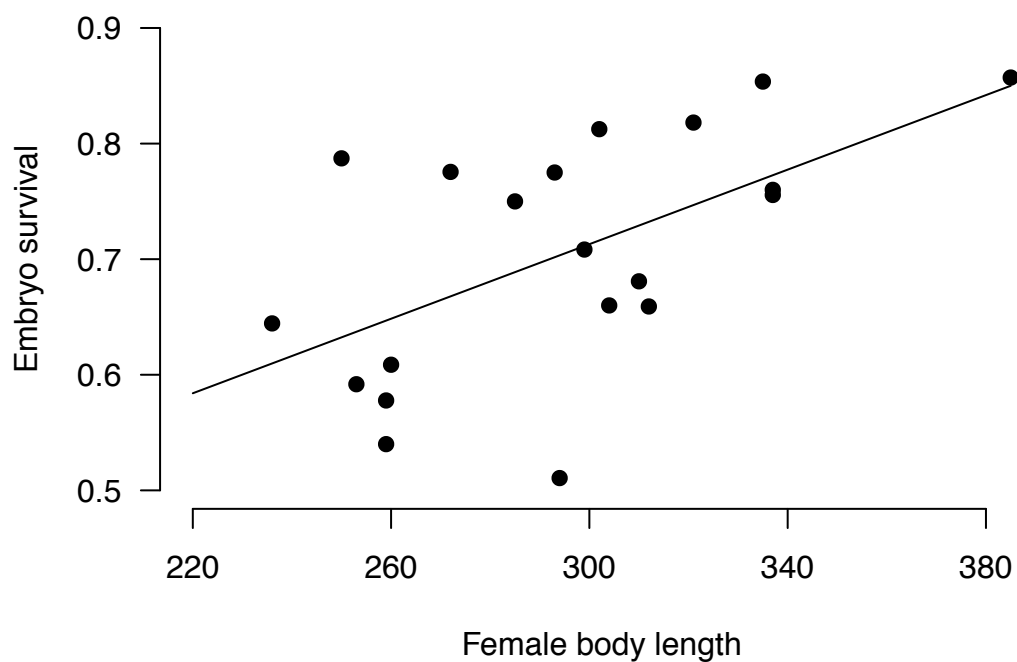
Appendix 1: Location, GPS coordinates, n of individuals sampled, and neutral genetic variation parameters of the study populations as determined from eleven microsatellite loci. These data are a subset of those presented in Stelkens *et al*. (2012a).

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

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

Appendix 2: Pairwise population comparisons of genetic differentiation (F_{ST}) at eleven microsatellite loci. All comparisons are significant after Bonferroni correction ($P < 0.005$). These data are a subset of those presented in Stelkens *et al*. (2012a).

	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					-

Supplementary Video S1: This video (51 seconds) shows the burial of an egg capsule into the river bed, and the later retrieval using a metal detector.
See http://youtu.be/VYhq9lM2G8g