


Sex-specific effects of inbreeding in juvenile brown trout

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Funding information

Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_159579 and 31003A_182265

Handling Editor: Andrew P. Kinziger

Abstract

Inbreeding depression, that is, the reduction of health and vigour in individuals with high inbreeding coefficients, is expected to increase with environmental, social, or physiological stress. It has therefore been predicted that sexual selection and the associated stress usually lead to higher inbreeding depression in males than in females. However, sex-specific differences in life history may reverse that pattern during certain developmental stages. In some salmonids, for example, female juveniles start developing their gonads earlier than males who instead grow faster. We tested whether the sexes are differently affected by inbreeding during that time. To study the effects of inbreeding coefficients that may be typical for natural populations of brown trout (*Salmo trutta*), and also to control for potentially confounding maternal or paternal effects, we sampled males and females from the wild, used their gametes in a block-wise full-factorial breeding design to produce 60 full-sib families, released the offspring as yolk-sac larvae into the wild, sampled them 6 months later, identified their genetic sex, and used microsatellites to assign them to their parents. We used whole-genome resequencing to calculate the kinship coefficients for each breeding pair and hence the expected average inbreeding coefficient per family. Juvenile growth could be predicted from these expected inbreeding coefficients and the genetic sex: Females reached lower body sizes with increasing inbreeding coefficient, while no such link could be found in males. This sex-specific inbreeding depression led to the overall pattern that females were on average smaller than males by the end of their first summer.

KEYWORDS

conservation genetics, ecological genetics, fish, inbreeding, population genetics – empirical

1 | INTRODUCTION

Inbreeding can lead to inbreeding depression, that is, to a reduction in health and vigour, because of the expression of deleterious recessive alleles and a general reduction of heterozygote advantages (Charlesworth & Willis, 2009). Males and females can be differently

affected by inbreeding, for example, because of sex-specific differences in the strength of sexual selection (Ebel & Phillips, 2016; Vega-Trejo et al., 2022). A general prediction is that males suffer more from inbreeding than females because the strength of sexual selection is usually higher for males (Janicke et al., 2013; Noel et al., 2019). Heterogamety has been discussed as a possible

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alternative explanation for sex-specific inbreeding depression (because deleterious mutations can be masked by dominant alleles in the homo- but not the heterogametic sex), but its relevance is still unclear (Connallon et al., 2022; Vega-Trejo et al., 2022). Little is known about other possible reasons for sex-specific effects of inbreeding such as differences in early life history (Vega-Trejo et al., 2022).

Most salmonid fish reach sexual maturity at the age of two or later, usually with no obvious sexual dimorphism before. However, the sexes differ in many aspects from very early stages. Sex-specific stress tolerances have already been observed at the embryo stage in different salmonids (Moran et al., 2016; Nusbaumer, Garaud, et al., 2021) and are possibly linked to the significant differences in gene expression that have been found at that stage (Guiguen et al., 2019; Maitre et al., 2017; Selmoni et al., 2019). Sex differences could also be found during the early juvenile stages when gonad formation starts. In grayling (*Thymallus thymallus*), genetic females start gonad formation earlier than males who instead grow faster during that time (Maitre et al., 2017). These sex differences peak around the first summer, possibly making female juveniles more susceptible to heat stress and thereby potentially explaining a correlation between water temperatures and male-biased sex ratios among adults (Wedekind et al., 2013). Analogous patterns have been observed in brown trout: females start gonad formation earlier than males, and captive-born males grow larger than captive-born females after their first months in the wild (Palejowski et al., 2022).

Inbreeding in wild populations is typically a consequence of low effective population sizes (Wang et al., 2002). It has been shown to negatively influence early life-history traits (Kincaid, 1976; Naish et al., 2013), disease resistance (Arkush et al., 2002), and reproductive traits (Naish et al., 2013; Paul et al., 2021; Waters et al., 2020) in diverse salmonid species, but other studies did not find significant and consistent negative effects of inbreeding (Houde et al., 2011; Johnson et al., 2015). Inbreeding effects in salmonid species can be influenced by environmental context (Gallardo & Neira, 2005) or temporal and regional genomic effects (Paul et al., 2021). Not much is known about sex-specific effects of inbreeding in salmonids, but a recent meta-analysis on other taxa (mostly insects) highlighted the potential sex-specific effects of inbreeding and concluded that they may mostly be due to differences in the strength of sexual selection (Vega-Trejo et al., 2022). The role of sex-specific life histories, however, remains unclear.

Here, we focus on juvenile brown trout around a time when the sexes are expected to differ at least in gonad formation and the physiological stress that may be associated with it, that is, around the end of their first summer. To study ecologically relevant inbreeding coefficients while experimentally controlling for potentially confounding maternal and paternal effects, we sampled adult males and females from the wild and used their gametes for in vitro fertilization in full-factorial breeding blocks. We released the larvae into the wild and sampled them 6 months later. Here, we (i) compare these captive-bred juveniles that had been stocked into the wild with the wild-born of the same cohort, and (ii) test whether there are sex-specific effects of inbreeding on fitness-relevant traits.

2 | METHODS

Adult brown trout were caught from the *Rotache* stream shortly before the spawning season. This rather fast-flowing stream is in the Swiss Pre-Alps in a sparsely populated area dominated by pasture and forest. In previous population comparisons, the *Rotache* has been used as a representative of rather pristine tributaries of the river Aare (Marques da Cunha et al., 2019; Nusbaumer, Marques da Cunha, & Wedekind, 2021). Its population of brown trout is genetically distinct from the neighbouring populations, including the Aare River population (Stelkens et al., 2012), but does not seem to suffer from elevated levels of inbreeding, as concluded from measurements of hybrid vigour (Clark et al., 2013; Stelkens et al., 2014).

The eggs of 12 females were stripped into large Petri dishes. Total egg weight per female was determined. The eggs of each female were then about equally distributed to five new Petri dishes. Each batch was fertilized with milt of one of in total 10 males in two full-factorial breeding blocks (6 × 5 each) to produce in total 60 full-sib families as described in Wilkins et al. (2017) (see also Table S1). Fin clips were stored in 70% ethanol at -20°C. After egg hardening (for two hours) and sampling 24 eggs per full-sib family for parallel laboratory studies on the possible effects of egg carotenoids on embryo stress tolerance (Marques da Cunha et al., 2018; Wilkins et al., 2017), photos of the remaining eggs were taken to later determine the number of eggs and hence the average egg weight per female (total egg weight/total egg number). In total 1925 remaining eggs (mean ± SD number per full-sib family = 32.1 ± 14.8) were then pooled and incubated under routine hatchery conditions at the cantonal *Fischereistützpunkt Reutigen* at a constant temperature of 8.5°C. Egg loss (unfertilized eggs and/or dead embryos) during that time was reported by the hatchery staff to be as low as usual for brown trout (i.e. "less than 15% in total"), confirming observations on the samples raised in parallel under laboratory conditions (Wilkins et al., 2017). At a late yolk-sac stage in early March, the hatchlings were evenly stocked along a 700m stretch of the *Mühlibach* streamlet (a small tributary to the *Rotache*; 46.804459°N, 7.690544°E). See Figure S1 for a map and photos.

About 6 months after release into the wild (i.e. in late August), electrofishing was used along the same 700m stretch of *Mühlibach* streamlet to catch as many brown trouts as possible ($N_{\text{total}} = 518$). The fish were narcotized (0.075 g/L tricaine methanesulfonate buffered with 0.15 g/L NaHCO₃) and photographed on a weighing scale to later extract fork length and body weight. Fin clips were collected and stored in 70% ethanol. After handling, all fish were released into the *Rotache* stream and not monitored further.

Fin clips of the adult breeders and a random subset of juveniles from the wild ($N = 376$) were used for microsatellite genotyping and genetic sexing. DNA was extracted using the BioSprint® 96 workstation following the manufacturer's protocol (Qiagen GmbH, Hilden, Germany). DNA was quantified using an HS dsDNA assay on a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA), and concentrations of up to 20 ng/μL were sent to *Ecogenics GmbH* (Balgach, Switzerland) for genotyping at 13 microsatellite loci and genetic sex determination using the protocol described in Palejowski et al. (2022). Briefly, amplification of the microsatellite loci was done in three

multiplex reactions, each containing primers for the amplification of four or five microsatellites (see Palejowski et al., 2022 for the primers and the PCR protocol). Fragment analyses were performed on a 3730XL DNA Analyser (Applied Biosystems, Foster City, CA, USA) with a GeneScan LIZ500 size standard (Applied Biosystems), and allele calling was performed using the GeneMarker V2.6.4 software (SoftGenetics LLC, State College, PA, USA). Parental assignment of the juveniles was based on the full-likelihood approach implemented in Colony v2.0.6.5 (Jones & Wang, 2010) with a threshold of 0.98. One male (ID="151") could not be genotyped at the microsatellite loci. However, Colony identified one male who had offspring with all six females of the first breeding block (Table S1) which allowed us to infer that this was male "151".

The sex-specific primers (Quéméré et al., 2014) were added to one multiplex. Genetic sexing was then based on the peak area ratios between the amplified sdY fragment and the autosomal MST-591 microsatellite marker. A threshold value of 0.25 was used to genetically sex the juveniles because Palejowski et al. (2022) had demonstrated, in a sample of 851 phenotypically sexed brown trout, that this threshold reduced the false assignment rate to 0 and 0.2% for males and females, respectively. A Bayesian model was used to separate 0+ juveniles from older ones based on the expected size gap between these age categories (see Supplementary Material).

For all but two breeders (one dam and one sire; Table S1), high-quality DNA extracts could be used for whole-genome resequencing to calculate the kinship coefficients for each breeding pair. Samples were sent to the NGS platform at the University of Bern (Switzerland) for library construction using the Illumina TruSeq DNA PCR-Free Library Prep Kit (Illumina Inc., San Diego, CA, USA) after mechanical shearing of the DNA. Electrophoresis-based size selection (150bp fragments) was used prior to library quantification, quality control, and paired-end sequencing using a NovaSeq 6000 S4 flow cell (Illumina Inc., San Diego, CA, USA). Adult samples from the current study were combined with samples from a parallel study to achieve an estimated coverage of 15x. The quality of raw sequence reads was assessed using FastQC v0.11.9 (Andrews, 2010). Trimmomatic v0.39 (Bolger et al., 2014) was subsequently used to remove adaptor sequences and remove low-quality reads (i.e. HEADCROP:6 LEADING:3 TRAILING:3 MINLEN:70 CROP:140). High-quality reads were aligned to the indexed reference genome of brown trout (Hansen et al., 2021) with BWA v0.7.17 (Li et al., 2009), and the obtained BAM files were further processed using Samtools v1.12 (Li et al., 2009) and Picard v2.24.0 (<http://broadinstitute.github.io/picard/>). BAM files were cleaned by soft-clipping beyond-end-of-reference alignment and setting MAPQ to 0 for unmapped reads, alignments were sorted by leftmost coordinates, mate coordinates were filled, and duplicated alignments were marked. The resulting clean, coordinate-sorted BAM files were indexed and ordered along the reference genome, and variants were called using the HaplotypeCaller function of GATK v4.2.0.0 (McKenna et al., 2010). Variants were subsequently hard filtered according to GATK best practices recommendations (i.e. QD < 2.0, QUAL < 30, SOR > 3.0, FS > 60.0, MQ < 40.0, MQRankSum < -12.5, ReadPosRankSum < -8.0) (Depristo et al., 2011; Van der Auwera & O'Connor, 2020). Further

filtering was performed to remove indels and SNPs with a sequencing depth < 10 and > 30, outside of Hardy-Weinberg equilibrium ($p < 10^{-8}$) and showing signs of strong linkage disequilibrium ($r^2 > .6$). Only SNPs present in at least 90% of individuals were retained. This led to a panel of 1,058,625 SNPs.

The *beta.dosage* function in the R package *Hierfstat* 0.04-30 (Goudet, 2005) was used to obtain individual inbreeding coefficients of the breeders and kinship coefficients of breeder pairs (i.e. the expected average inbreeding coefficient per full-sib family prior to selection). The kinship coefficient r^{β} as described in Goudet et al. (2018) was determined using allele dosage data to estimate the relative pairwise kinship coefficients, that is, the inbreeding and the kinship coefficients are both calculated relative to the current population and can therefore be negative (for example, for pairs of individuals that share fewer alleles than the population average, Goudet et al., 2018). Relative estimates have the advantage that reference allele frequencies do not need to be estimated. Pairwise kinship coefficients were also determined using the 13 microsatellite genotypes following Wang's estimators of relatedness (that is twice the kinship, see Wang, 2002, 2017) implemented in the Coancestry software (Wang, 2011), to assess the correlation between SNP- and microsatellite-based estimates.

Statistical analyses were done in JMP Pro17 and R 4.0.2 (R Development Core Team, 2015). The Akaike information criterion (AIC) was used to describe the fit of different distribution models on juvenile sizes. Standard F-tests were used to compare means when visual examination of the distributions suggested similar variances. Welch's F-tests were used when this model assumption seemed violated. Likelihood ratio tests were used to compare frequencies. Linear mixed-effect models (LMM) were used to evaluate the combined effects of sex and kinship on juvenile body length and weight, after excluding one family with an extremely high kinship coefficient to avoid violating the assumptions of the LMM (see Results). In these models, sex was entered as a fixed factor and the kinship coefficient as a covariate. Dam and sire identities were entered as random factors after visual inspection of the length and weight distributions and of the corresponding Q-Q plots suggested that the model assumptions were not significantly violated (see Figure S2 for body lengths, the distributions looked similar for body weights). Non-parametric Spearman correlation coefficients r_s were used to test for correlations between parental inbreeding coefficients, kinship coefficients, and family sex ratios.

3 | RESULTS

Figure 1a shows the bimodal size distribution of the 518 juveniles that could be caught from the wild. As expected, the random sample of 375 juveniles that were genotyped for parental assignment did not significantly differ in body lengths from the non-genotyped fish (Welch's $F_{1,297.4} = 1.7$, $p = .19$). In total, 301 (80.3%) of these 375 wild-caught juveniles could be assigned to 56 of the 60 experimental sib groups. Their average (\pm SD) body lengths and weights were 95.6 ± 10.6 mm and 11.3 ± 3.9 g, respectively,

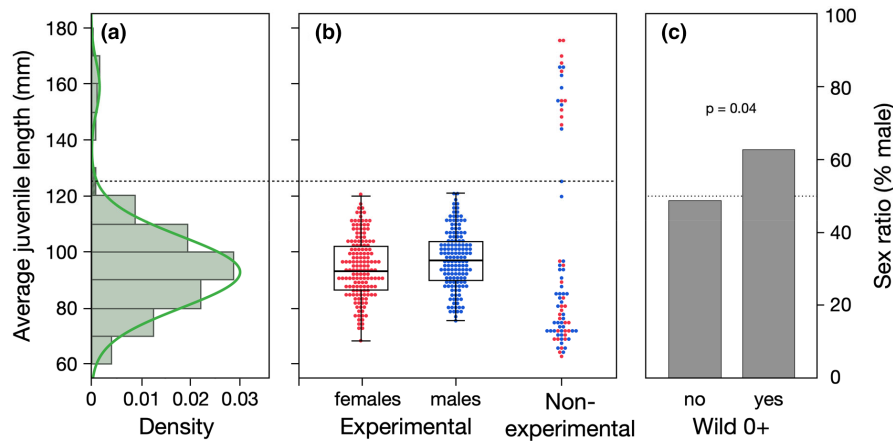


FIGURE 1 Sizes of trout sampled from the wild. (a) Size distribution of all trout ($N=518$) with the bimodal normal distribution (green line; $AIC=4278.3$) that fit the data better than a normal distribution ($AIC=4465.1$). (b) Sizes of trout that were genetically sexed and assigned to one of the experimental families or to the non-experimental ones (blue = male, red = female). The hatched line indicates the largest size of 0+ fish based on a Bayesian mixture model (Supplementary Material) and that turned out to be supported by the genetic assignments of known 0+. (c) Sex ratio (% males) among captive-bred and wild-born that were identified as 0+. The dotted line indicates the 50% male ratio. See text for statistics.

and were always below the 125 mm that the Bayesian mixture model (see Supplementary Material) had identified as upper size for 0+ fish (Figure 1b). All but one of the offspring could be genetically sexed. The overall percentage of males among these experimentally bred juveniles was 48.3% and not significantly biased ($\chi^2=0.33$, d.f. = 1, $p=.56$).

The Bayesian model identified 57 of the remaining 74 genotyped fish as wild-born 0+ juveniles and 17 (the largest ones) as 1+ or older (Figure 1b). The wild-born 0+ were on average 17.6 mm smaller than the experimentally bred 0+ (Figure 1b; $F_{1,356}=122.5$, $p<.001$). Among the experimentally bred 0+, males were on average 2.6 mm larger than females ($F_{1,298}=4.5$, $p=.03$; Figure 1b). Among the wild-born 0+, males were on average 3.0 mm larger than females, which was in this smaller sample not statistically significant ($F_{1,55}=1.6$, $p=.21$). However, the wild-born 0+ had a male-based sex ratio (63.2% males) that was not observed in the experimentally bred 0+ ($\chi^2=4.3$, d.f. = 1, $p=.04$; Figure 1c).

We obtained 49 kinship coefficients (mean \pm SD = -0.001 ± 0.04). One full-sib family with a kinship coefficient of 0.226 was classified as extreme (because $\gg 3$ SDs away from the mean, following the three-sigma rule; see also Figure S3). The nine offspring from this family were therefore excluded from all further analyses (Figure S4 gives their sizes relative to their maternal half-siblings), leaving 251 genetically sexed juveniles of 46 full-sib families for the final analyses (Table S1; no juveniles could be sampled from two full-sib families). The kinship coefficients, that is, the expected average inbreeding coefficients per family, could partly be predicted from the inbreeding coefficients of the dams or the sires: Higher parental inbreeding coefficients led to higher average kinship coefficients between a parental individual and all of its mates ($r_s=.61$, $n=20$, $p=.004$; Figure S5). These SNP-based kinship coefficients were, however, not significantly correlated to the kinship coefficients calculated from the 13 microsatellites ($r_s=.19$, $n=44$, $p=.23$; Figure S3).

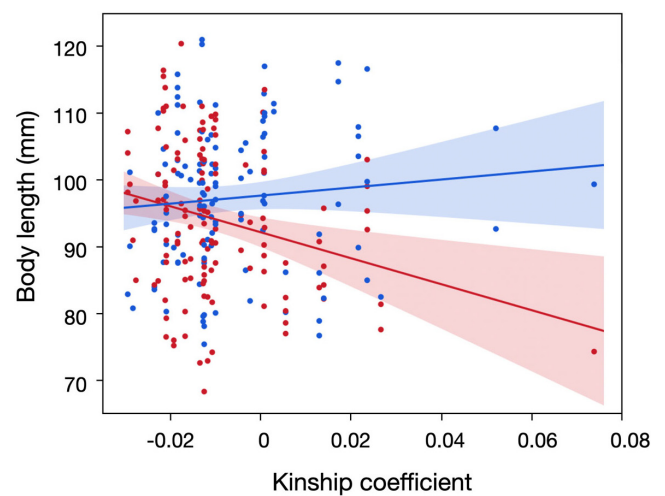


FIGURE 2 Body length of wild-caught juvenile brown trout predicted by the kinship coefficient (i.e. the expected average inbreeding coefficient per full-sib family) in male (blue dots and regression line) and female offspring (red). The shaded areas give the 95% confidence intervals for the linear regressions. Female but not male size declines with increased kinship coefficients (Table 1; this is also the case if kinship coefficients >0.03 are excluded, see Figure S5 and Table S2).

The body sizes of female juveniles declined with increased kinship coefficients, while the body sizes of male juveniles were not significantly correlated to kinship coefficients (Figure 2; Table 1; Figure S6; Table S2). There were also significant dams but not sire effects on juvenile body size (Table 1). These dam effects on juvenile size could, however, not be explained by mean egg weight per dam (mean female juvenile size: $r_s=.43$, $n=12$, $p=.17$; mean male juvenile size: $r_s=.44$, $p=.15$).

The recapture rates per full-sib family, that is, the mean number of recovered juveniles per number of released larvae, varied

TABLE 1 Linear mixed model on juvenile length and weight when predicted by sex (baseline: females) and mean kinship coefficient per family.

Effects	Body length				Body weight			
	d.f.	F	Variance component	p	d.f.	F	Variance component	p
Fixed effects								
Sex	1, 242.1	5.4		.02	1, 240.5	9.1		.003
Kinship	1, 42.9	0.3		.58	1, 43.4	0.2		.66
Sex × kinship	1, 242.1	8.3		.004	1, 240.8	8.8		.003
Random effects								
Dam ^a			27.3 ± 14.0	.05			3.6 ± 1.9	.05
Sire ^a			3.1 ± 3.3	.35			0.4 ± 0.5	.34
Residual			83.4 ± 7.7				10.5 ± 1.0	

Note: Parental identities were included as random factors. Significant *p*-values are highlighted in bold.

^aREML unbounded variance components ± standard error, Wald *p*-values.

between 0 and 0.68 and were not correlated with the kinship coefficients ($r_s = .13$, $n = 48$, $p = .38$) nor with the inbreeding coefficients of the dams ($r_s = .39$, $n = 11$, $p = .23$) or the sires ($r_s = .05$, $n = 9$, $p = .90$). Recapture rates were also not significantly correlated to mean egg size per dam ($r_s = -.13$, $n = 12$, $p = .70$).

The number of recovered juveniles per experimental sib group that was represented in our sample varied from 1 to 17 (mean = 5.3, SD = 3.1), that is, sex ratios per full-sib family could mostly not be determined due to low *N*. However, the number of recovered juveniles per dam varied from 5 and 52 (mean = 24.3, SD = 10.6). Family sex ratio differed among the maternal sib groups ($\chi^2 = 22.5$, d.f. = 11, $p = .02$) but could not be predicted by mean egg size per dam ($r_s = -.31$, $n = 12$, $p = .33$), maternal inbreeding coefficients ($r_s = -.19$, $p = .57$), nor the average kinship per maternal sib group ($r_s = .30$, $p = .37$). The number of recovered juveniles per sire varied from 17 to 51 (mean = 29.2, SD = 10.6). Family sex ratio did not differ among the paternal sib groups ($\chi^2 = 10.8$, d.f. = 9, $p = .29$) and was not correlated with paternal inbreeding coefficients ($r_s = .65$, $n = 9$, $p = .06$) or average kinship per paternal sib group ($r_s = .37$, $n = 9$, $p = .33$).

4 | DISCUSSION

Using molecular markers, we could identify captive-bred juveniles of 56 experimentally produced families and compare them to wild-born juveniles of the same cohort. The captive-bred 0+ dominated in number (by a factor of 5.3), had a significantly more balanced sex ratio, and were on average larger than wild-born 0+. The reason for these differences remains unclear but could be linked, for example, to different stress levels during embryogenesis, the timing of stocking relative to the timing of emergence of wild-born, size differences at the time when exogenous feeding starts, or different parental characteristics. Given these many possible reasons, it may even be a general rule that captive-bred and wild-born fish of the same cohort usually differ in growth and survival (Palejowski et al., 2022).

We used a panel of >1 million SNPs to calculate the kinship coefficients of 49 parental combinations that resulted from our experimental breeding. These 49 kinship coefficients (minus one statistical outlier that remained unexplained) may well reflect the expected average inbreeding coefficient per experimental full-sib family in a population that does not seem to suffer from elevated levels of inbreeding, as concluded from measurements of hybrid vigour that included our study population (Clark et al., 2013; Stelkens et al., 2014) in crosses of populations that are genetically distinct (Stelkens et al., 2012). Nevertheless, the variation in kinship coefficients that we observed could be used to predict female growth in the wild. Females reached smaller body sizes with increasing expected average inbreeding coefficients. No such effects could be observed among males. We conclude that the effects of inbreeding on growth are sex-specific at this juvenile stage.

We sampled the juveniles about 6 months after release into the wild. Sexual maturity and first breeding are expected at the end of their second or third year of life. Therefore, the sex-specific effects of inbreeding that we observed cannot be explained by the sex-specific stress that is expected during the mating season. Moreover, because sex chromosomes of brown trout are largely homomorphic (Guiguen et al., 2019), as is typical for lower vertebrates (Beukeboom & Perrin, 2014), they are not expected to contribute significantly to sex-specific inbreeding depression (Vega-Trejo et al., 2022). The effects we found here may therefore be best explained by sex differences in life histories. In the case of brown trout, these sex differences are rather cryptic. Little is known about sex differences in morphometry or behaviour at such early life-history stages even though the brown trout is a common and well-studied species. However, recent studies on brown trout and grayling revealed that the sexes differ at least in the timing of gonad development. Females generally develop their gonads earlier than males while males in turn grow faster than females during that time (Maitre et al., 2017; Palejowski et al., 2022). The size difference that was found before in other populations (Palejowski et al., 2022) could be confirmed in the present study but was overall

small (around 3%). This difference was linked to the variance in kinship coefficients: The sex difference in size was not apparent in families with small expected average inbreeding coefficients. We therefore predict that increased inbreeding within a population will accentuate the sex difference in size.

The overall sex ratio in our recaptured sample was about equal, and there was no significant effect of the expected average inbreeding coefficient on recapture rates for the different families. It remains to be evaluated, however, whether and under what circumstances sex-specific effects of inbreeding can affect population sex ratios. Based on the percentage of experimentally produced fish among the sampled ones (80.3%), the total number of fish that could be sampled, and the number of fertilized eggs that were used for this study, the overall apparent mortality of the experimentally produced fish over their first 9 months of their life was 78.4% or less if some fish had escaped sampling. Because embryo mortality was low during hatchery rearing and in a parallel study on the same families (Wilkins et al., 2017), the mortality in the present study may reflect the acute stress during stocking and/or the selection during the fish's first spring and summer in the wild. Somewhat comparable levels of apparent mortality during these first months have been observed in nearby populations of brown trout (Palejowski et al., 2022), that is, the level of selection in our study system seems not extraordinary.

When sampling by electrofishing, capture probability is often size-dependent, with larger fish being more likely caught than smaller ones (Richter et al., 2022). It is therefore possible that our recapture rates overestimate mortality. It is even possible that a size-biased sampling leads to underestimating the effects of sex-linked inbreeding depression if small, inbred females are less likely sampled than large, inbred females. However, the near-equal sex ratio among the captive-born fish suggests that such a possible bias is small.

As is typical for studies on wild populations, quantifying likely effects of emigration remained difficult. In our study system, upstream emigration was not possible (except few meters into an underground pipe). Downstream emigration into the larger stream (Rotache) was possible. However, if migration happens in this species, it typically starts at later developmental stages and is then often sex-biased, with females being more likely to migrate than males (Forseth et al., 1999; Nevoux et al., 2019). The most parsimonious explanation for the observed overall equal sex ratio in the captive-born fish, and the non-significant correlations between recapture rates and inbreeding, is therefore that there was no sex-specific mortality and no sex-specific emigration, and that inbreeding depression only affected size at age but did not lead to increased mortality or emigration in the hatchery-produced fish. The pattern was different in the wild-born 0+ who reached smaller sizes than the hatchery-produced 0+ and had a male-bias sex ratio, suggesting that wild-born females suffered from a higher mortality than wild-born males during their first spring and summer. It is possible that a combination of sex-specific inbreeding and strong competition by larger hatchery-born competitors led to sex-specific mortality among the wild-born. However, we cannot exclude the possibility that large wild-born females are more likely to emigrate than large wild-born males even at these early developmental stages.

Inbreeding coefficients can show significant heritability in small and structured populations (Neff & Pitcher, 2008; Nietlisbach et al., 2016). This prediction is supported by the significant correlation that we found between parental inbreeding coefficients and the average kinship coefficients, that is, the expected average inbreeding coefficients of their offspring. If parental inbreeding coefficients predict offspring inbreeding coefficients, and if inbreeding depression during the spawning season affects intra- and inter-sexual selection (i.e. giving less inbred individuals a selective advantage), natural spawning would be expected to reduce the average inbreeding coefficient of the next generation. However, the one extreme kinship coefficient that we observed would not be avoided through the effects of inbreeding depression on sexual selection. The parents of this sib group had average and very similar inbreeding coefficients, suggesting that they were close relatives who would need kin recognition to avoid each other as mating partners.

Our experimental breeding also allowed us to test for general maternal and paternal effects on juvenile growth. We found significant maternal but no paternal effects, suggesting that juvenile growth is affected by maternal environmental effects linked to egg quality, even if mean egg size did not significantly predict recapture rates nor male or female size after 6 months in the wild. The absence of significant paternal effects is either due to limited statistical power or suggests that heritability of growth is small when measured in juveniles recaptured from the wild. Paternal effects on offspring growth are, however, frequently observed in brown trout larvae when studied under controlled laboratory conditions, revealing significant heritability of growth in this species (Marques da Cunha et al., 2019; Nusbaumer et al., 2019). The limited number of recaptured juveniles for each of the 60 full-sib families did not allow to test for possible effects of dam \times sire interactions on growth.

In conclusion, the observed kinship coefficients of breeding pairs, that is, the expected average inbreeding coefficients per full-sib family, did not significantly affect mortality of juvenile brown trout that had been stocked into the wild as larvae. However, female growth during their first spring and summer in the wild was reduced with increased kinship coefficients. No such effect could be observed in males who even grew larger than females during that time. Effects of inbreeding on growth are hence sex-specific around the time of gonad formation and long before intra- or inter-sexual selection are expected to cause sex-specific inbreeding depression. It remains to be shown whether and to what extent this sex-specific inbreeding depression is linked to gonad development.

AUTHOR CONTRIBUTIONS

Jonas Bylemans, Lucas Marques da Cunha, and Claus Wedekind designed and supervised the study. Lucas Marques da Cunha, David Nusbaumer, Anshu Uppal, and Claus Wedekind did the experimental breeding. Lucas Marques da Cunha, David Nusbaumer, and Anshu Uppal sampled the juveniles from the wild and Lucas Marques da Cunha determined their growth. Jonas Bylemans and Lucas Marques da Cunha were responsible for the parental assignments and the genetic sexing. Jonas Bylemans and Sonia Sarmiento Cabello performed

bioinformatic analysis. Jonas Bylemans and Claus Wedekind performed the statistical analyses and wrote the manuscript. All authors revised and approved the final manuscript for publication.

ACKNOWLEDGEMENTS

We dedicate this paper to U. Gutmann and thank him for the valuable advice and support he provided in this and many other collaborations over 20 years until his retirement. We also thank T. Bösch, B. Bracher, U. Gutmann de Guttry, M. Escher, J. Kast, A. Knutti, C. Küng, E. Longange, E. Pereira-Alvarez, L. Wilkins, and the members of the *Pachtverein Rotache* for assistance and discussion. The study was funded by the Swiss National Science Foundation (31003A_159579 & 31003A_182265). Open access funding provided by Universite de Lausanne.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data used for this study have been deposited in the Dryad depository. doi: [10.5061/dryad.2ngf1vhv4](https://doi.org/10.5061/dryad.2ngf1vhv4) (Bylemans et al., 2023).

ETHICS STATEMENT

The sampling and handling of wild adults, the experimental breeding, the raising of offspring, the stocking into the wild, and sampling and handling of juveniles caught from the wild were approved by the fishery inspectorate and the veterinary office of the Bern canton (permit BE118/14).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bylemans, J., Marques da Cunha, L., Sarmiento Cabello, S., Nusbaumer, D., Uppal, A., & Wedekind, C. (2024). Sex-specific effects of inbreeding in juvenile brown trout. *Molecular Ecology*, 33, e17298. <https://doi.org/10.1111/mec.17298>

Supplemental Information for:

Sex-specific effects of inbreeding in juvenile brown trout

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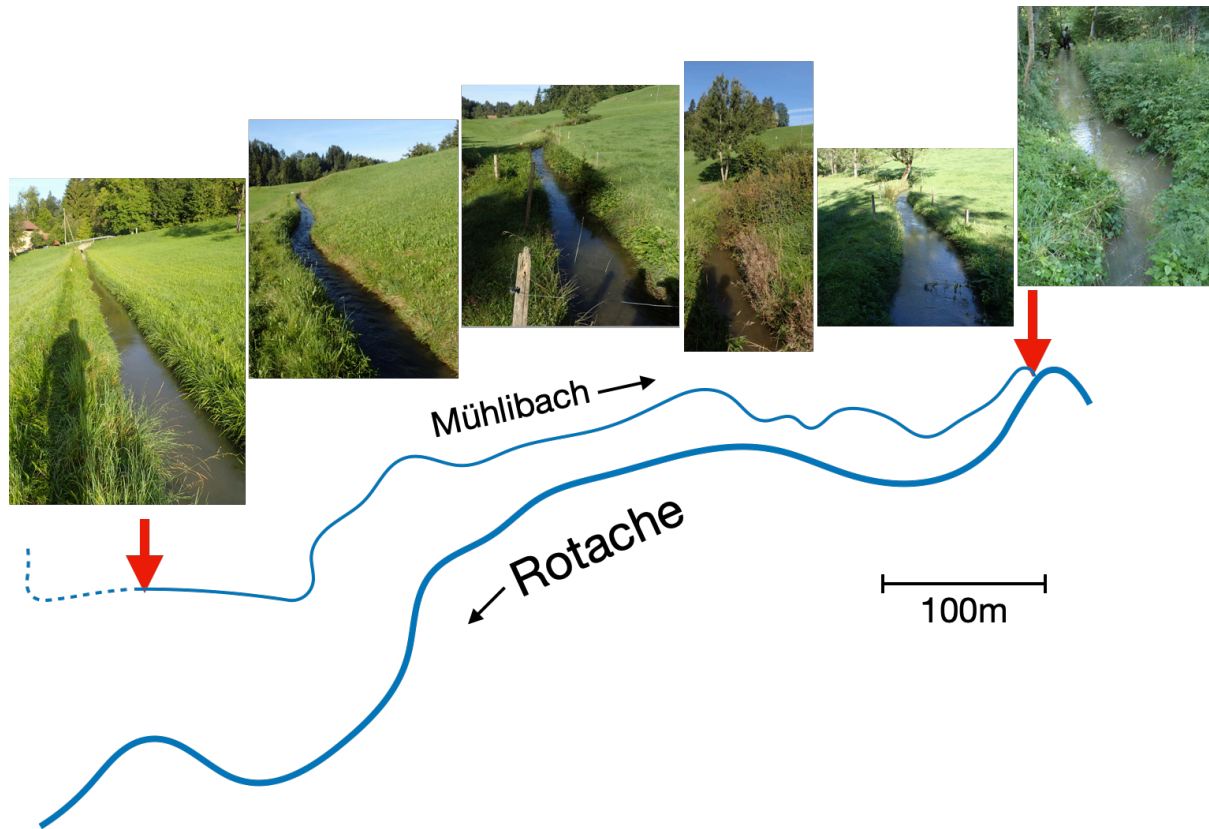
1. Bayesian mixed model to separate 0+ fish from older ones

When plotting the distribution of the fork length data of all juvenile brown trout that were sampled in the present study (i.e., juveniles from the *Mühlbach* streamlet) and 16 further streamlets that had been stocked and sampled in a parallel study on general stocking success (Bylemans et al. in prep.), two clear peaks could be observed in the *Mühlbach* sample (Figure 1) and for most other streamlets. These distinct size gaps suggest that both 0+ and older age classes were sampled.

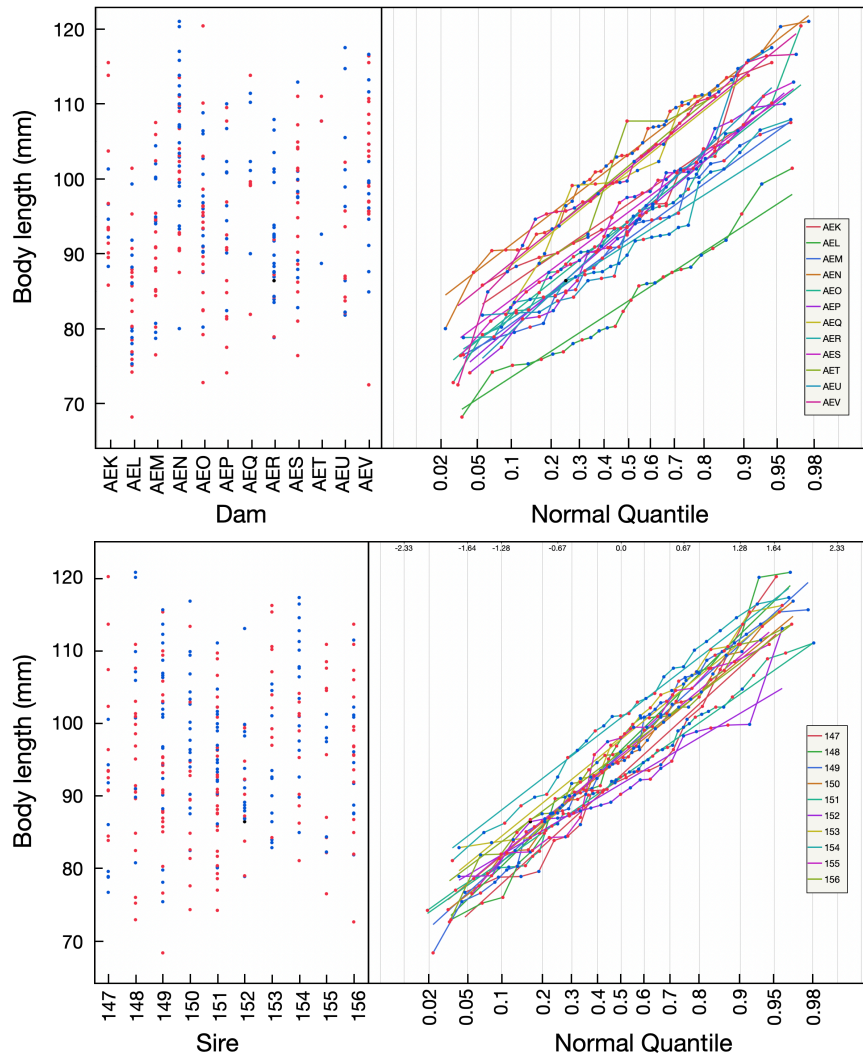
To identify the 0+ for further analyses, a hierarchical Bayesian mixture model was used to determine, for each juvenile, the probability of it belonging to the 0+ age class. A two-component Bayesian mixture model was used with the length distribution of all fish (Bylemans et al. in prep.). For each streamlet, juvenile fish length was modelled as coming from two normal distributions, one describing the length distribution for the 0+ individuals and the other describing the length distribution for individuals from older age classes. For each streamlet, two observations were used to anchor the distributions of the two age classes with the shortest individual being assigned to the 0+ class and the largest individual being assigned to the older age class. Informative priors were used with the mean length of the young-of-year age class drawn from a normal distribution (mean = 9, variance = 10) and an upper limit being equal to the largest individual that was characterized as ‘captive bred’ based on the parental assignments. The prior means for the older age class were drawn from a normal distribution (mean = 15, variance = 10) and a lower limit being set to a length of 10 cm based on a visual inspection of the data. Standard deviations were drawn from a positively truncated normal distribution (mean = 0, variance = 2). The model was run in JAGS using 3 chains with 1,000 adaptation iterations followed by 20,000 iterations and discarding the first 10,000 iterations as a burn-in. Final model outputs were thinned by retaining every 5th value and outputs were used to determine for each streamlet and each fish the probability of it belonging to the young-of-year age class.

The probabilities of each fish belonging to the young-of-year age class was plotted on the distribution of the fork lengths and for further analyses, only those fish which had a probability of belonging to the young-of-year above 0.25 were considered. This threshold value was chosen as it provides a good split between the two peaks in the fork length distribution data for most sampled streamlets. For the *Mühlbach* streamlet, all the 301 fish that could be assigned to the experimental families were also categorized as belonging to the 0+ class (Figure 1).

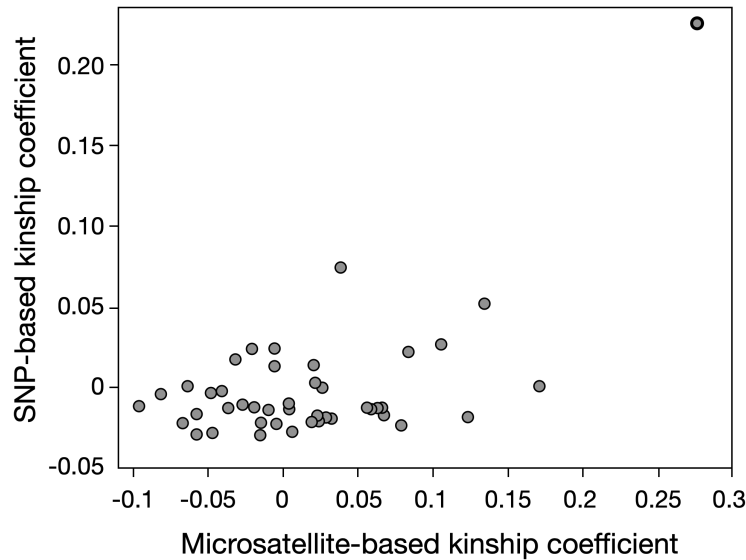
MOLECULAR ECOLOGY



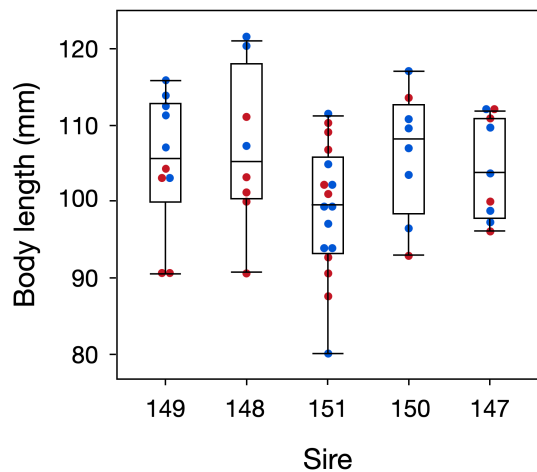
Supplementary Figure S1. The streamlet *Mühlbach* as a tributary of the *Rotache*. The red arrows indicate the start and the end of the ca 700 m stretch into which the hatchery-born fish were stocked in early March, and where electrofishing happened in late August. Hatched line = streamlet underground. The black arrows indicate the direction of the water flow. The photos were taken during the electrofishing and are used here to illustrate the ecology downstream (left to right).



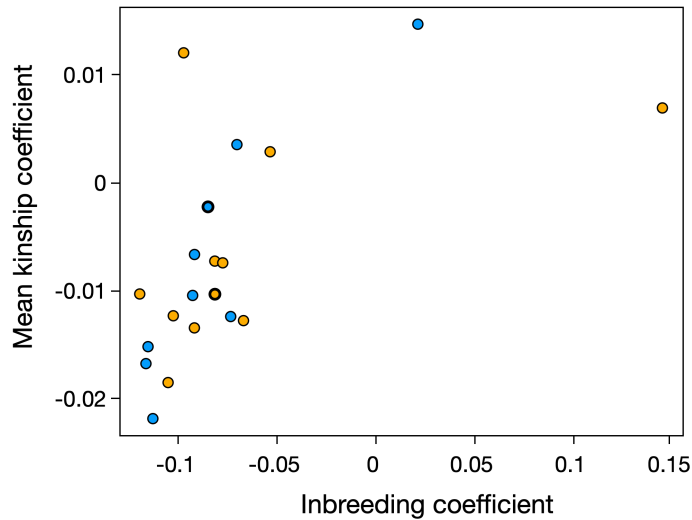
Supplementary Figure S2. Distributions and Q-Q plots of body lengths of 0+ juveniles relative to sex (males = blue, female = red symbols), dam (mother) identity, and sire (father) identity.



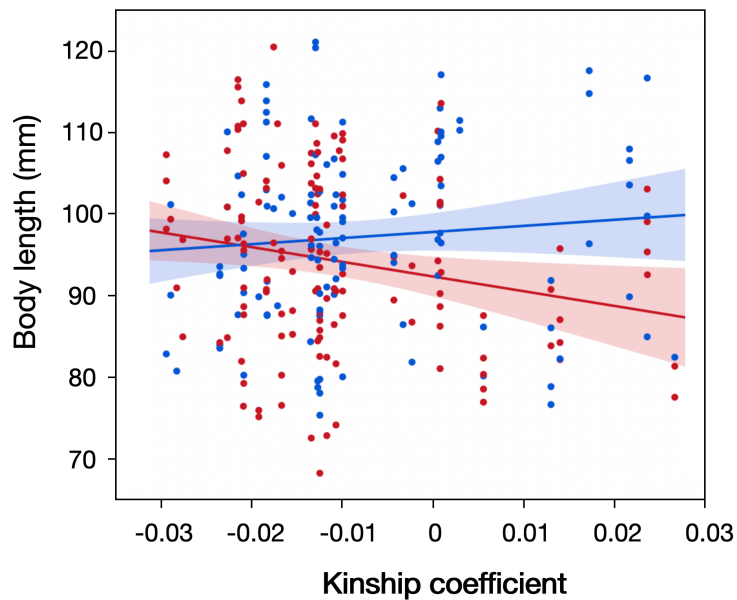
Supplementary Figure S3. Kinship coefficients as calculated from >1 million SNPs predicted by kinship coefficients calculated from 13 microsatellite markers. Family “AEN-147” (highlighted) appeared extreme in both estimates, but the two estimates of kinship are not significantly correlated (see main text for statistics).



Supplementary Figure S4. Body lengths of juvenile offspring of dam "AEN" crossed with sires “147” to “151” (families ordered by the kinship coefficient, from lowest to highest). Sib group “AEN-147” had a kinship coefficient of 0.226 that was classified as extreme and therefore excluded from analyses that (see text). The average juvenile body length of this sib group was, however, not significantly different from the other maternal half-sib groups (ANOVA, $F = 1.9$, d.f. = 4, $p = 0.13$). Tukey box plots with whiskers and jittered individual observations for males (blue) and females (red).



Supplementary Figure S5. Mean SNP-based kinship coefficients per paternal (light blue) and maternal (orange) inbreeding coefficient. The parents whose joint offspring had the extreme kinship coefficient of 0.226 are highlighted (this extreme kinship coefficient was excluded from the calculation of the mean kinship coefficients). See text for statistics.



Supplementary Figure S6. Body length of wild-caught juvenile brown trout predicted by the kinship coefficient (i.e., the expected average inbreeding coefficient per full-sib family) in males (blue dots and regression line) and females (red dots and regression line) when 4 fish with kinship coefficients > 0.03 are excluded. The shaded areas give the 95% confidence intervals for the linear regressions. See Supplementary Table S1 for statistics.

Supplementary Table S1. Breeding design, parental inbreeding coefficients (F_{β}), kinship coefficient per full-sib family (r^{β}), and number of recovered offspring from the wild per total number of freshly fertilized eggs used for hatchery rearing and stocking. Sire and dam IDs are given with three-digits numbers and three letters, respectively. “NA” = inbreeding or kinship coefficient was not available. The kinship coefficient that was considered an outlier is marked in bold.

1st breeding block	Sire “147” ($F_{\beta} = -0.0849$)	Sire “148” ($F_{\beta} = -0.116$)	Sire “149” ($F_{\beta} = -0.0732$)	Sire “150” ($F_{\beta} = -0.0696$)	Sire “151” ($F_{\beta} = -0.0925$)	Total recapture rates
Dam “AEK” ($F_{\beta} = \text{NA}$)	3 of 21 $r^{\beta} = \text{NA}$	1 of 19 $r^{\beta} = \text{NA}$	4 of 32 $r^{\beta} = \text{NA}$	2 of 20 $r^{\beta} = \text{NA}$	7 of 34 $r^{\beta} = \text{NA}$	17 of 126 (13.5%)
Dam “AEL” ($F_{\beta} = -0.0534$)	6 of 29 $r^{\beta} = 0.0131$	4 of 17 $r^{\beta} = -0.0191$	9 of 20 $r^{\beta} = -0.0124$	2 of 25 $r^{\beta} = 0.0740$	7 of 26 $r^{\beta} = 0.0057$	28 of 117 (23.9%)
Dam “AEM” ($F_{\beta} = -0.0916$)	7 of 28 $r^{\beta} = -0.0126$	2 of 14 $r^{\beta} = -0.0282$	8 of 54 $r^{\beta} = -0.0166$	6 of 41 $r^{\beta} = -0.0042$	4 of 43 $r^{\beta} = -0.0154$	27 of 180 (15.0%)
Dam “AEN” ($F_{\beta} = -0.0813$)	9 of 57 $r^{\beta} = 0.2256$	8 of 58 $r^{\beta} = -0.0129$	10 of 68 $r^{\beta} = -0.0183$	8 of 69 $r^{\beta} = 0.0010$	17 of 71 $r^{\beta} = -0.0099$	52 of 323 (16.1%)
Dam “AEO” ($F_{\beta} = -0.0666$)	3 of 28 $r^{\beta} = -0.0175$	7 of 53 $r^{\beta} = -0.0116$	6 of 29 $r^{\beta} = 0.0006$	9 of 35 $r^{\beta} = -0.0124$	10 of 47 $r^{\beta} = -0.0208$	35 of 192 (18.2%)
Dam “AEP” ($F_{\beta} = -0.0776$)	1 of 20 $r^{\beta} = 0.0237$	6 of 44 $r^{\beta} = -0.0226$	7 of 58 $r^{\beta} = -0.0108$	3 of 29 $r^{\beta} = 0.0268$	6 of 36 $r^{\beta} = -0.0106$	23 of 187 (12.3%)
Total recapture rates	29 of 183 (15.8%)	28 of 205 (13.7%)	44 of 261 (16.9%)	30 of 219 (13.7%)	51 of 257 (19.8%)	

2nd breeding block	Sire “152” ($F_{\beta} = \text{NA}$)	Sire “153” ($F_{\beta} = -0.1125$)	Sire “154” ($F_{\beta} = 0.0223$)	Sire “155” ($F_{\beta} = -0.0916$)	Sire “156” ($F_{\beta} = -0.1154$)	Total recapture rates
Dam “AEQ” ($F_{\beta} = -0.105$)	0 of 13 $r^{\beta} = \text{NA}$	3 of 29 $r^{\beta} = -0.0289$	2 of 25 $r^{\beta} = 0.0030$	0 of 23 $r^{\beta} = -0.0142$	5 of 26 $r^{\beta} = -0.0210$	10 of 116 (8.6%)
Dam “AER” ($F_{\beta} = -0.1194$)	9 of 35 $r^{\beta} = \text{NA}$	6 of 24 $r^{\beta} = -0.0235$	4 of 25 $r^{\beta} = 0.0218$	3 of 28 $r^{\beta} = -0.0134$	5 of 30 $r^{\beta} = -0.0182$	27 of 142 (19.0%)
Dam “AES” ($F_{\beta} = -0.1022$)	9 of 39 $r^{\beta} = \text{NA}$	4 of 35 $r^{\beta} = -0.0294$	10 of 30 $r^{\beta} = 0.0009$	4 of 32 $r^{\beta} = -0.0208$	2 of 31 $r^{\beta} = -0.0275$	29 of 167 (17.4%)
Dam “AET” ($F_{\beta} = -0.0968$)	0 of 4 $r^{\beta} = \text{NA}$	0 of 8 $r^{\beta} = -0.0221$	2 of 6 $r^{\beta} = 0.0523$	1 of 9 $r^{\beta} = -0.0103$	2 of 26 $r^{\beta} = -0.0171$	5 of 53 (9.4%)
Dam “AEU” ($F_{\beta} = 0.1463$)	2 of 24 $r^{\beta} = \text{NA}$	3 of 30 $r^{\beta} = -0.0032$	3 of 37 $r^{\beta} = 0.0173$	5 of 34 $r^{\beta} = 0.0141$	4 of 26 $r^{\beta} = -0.0022$	17 of 151 (11.3%)
Dam “AEV” ($F_{\beta} = -0.0811$)	3 of 31 $r^{\beta} = \text{NA}$	7 of 24 $r^{\beta} = -0.0214$	6 of 41 $r^{\beta} = 0.0237$	4 of 31 $r^{\beta} = -0.0127$	11 of 44 $r^{\beta} = -0.0134$	31 of 171 (18.1%)
Total recapture rates	23 of 146 (15.8%)	23 of 150 (15.3%)	27 of 164 (16.5%)	17 of 157 (10.8%)	29 of 183 (15.8%)	

Supplementary Table S2. Linear mixed model on juvenile length and weight when predicted by sex and mean kinship coefficient per family, excluding families with a kinship coefficient > 0.03. Parental identities were included as random factors. Significant p-values are highlighted in bold.

Effects	Body length				Body weight			
	d.f.	F	Variance component	p	d.f.	F	Variance component	p
Fixed effects:								
Sex	1, 237	4.5		0.03	1, 235.5	8.1		0.005
Kinship	1, 29.7	0.2		0.63	1, 30.2	0.2		0.64
Sex x kinship	1, 234	6.3		0.01	1, 232.7	8.0		0.005
Random effects:								
Dam ¹			30.1±18.8	0.056			4.0±2.1	0.055
Sire ¹			3.8±4.0	0.34			0.5±0.5	0.32
Residual			83.2±7.8				10.5±1.0	

¹REML unbounded variance components ± standard error, Wald p-values