Sex-specific effects of inbreeding in juvenile brown trout

Jonas Bylemans[1], Lucas Marques da Cunha[1], Sonia Sarmiento Cabello[1], David Nusbaumer[1], Anshu Uppal[1], and Claus Wedekind[1]

[1]University of Lausanne

June 23, 2023

Abstract

Inbreeding depression, i.e., the reduction of health and vigour in individuals with high inbreeding coefficients, is expected to increase with environmental, social, or physiological stress. Differences in the strength of sexual selection are therefore predicted to 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 salmonids, for example, female juveniles start developing their gonads earlier than males who instead grow faster during that time. 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 breeding design to produce 60 full-sib families, released the offspring as yolk-sac larvae into the wild, caught them back 6 months later, identified their genetic sex, and used microsatellites to assign them to their parents. We calculated the average inbreeding coefficient per family based on a panel of >1 million SNPs. Juvenile growth could be predicted from these inbreeding coefficients and the genetic sex: Females grew slower 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 grew on average slower than males during the time of gonad formation.

Sex-specific effects of inbreeding in juvenile brown trout

Jonas Bylemans[1,2#], Lucas Marques da Cunha[1#], Sonia Sarmiento Cabello[1], David Nusbaumer[1], Anshu Uppal[1], Claus Wedekind[1*]

Shared first authors

* Corresponding author; claus.wedekind@unil.ch

[1] Department of Ecology and Evolution, Biophore, University of Lausanne, 1015, Lausanne, Switzerland

[2] University of Savoie Mont Blanc, INRAE, CARRTEL, 74200 Thonon-les-Bains, France

Abstract

Inbreeding depression, i.e., the reduction of health and vigour in individuals with high inbreeding coefficients, is expected to increase with environmental, social, or physiological stress. Differences in the strength of sexual selection are therefore predicted to 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 salmonids, for example, female juveniles start developing their gonads earlier than males who instead grow faster during that time. 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 breeding design to produce 60 full-sib families, released the offspring as yolk-sac larvae into the wild, caught them back 6 months later, identified their

genetic sex, and used microsatellites to assign them to their parents. We calculated the average inbreeding coefficient per family based on a panel of >1 million SNPs. Juvenile growth could be predicted from these inbreeding coefficients and the genetic sex: Females grew slower 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 grew on average slower than males during the time of gonad formation.

Introduction

Inbreeding can lead to inbreeding depression, i.e., 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 alternative explanation for sex-specific inbreeding depression, 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 2 or later, usually with no obvious sexual dimorphism before. However, the sexes differ in many aspects from very early stages on. Sex-specific gene expression could be observed in grayling embryos (*Thymallus thymallus*) (Maitre *et al.* 2017; Selmoni *et al.* 2019) and around hatching in rainbow trout (*Onchorhynchus mykiss*) (Guiguen*et al.* 2019). Embryos can even show sex-specific stress tolerance (Moran *et al.* 2016; Nusbaumer *et al.* 2021). Sex differences could also be found during the early juvenile stages, i.e., within the first few months when gonad formation starts. In grayling, 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 during summer and thereby explaining the observed link between climate warming and male-biased sex ratios among adults (Wedekind *et al.* 2013). Analogous sex differences in the timing of gonad formation could be 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 often a consequence of adaptive responses to local conditions, especially in salmonids (Wang *et al.* 2002). It has been shown to influence early life-history traits (Kincaid 1976; Naish *et al.* 2013), disease resistance (Arkush*et al.* 2002), and reproductive traits (Naish *et al.* 2013; Waters *et al.* 2020; Paul *et al.* 2021) 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 difference in the strength of sexual selection while heterogamety seem to play no significant role (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 differ in gonad formation and the physiological stress that may be associated to this, i.e., 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 use their gametes for *in vitro* fertilizations in full-factorial breeding blocks. The resulting 60 full-sib families differed in their mean inbreeding coefficients that were, however, mostly low as expected for the study population. 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.

Methods

Adult brown trout were caught from the *Rotache* stream (a rather pristine tributary of the Aare river, Marques da Cunha *et al.*2019) shortly before the spawning season. The eggs of 12 females were stripped and fertilized with milt of 10 males in two full-factorial breeding blocks (6 x 5 each) to produce in total 60 full-sib families as described in Wilkins et al. (2017). Fin clips were stored in 70 % ethanol at -20 °C.

After egg hardening and sampling eggs for parallel laboratory studies on embryo stress tolerance (Wilkins *et al.* 2017; Marques da Cunha*et al.* 2018), a total of 1,925 eggs (mean±SD number per full-sib family = 32.1±14.8) were incubated under routine hatchery conditions at the cantonal *Fischereistützpunkt Reutigen* at constant temperature of 8.5°C and stocked into the *Mühlibach* streamlet (a tributary to the *Rotache* ; 46.804459°N, 7.690544°E) at a late yolk-sac stage in early March.

About 6 months after release into the wild (i.e., in late August), electrofishing was used along the *Mühlibach* streamlet to catch as many brown trout as possible (N_{total} = 518). The fish were narcoticized (0.075 g/L tricaine methanoesulfonate buffered with 0.15 g/L NaHCO3) and photographed on a weighting scale to later extract fork length and body weight. Fin clips were collected and stored in 70 % ethanol. After handling, all fish were returned to the wild.

Fin clips of 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 a 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). Parental assignment of the juveniles was based on the full-likelihood approach implemented in Colony v2.0.6.5 (Jones and Wang, 2010) with a threshold of 0.98.

For all but two breeders (one dam and one sire) high quality DNA extracts or tissue samples 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 bases 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, 2013) and the obtained BAM files were further processed using Samtools v1.12 (Li*et al.* 2009) and Picard version 2.24.0 ("Picard Tools - By Broad Institute," n.d.). 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, a minor allele frequency <0.01, outside of Hardy-Weinberg equilibrium ($p<10^{-8}$) and showing signs of strong linkage disequilibrium (r2 > 0.6). Only SNPs present in at least 90% of individuals were retained and the R package Hierfstat (Goudet 2005) was used to obtain marker-based estimates of kinship between all parental breeding pairs using a panel of 1,058,625 SNPs (Goudet *et al.* 2018).

Statistical analyses were done in JMP Pro17 and R 4.0.2 (R Development Core Team 2015). The Akaike information criterion (AIC) were 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 were used to evaluate the combined effects of sex and kinship on growth indicators. 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 was genotyped for parental assignment did not significantly differ in body lengths from the non-genotyped fish (Welch's F = 1.7, p = 0.19). In total 301 (80.3%) of these 375 wild-caught juveniles could be assigned to 56 of the 60 experimental sib groups. Their average (± SD) body lengths and weights were 95.6 ± 10.6 mm and 11.3 ± 3.9 g, respectively, and were always below the 125 mm that the Bayesian mixture model had identified as upper size for 0+ fish (Fig. 1b). All but one of them could be genetically sexed. The overall male ratio was 48.3% and not significantly biased (χ^2 = 0.33, p = 0.56).

The Bayesian model identified 57 of the remaining 74 genotyped fish as wild-born 0+ juveniles and the larger ones as 1+ or older (Fig. 1b). The wild-born 0+ were on average 17.6 mm smaller than the experimentally bred 0+ (Fig. 1b; F = 122.5, p < 0.001). Among the experimentally bred 0+, males were on average 2.6 mm larger than females (F = 4.4, p = 0.03; Fig. 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 = 1.6, p = 0.21). However, the wild-born 0+ had a male-based sex ratio (63.2% males) that was not observed in the experimentally bred 0+ (χ^2 = 4.3, p = 0.04; Fig. 1c).

We obtained 47 kinship coefficients (mean ± SD = -0.0003 ± 0.04). One full-sib family with a kinship coefficient of 0.226 was classified as outlier (because ⩾3 SDs away from the mean, following the three-sigma rule) and excluded from all further analyses, leaving 251 genetically sexed juveniles of 46 full-sib families for the final analyses. The kinship coefficients of these sib groups could partly be predicted by the parental inbreeding coefficients: Higher parental inbreeding coefficients led to higher average kinship coefficients among their offspring (r_s = 0.61, n = 20, p = 0.004; Supplementary Figure S1).

The body sizes of female juveniles declined with increased kinship coefficients, while the body sizes of male juveniles did not seem to be affected (Figure 2; Table 1; Supplementary Figure S2; Supplementary Table S1). Juvenile body size also varied among maternal but not paternal sib groups (Table 1).

The recapture rates per full-sib family, i.e., the mean number of recovered juveniles per number of released larvae, varied between 0.0 and 0.68 and were not correlated with the kinship coefficients (r_s = 0.13, n= 48, p = 0.38) nor with the inbreeding coefficients of the dams (r_s = 0.39, n= 11, p = 0.23) or the sires (r_s = 0.05, n= 9, p = 0.90).

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), i.e., sex ratios per individual 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 (χ^2 = 22.5, d.f. = 11, p = 0.02) but could not be predicted by maternal inbreeding coefficients (r_s = -0.19, p = 0.57) nor the average kinship per maternal sib group (r_s = 0.30, p = 0.37). 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 (χ^2 = 10.8, d.f. = 9, p = 0.29) and was not correlated with paternal inbreeding coefficients (r_s = 0.65, p = 0.06) or average kinship per paternal sib group (r_s = 0.37, p = 0.33).

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 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, or size differences at the time when exogenous feeding starts. Given these many possible reasons, it may even be a general rule that captive-bred and wild-born fish of the same cohort differ in growth and survival because of differences in early life history (Palejowski *et al.* 2022).

We used a panel of > 1 million SNPs to calculate the kinship coefficients of 47 parental combinations that resulted from our experimental breeding. These 47 kinship coefficients (minus one outlier) may well reflect the 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 grew slower with increasing inbreeding coefficients. No such effects could be observed among males. We conclude that the susceptibility to inbreeding depression is 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 are therefore 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 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 and may even be caused by the variance in kinship coefficients because the sex difference in size was not apparent in families with small inbreeding coefficients. We therefore predict that increased inbreeding within a population will accentuate the sex difference in growth.

The overall sex ratio in our re-captured sample was about equal, and there was no significant effect of inbreeding on recapture rates for the different families. Based on the rate 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 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 in a parallel study on the same families (Wilkins *et al.* 2017), the mortality in the present study reflects the acute stress during stocking and/or the selection during the fish's first spring and summer in the wild. Somewhat comparable levels of mortality during these first months have been observed in nearby populations of brown trout (Palejowski *et al.* 2022), i.e., the level of selection in our study system seems not extra-ordinary.

As is typical for studies on wild populations, quantifying likely effects of emigration remained difficult. In our study system, upstream emigration was not possible. 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 growth but did not lead to increased mortality in the hatchery-produce fish. The pattern was different in the wild-born 0+ who grew smaller 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 larger hatchery-born competitors led to sex-specific mortality among the wild-born.

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, i.e., the expected 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 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 recorded would not be avoid through effects of inbreeding on health and vigour because the parents of this sib group had average and very similar inbreeding coefficients, suggesting that they could have been brother and sister that would not be able to avoid each other without some form in kin recognition.

Our experimental breeding also allowed to test for general maternal and paternal effects on juvenile growth. We found significant maternal but not paternal effects, suggesting that juvenile growth is affected by maternal environmental effects such as egg size and egg content. 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 per each of the 60 full-sib families did not allow to test for possible effects of dam x sire interactions on growth.

In conclusion, inbreeding 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 increasing inbreeding coefficients. No such effect could be observed in males who even could grew on average about 3% larger than females during that time. Inbreeding depression is hence sex-specific around the time of gonad formation and long before intra- or inter-sexual selection are expected to cause sex-specific (male-biased) inbreeding depression.

Data availability statement

All data used here will be made available upon acceptance of the manuscript.

Author statement

JB, LMC, and CW designed and supervised the study. LMC, DN, AU & CW did the experimental breeding. LMC, DN & AU sampled the juveniles from the wild and LMC determined their growth. JB & LMC were responsible for the parental assignments and the genetic sexing. JB & SSC performed bio-informatic analysis. JB & CW performed the statistical analyses and wrote the manuscript. All authors revised and approved the final manuscript for publication.

Declaration of competing interest

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

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).

Acknowledgements

We thank T. Bösch, B. Bracher, U. Gutmann, C. 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/or discussion. The study was funded by the Swiss National Science Foundation (31003A_159579 & 31003A_182265).

References

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data, p. http://www.bioinformatics.babraham.ac.uk/projects/fastqc. Babraham Bioinformatics, Cambridge, UK.

Arkush KD, Giese AR, Mendonca HL*, et al.* (2002) Resistance to three pathogens in the endangered winter-run chinook salmon (*Oncorhynchus tshawytscha*): effects of inbreeding and major histocompatibility complex genotypes. *Canadian Journal of Fisheries and Aquatic Sciences* **59** , 966-975.

Beukeboom LW, Perrin N (2014) *The evolution of sex determination.*Oxford University Press, Oxford.

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30** , 2114-2120. 10.1093/bioinformatics/btu170

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression.*Nature Reviews Genetics* **10** , 783-796. 10.1038/nrg2664

Clark ES, Stelkens RB, Wedekind C (2013) Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. *PLoS ONE* **8** , e57832. 10.1371/journal.pone.0057832

Connallon T, Beasley IJ, McDonough Y, Ruzicka F (2022) How much does the unguarded X contribute to sex differences in life span? *Evolution Letters* **6** , 319-329. 10.1002/evl3.292

Ebel ER, Phillips PC (2016) Intrinsic differences between males and females determine sex-specific consequences of inbreeding. *BMC Evolutionary Biology* **16** . 10.1186/s12862-016-0604-5

Forseth T, Naesje TF, Jonsson B, Harsaker K (1999) Juvenile migration in brown trout: a consequence of energetic state. *Journal of Animal Ecology* **68** , 783-793. 10.1046/j.1365-2656.1999.00329.x

Gallardo JA, Neira R (2005) Environmental dependence of inbreeding depression in cultured Coho salmon (*Oncorhynchus kisutch*): aggressiveness, dominance and intraspecific competition. *Heredity***95** , 449-456. 10.1038/sj.hdy.6800741

Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* **5** , 184-186. 10.1111/j.1471-8286.2004.00828.x

Goudet J, Kay T, Weir BS (2018) How to estimate kinship. *Molecular Ecology* **27** , 4121-4135. 10.1111/mec.14833

Guiguen Y, Bertho S, Herpin A, Fostier A (2019) Sex determination and sex control in Salmonidae. In: *Sex Control in Aquaculture* (eds. Wang H-P, Piferrer F, Chen S-L, Shen Z-G), pp. 251-280. John Wiley & Sons Ltd., Hoboken, NJ, USA.

Hansen T, Fjelldal PG, Lien S*, et al.* (2021) The genome sequence of the brown trout, *Salmo trutta* Linnaeus 1758. *Wellcome Open Research* **6** , 108. 10.12688/wellcomeopenres.16838.1

Houde ALS, Fraser DJ, O'Reilly P, Hutchings JA (2011) Relative risks of inbreeding and outbreeding depression in the wild in endangered salmon.*Evolutionary Applications* **4** , 634-647. 10.1111/j.1752-4571.2011.00186.x

Janicke T, Vellnow N, Sarda V, David P (2013) Sex-specific inbreeding depression depends on the strength of male-male competition.*Evolution* **67** , 2861-2875. 10.1111/evo.12167

Johnson K, Butts IAE, Smith JL, Wilson CC, Pitcher TE (2015) The effects of inbreeding on sperm quality traits in captive-bred lake trout,*Salvelinus namaycush* (Walbaum, 1972). *Journal of Applied Ichthyology* **31** , 62-70. 10.1111/jai.12734

Kincaid HL (1976) Effects of inbreeding on rainbow trout populations. *Transactions of the American Fisheries Society* **105** , 273-280. 10.1577/1548-8659(1976)105<273:Eoiort>2.0.Co;2

Li H, Handsaker B, Wysoker A*, et al.* (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* **25** , 2078-2079. 10.1093/bioinformatics/btp352

Maitre D, Selmoni OM, Uppal A*, et al.* (2017) Sex differentiation in grayling (Salmonidae) goes through an all-male stage and is delayed in genetic males who instead grow faster. *Scientific Reports***7** , 15024. 10.1038/s41598-017-14905-9

Marques da Cunha L, Wilkins LGE, Menin L*, et al.* (2018) Consumption of carotenoids not increased by bacterial infection in brown trout embryos (*Salmo trutta*). *PLoS ONE* **13** , e0198834. 10.1371/journal.pone.0198834

Marques da Cunha L, Uppal A, Seddon E*, et al.* (2019) No additive genetic variance for tolerance to ethynylestradiol exposure in natural populations of brown trout (*Salmo trutta*). *Evolutionary Applications* **12** , 940-950. 10.1111/eva.12767

McKenna A, Hanna M, Banks E*, et al.* (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* **20** , 1297-1303. 10.1101/gr.107524.110

Moran P, Labbe L, Garcia de Leaniz C (2016) The male handicap: male-biased mortality explains skewed sex ratios in brown trout embryos.*Biology Letters* **12** . 10.1098/rsbl.2016.0693

Naish KA, Seamons TR, Dauer MB, Hauser L, Quinn TP (2013) Relationship between effective population size, inbreeding and adult fitness-related traits in a steelhead (*Oncorhynchus mykiss*) population released in the wild. *Molecular Ecology* **22** , 1295-1309. 10.1111/mec.12185

Neff BD, Pitcher TE (2008) Mate choice for non-additive genetic benefits: A resolution to the lek paradox. *Journal of Theoretical Biology* **254** , 147-155. 10.1016/j.jtbi.2008.05.019

Nevoux M, Finstad B, Davidsen JG*, et al.* (2019) Environmental influences of life history strategies in partial anadromous brown trout (*Salmo trutta* , Salmonidae). *Fish and Fisheries***20** , 1051-1082. 10.1111/faf.12396

Nietlisbach P, Keller LF, Postma E (2016) Genetic variance components and heritability of multiallelic heterozygosity under inbreeding.*Heredity* **116** , 1-11.

Noel E, Fruitet E, Lelaurin D*, et al.* (2019) Sexual selection and inbreeding: Two efficient ways to limit the accumulation of deleterious mutations. *Evolution Letters* **3** , 80-92. 10.1002/evl3.93

Nusbaumer D, Marques da Cunha L, Wedekind C (2019) Sperm cryopreservation reduces offspring growth. *Proceedings of the Royal Society of London Series B-Biological Sciences* **286** , 20191644. 10.1098/rspb.2019.1644

Nusbaumer D, Garaud L, Ançay L, Wedekind C (2021) Sex-specific stress tolerance in embryos of lake char (*Salvelinus umbla*).*Frontiers in Ecology and Evolution* **9** , 768263. 10.3389/fevo.2021.768263

Palejowski H, Bylemans J, Ammann V*, et al.* (2022) Sex-specific life history affected by stocking in juvenile brown trout.*Frontiers in Ecology and Evolution* **10** , 869925. 10.3389/fevo.2022.869925

Paul K, D'Ambrosio J, Phocas F (2021) Temporal and region-specific variations in genome-wide inbreeding effects on female size and reproduction traits of rainbow trout. *Evolutionary Applications* . 10.1111/eva.13308

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Selmoni OM, Maitre D, Roux J*, et al.* (2019) Sex-specific changes in gene expression in response to estrogen pollution around the onset of sex differentiation in grayling (Salmonidae). *BMC Genomics***20** , 583. 10.1186/s12864-019-5955-z

Stelkens RB, Jaffuel G, Escher M, Wedekind C (2012) Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Molecular Ecology* **21** , 2896-2915. 10.1111/j.1365-294X.2012.05581.x

Stelkens RB, Pompini M, Wedekind C (2014) Testing the effects of genetic crossing distance on embryo survival within a metapopulation of brown trout (*Salmo trutta*). *Conservation Genetics* **15** , 375-386. 10.1007/s10592-013-0545-0

Vega-Trejo R, de Boer RA, Fitzpatrick JL, Kotrschal A (2022) Sex-specific inbreeding depression: A meta-analysis. *Ecology Letters* **25** , 1009-1026. 10.1111/ele.13961

Wang SZ, Hard J, Utter F (2002) Salmonid inbreeding: a review.*Reviews in Fish Biology and Fisheries* **11** , 301-319.

Waters CD, Hard JJ, Fast DE*, et al.* (2020) Genomic and phenotypic effects of inbreeding across two different hatchery management regimes in Chinook salmon. *Molecular Ecology* **29** , 658-672. 10.1111/mec.15356

Wedekind C, Evanno G, Szekely T*, et al.* (2013) Persistent unequal sex ratio in a population of grayling (Salmonidae) and possible role of temperature increase. *Conservation Biology* **27** , 229-234. 10.1111/j.1523-1739.2012.01909.x

Wilkins LGE, Marques da Cunha L, Menin L*, et al.* (2017) Maternal allocation of carotenoids increases tolerance to bacterial infection in brown trout. *Oecologia* **185** , 351-363. 10.1007/s00442-017-3952-y

Table 1. Linear mixed model (REML, unbounded variance components) on juvenile length and weight when predicted by sex and mean kinship coefficient per family. Parental identities were included as random factors. Significant p-values are highlighted in bold.

		Body length	Body length	Body length	Body length		Body weight
Effects	d.f.	F	Variance component	p		F	F
Fixed effects:	Fixed effects:						
Sex	1	5.4		**0.02**		9.1	9.1
Kinship	1	0.3		0.58		0.2	0.2
Sex x kinship	1	8.3		**0.004**		8.8	8.8
Random effects:							
Dam[1]			27.3±14.0	**0.05**			
Sire[1]			3.1±3.3	0.35			
Residual			83.4±7.7				

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

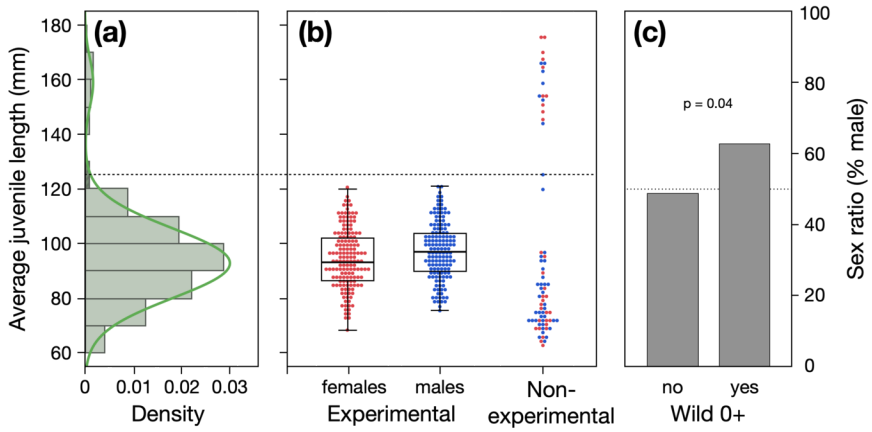

Figure 1. Sizes of trout sampled from the wild. (A) Size distribution of all trout (N = 518) with the bimodal normal distribution (green line; AIC = 4278.3) that fit the data better than a normal distribution (AIC = 4465.1). (B) Sizes of trout that were genetically sexed and assigned to one of the experimental 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 is 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.

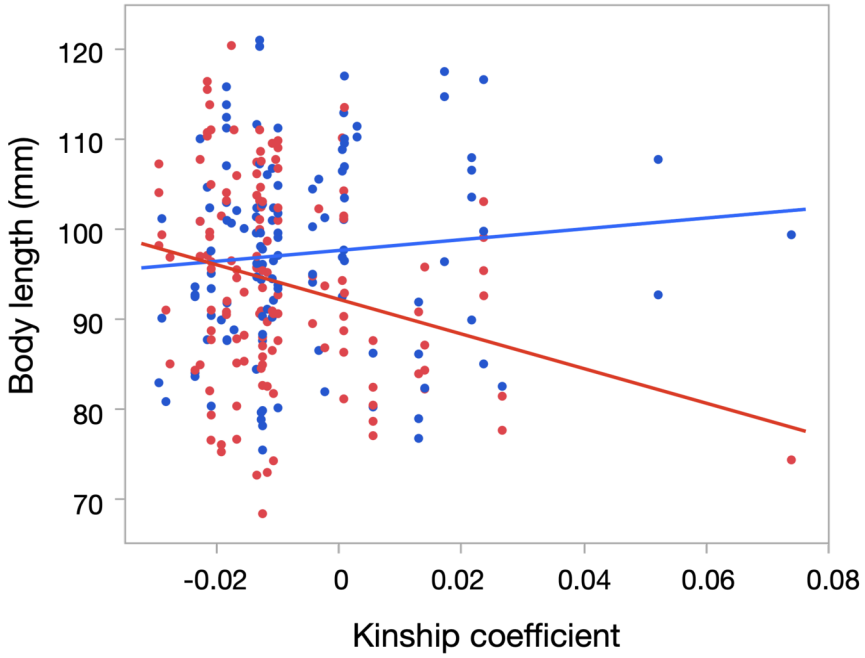

Figure 2. Body length of wild-caught juvenile brown trout in relation to the kinship coefficient in males (blue dots and regression line) and females (red). Female but not male size declines with increased kinship coefficients (Table 1; this is also the case if kinship coefficients > 0.03 are excluded, see Supplementary Figure S1 & Table S1).