Sex-specific overdominance at the maturation *vgll3* gene for reproductive fitness in wild Atlantic salmon

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Abstract

Linking reproductive fitness with adaptive traits at the genomic level can shed light on the mechanisms that produce and maintain sex-specific selection. Here, we construct a multigenerational pedigree to investigate sex-specific selection on a maturation gene, *vgll3*, in a wild Atlantic salmon population. The *vgll3* locus is responsible for ~40% of the variation in maturation (sea age at first reproduction). Genetic parentage analysis was conducted on 18,265 juveniles (parr) and 685 adults collected at the same spawning ground over eight consecutive years. A high proportion of females (26%) were iteroparous and reproduced 2-4 times in their lifetime. A smaller proportion of males (9%) spawned at least twice in their lifetime. Sex-specific patterns of reproductive fitness were related to *vgll3* genotype. Females showed a pattern of overdominance where *vgll3**EL genotypes had three-fold more total offspring than homozygous females. In contrast, males demonstrated that late-maturing *vgll3**LL individuals had 2-fold more offspring than either *vgll3**EE or *vgll3**EL males. Taken together, these data suggest that balancing selection in females likely maintains variation in this locus via increased fitness of iteroparous *vgll3**EL females. This study demonstrates the utility of multigenerational pedigrees for uncovering complex patterns of reproduction, sex-specific selection, and the maintenance of genetic variation.

Introduction

Sex-specific selection arises when males and females have discordant selection on fitness and may lead to sexual conflict (Connallon et al. 2010; Wright et al. 2018). Sex-specific selection can shape fitness, gene expression, genomic architecture, and the maintenance of genetic variation (Johnston et al. 2013; Mank 2017; Mérot et al. 2020; Wright et al. 2018). The mechanisms underlying sex-specific selection are not well understood, partly because fitness may be affected by several interacting life-history traits such as survival, growth, sexual maturation, and offspring survivorship (Mérot et al. 2020). This selection can vary through different periods of time due to spatial and temporal environmental heterogeneity (Brown and Kelly 2018) and potentially shift during different life history stages.

Recent studies that investigate the genetic basis of sex-specific selection are beginning to shed light on the topic. For example, sex-specific selection on a single locus maintains a polymorphism in horn size of male Soay sheep (Johnston et al. 2013). Here, a life history trade-off between survival and fitness drives antagonistic pleiotropy at the locus in males. The product of this trade-off results in a pattern of overdominance, or heterozygote advantage maintaining polymorphism in the locus (Johnston et al. 2013). In the seaweed fly, *Coelopa frigida*, a chromosomal inversion polymorphism underlies a life-history trade-off between larval survival and adult reproduction. Antagonistic pleiotropy on this inversion results in a pattern of overdominance that maintains this polymorphism (Mérot et al. 2020). In Atlantic salmon, *Salmo salar*, sex-dependent dominance and sexual antagonism at a large effect locus is thought to maintain variation in maturation age (Barson et al. 2015). Yet, questions remain concerning the genetic basis of sex-specific selection in species with complex life histories. For example, how is sex-specific selection maintained in species that have multiple reproductive events in their lifetime? Moreover, what are the sex-specific fitness consequences of different reproductive strategies? These questions are particularly relevant in natural populations where selection may differ during different life stages and may mask the root causes of sex-specific selection.

Atlantic salmon is an interesting model system to investigate the nature of sex-specific selection and the link to reproductive fitness (Barson et al. 2015; Mank 2017; Mobley et al. 2021). On average, male and female Atlantic salmon have different optimal life history strategies, including differences in maturation timing and reproductive fitness that may ultimately drive differences in reproductive optima (Mobley et al. 2021; Mobley et al. 2020). Most Atlantic salmon populations are anadromous, whereby they reproduce in fresh water and the juveniles (parr) spend a number of years in fresh water before migrating to sea (Erkinaro et al. 2019; Jonsson and Jonsson 2011; Mobley et al. 2021; Økland et al. 1993). Female and male parr spend similar amounts of time in fresh water before migrating to the sea (Mobley et al. 2020). However, females have a negative correlation in pre- and post-marine migration growth (Einum et al. 2002) and females that spend more time in fresh water spend less time at sea before return migrating to spawn (Erkinaro et al. 1997; Mobley et al. 2020). Atlantic salmon spend one or several years feeding and growing at sea prior to returning to fresh water to spawn (Fleming 1998; Fleming 1996; Jonsson and Jonsson 2011; Mobley et al. 2021). The time spent at sea prior to returning to spawn, known as sea age or sea age at maturity, is commonly measured in sea winters (SW). Males usually mature after 1SW even though reproductive fitness is higher in older and larger males (Mobley et al. 2020). Females, on the other hand, spend more time at sea prior to returning to spawn than males (Mobley et al. 2020). Females that spawn after spending only one year at sea have lower reproductive fitness than those that spend at least two years at sea (Mobley et al. 2020) demonstrating that females should delay maturation in order to maximize reproductive fitness. The optimal time spent at sea differs between male and female Atlantic salmon theoretically to maximize reproductive fitness (Mobley et al. 2021; Mobley et al. 2020).

Atlantic salmon commonly reproduce only once in their lifetime prior to death, a strategy known as semelparity. However, a small proportion of anadromous Atlantic salmon are iteroparous and may reproduce in several different years (Birnie-Gauvin et al. 2023; Bordeleau et al. 2020; Fleming 1998; Fleming and Reynolds 2004; Fleming and Einum 2011; Jonsson and Jonsson 2011; Mobley et al. 2021; Persson et al. 2023). Iteroparity, or repeat-spawning, can be advantageous in that individuals may gain additional reproductive fitness in subsequent spawning years. Iteroparous individuals are more likely to be earlier-maturing salmon that invest proportionally less into reproduction than later-maturing salmon after controlling for sea age (Aykanat et al. 2019). Iteroparity safeguards against periods of low adult recruitment (Bordeleau et al. 2020) and therefore is important for long-term population viability.

Further variation in reproductive strategies is achieved in males, as male parr may become sexually mature without a marine migration and spawn with anadromous females (Fleming 1996; reviewed in Hutchings and Myers 1994; Mobley et al. 2021). This phenomenon represents an alternative mating strategy in which mature male parr may gain reproductive fitness. The amount of male parr maturation and contributions to reproductive fitness is highly variable and appears to be population specific (Hutchings and Myers 1988; Jones and Hutchings 2002; Mobley et al. 2021).

Up to 39% of the variation in sea age for Atlantic salmon males and females is explained by a single large-effect locus, *vgll3* (vestigial-like family member 3), identified by genome-wide association mapping studies (Barson et al. 2015). The *vgll3* locus encodes a transcription cofactor involved in adipogenesis regulation in mice (Halperin et al. 2013) and influences reproductive axis gene expression in Atlantic salmon testes (Ahi et al. 2022; Verta et al. 2020). In Atlantic salmon, the *vgll3* locus is linked with body condition (fatness) (Debes et al. 2021; House et al. 2023), aerobic scope (Prokkola et al. 2022), and juvenile aggressive behavior (Bangura et al. 2022). Alternative alleles in the *vgll3* locus are associated with either early (*vgll3**E) or late (*vgll3**L) maturation in the wild (Barson et al. 2015) and this association has been validated in controlled conditions in multiple populations (Debes et al. 2021; Sinclair-Waters et al. 2022; Åsheim et al. 2023). The *vgll3* locus exhibits incomplete sex-specific dominance that may help to explain the maintenance of variation at this locus (Barson et al. 2015). Males and females that are homozygous for the *E* allele (*vgll3**EE) most often mature after 1SW while *vgll3**LL adults mature after two or more SW (Barson et al. 2015). Due to complete dominance of the *vgll3**E allele in males, the sea age of *vgll3**EL males is similar to *vgll3**EE males, and both these genotypes return to spawn most often after 1SW (Barson et al. 2015). On the other hand, *vgll3**EL females return to spawn at an intermediate age between both homozygotes (Barson et al. 2015). Differing phenotypic optima for males and females over sea age and body size at maturation may result in the potential for sex-specific selection and sexual conflict between the *vgll3**E and *vgll3**L alleles at the locus (Barson et al. 2015; Mank 2017; Mobley et al. 2021). Indeed, *vgll3* alleles are maintained at intermediate frequencies in many populations (Barson et al. 2015). This may indicate that balancing selection is operating at this locus as predicted under intra-locus sexual conflict (Connallon and Clark 2014) and is supported by simulations and empirical data (Czorlich et al. 2018; Kuparinen and Hutchings 2019). Iteroparity is also linked to the *vgll3* locus (Aykanat et al. 2019). In particular, the odds ratio of survival until second reproduction was 2.4 times higher for *vgll3**EE compared to *vgll3**LL individuals (Aykanat et al. 2019).

In this study, we investigate sex-specific effects of the *vgll3* gene on reproductive fitness in Atlantic salmon. Differences in sea age, dominance patterns, and iteroparity at the *vgll3* locus, lead us to the prediction that alternative *vgll3* genotypes have sex-specific effects on reproductive fitness. To test this hypothesis, we reconstructed a multigenerational pedigree based on parentage assignment using single nucleotide polymorphism (SNP) data from adults and offspring collected over eight consecutive years from a population of wild Atlantic salmon from northern Finland. We then estimated reproductive success (i.e., the total number of assigned offspring) as a proxy for reproductive fitness for individual sires and dams for discrete reproductive events (i.e., spawning in different years).

We first investigated whether there were sex differences in reproductive success, iteroparity, and the maximum number of reproductive events. To measure the overall contributions of the *vgll3* locus on reproductive success, we tested for a sex-specific relationship between specific *vgll3* genotypes on the cumulative number of offspring over all reproductive events (total reproductive success) and for first reproductive event (age at first reproduction). We also compared reproductive success of semelparous and iteroparous individuals in the first reproductive event to see whether there was a sex-specific reproductive advantage to spawning earlier. In this manner, we can ascertain whether there was a fitness advantage between these two life-history strategies mediated by the *vgll3* locus. Finally, we test additive and dominance models of *vgll3* genotypes on reproductive fitness for each sex separately to help understand the nature of sex-specific selection. The combined goal of these investigations is to understand the effect of sex-specific selection and the underlying genetic architecture on reproductive fitness in a species under natural conditions.

Methods

Anadromous adults were collected in September-October 2011-2018 from the lower Utsjoki River, approximately 1 to 2 weeks before the commencement of spawning. The lower Utsjoki sampling site is located at the mouth of the Utsjoki tributary of the Teno River in northern Finland (69°54'28.37''N, 27°2'47.52''E, see Mobley et al. (2019) for further details on sampling location and methods). Adults were sexed, weighed (g), and total length (cm) was recorded. Adults were tagged with a unique alphanumeric-coded anchor dart tag in the musculature at the base of the dorsal fin. Scales were collected for age determination (see age determination below) and a small piece of the adipose fin was collected for genetic analysis prior to release near the site of capture.

Juvenile parr were sampled by electrofishing shallow areas along a 2km tract of the lower Utsjoki River upstream and downstream of the adult collection area in September 2012-2019 (Mobley et al. 2019). Parr were assigned an age class (0+, 1+, 2+, 3+) based on total length distributions that correspond to their age in years since hatching. Parr were age classed as follows: 0+: <6cm, 1+: 6-9cm, 2+: 9-11.5cm, 3+ >11.5cm. To track yearly cohorts, only 0+ parr were collected in 2012, 0+ and 1+ were collected in 2013, 0+, 1+ and 2+ were collected in 2014, and from 2015 onward all parr were collected. Total length was measured, and scale samples were collected on a few 0+ and 1+ individuals and all 2-3+ class individuals from 2014 forward to verify age as determined by scale analysis. Parr sexual maturity was determined on all larger individuals sampled in the field from 2014 onward. Individuals that expressed seminal fluid after stripping were considered mature male parr. Genetic samples were collected from all parr by removing a small piece of adipose and/or anal fins, after which parr were immediately returned to the river (Mobley et al. 2020; Mobley et al. 2019).

Age determination

Freshwater age, defined as the number of years spent in fresh water prior to migrating to sea, and sea age, defined as the number of years an individual overwintered at sea before returning to spawn, was determined for adults sampled on the spawning ground using scale growth readings as outlined in Aykanat et al. (2015) and Erkinaro et al. (2019). Hatch year (i.e., the year when alevins hatched from eggs) for adults was then calculated as year of collection – (freshwater age + sea age). A small proportion of adults were missing scales and/or age could not be determined for all life history stages. For adults that did not have sea age (n=11), sea age was interpolated using the means of sea age regressed on weight for each sex separately (Mobley et al. 2019). For adults that did not have freshwater age (n=53) due to either missing scales or inconclusive scale analysis, it was assumed that freshwater age was 4 reflecting the means of freshwater age of adult males and females (females: mean 3.52 ± 0.06, males: mean 3.53 ± 0.02). Hatch year of parr was calculated as the year of collection minus the age class (0+ = same year, 1+ = -1, 2-3+ = -2). Because parr were sampled as a combined class 2-3+ for years 2018 and 2019, 2+ and 3+ individuals were pooled for each year and assumed to have a hatch year = year of collection -2. Individuals that show previous returns as adults to fresh water based on scale data were considered iteroparous.

The life history strategy of each adult is characterized by a combination of freshwater age and sea age. For example, an individual that spent four years in fresh water and one year at sea before returning to spawn would be designated as 4-1. Iteroparous individuals have an additional designation first spawning (S) and reconditioning period at sea (variable number of years). Therefore, an individual 4-1S1 would have first spent four years in fresh water, spawned after spending one year at sea, and then would have returned two years later to spawn a second time.

DNA extraction and SNP genotyping

DNA extraction was carried out according to protocols outlined in Mobley et al. (2019). Genotyping was accomplished using a 176 single nucleotide polymorphisms (SNPs) of a panel originally described in Aykanat et al. (2016) with modifications to enable sequencing using Illumina platform (MiSeq or Next-Seq) sequencers (Aykanat et al. 2020). Five loci were removed from the final analysis due to high linkage ($r > 0.5$) using the LD function in the 'genetics' package (Warnes 2012) in R (R Core Team 2022)(Table S1). Two additional loci were excluded due to low sequencing success (>40% missing data: TN_423, 58%; TN_1088, 45%). The remaining 169 SNPs were filtered to remove individuals with low SNP coverage (>5% missing genotypes).

The *vgll3* locus was scored using the *vgll3*$_{\mathrm{TOP}}$ SNP (Aykanat et al. 2020; Barson et al. 2015) and sex was determined by estimating read counts of the sex determining region, *sdY* locus, using a read-depth threshold to distinguish males (high read number) and females (low read number) (Aykanat et al. 2016; Aykanat et al. 2020).

Duplicate genotypes were identified with SEQUOIA version 1.3.3 (Huisman 2017) and removed prior to parentage analyses. Of the duplicated genotypes, seven were adult males recaptured in a later year (confirmed by tag ID), six were parr recaptured as adults, 21 were parr recaptured in the same year, 68 were parr recaptured in different years, and nine were collection or DNA extraction errors (i.e., identical genotypes in consecutive samples).

Parentage analysis

Parentage was determined with SEQUOIA to reconstruct a maximum-likelihood, multigenerational pedigree from SNP genotypes, sex and back-calculated hatch years. The sex determining locus, *sdY* (SDY_ion2), was used for sex assignment. Parentage analysis was conducted using three methods: 1) program defaults, 2) informed parentage with priors set to zero for age at maturity of 0-1 for males, and 0-3 for females, and 3) conservative parentage where all priors <0.1 set to zero to exclude all the most improbable relationships. Age structure was based on back-calculated hatch dates such that offspring cannot be assigned to individuals prior to their hatch year. Adults could be assigned as offspring and vice versa.

Reproductive fitness

Reproductive fitness was quantified as reproductive success, or the number of offspring assigned to a sire or dam. We determined how many reproductive events (i.e., how many different years an individual spawned) and the reproductive success of each reproductive event by grouping offspring assigned by parentage into discrete hatch years based on back-calculated hatch year of offspring. Results were then visualized, and major hatch years were identified based on the large number of offspring assigned to a particular hatch year. Assigned offspring that fell between major hatch years were assumed to be incorrectly assigned to an age class and were grouped with the first hatch year or, in the case of iteroparous individuals, grouped with the proceeding hatch year. The first reproductive event for each sire and dam was calculated as the first major hatch year. Offspring that were iteroparous were either assigned a first reproductive event corresponding to the first hatch year identified by scale analysis or by the first hatch year identified by parentage. No individual offspring was assigned to more than one reproductive event.

Comparison of parentage analysis methods

To assess the performance of the three parentage methods, we compared the age at first reproduction of adults to the age of first reproduction of parr assigned as parents. Age at first reproduction was calculated as the hatch year of the parent – hatch year of assigned offspring and were considered adults if age at first reproduction was > 4. Sires that had an age of first reproduction ≤ 3 years were considered mature male parr. We used the conservative method of parentage analysis due to some cases of unrealistic assignments in the default and informed parentage analysis methods (Supplementary Information, Fig. S1).

Reproductive fitness

We constructed two datasets to test the sex-specific effects of *vgll3* genotype on reproductive fitness: 1) total reproductive success was calculated as the sum of all offspring assigned to an adult across all reproductive events, and 2) reproductive success for each adult's first reproductive event. In addition, we partitioned the first reproductive event into iteroparous individuals (respawners with offspring in more than one reproductive event) and ostensibly semelparous individuals with only one known reproductive event.

Statistical analyses

A χ^2 test was employed to test for differences in the frequencies in offspring age class assigned by parentage between dams and sires. General linear models (GLM) were constructed using lme4 version 1.1.29 (Bates et al. 2015) package to test for differences in sea age between the sexes *vgll3* genotypes for 1) adults collected on the spawning ground and 2) sires and dams assigned with the conservative method of parentage analysis.

A GLM was constructed to test for differences in total reproductive fitness between the sexes with respawner (semelparous/iteroparous) and the maximum number of reproductive events as factors. All models of reproductive success were run with a negative binomial error distribution and a log link function based on variance distributions of model residuals in the MASS package in R (Venables and Ripley 2002).

To test for differences in sex-specific effects of the *vgll3* gene on reproductive success, negative binomial GLMs were constructed with reproductive fitness as the response variable and *vgll3* genotype as a fixed effect and sea age as a covariate. GLMs were fitted in males and females separately due to differences in the distribution of reproductive success between the sexes using a restricted dataset. The restricted dataset excluded sires and dams that had offspring assigned in reproductive events prior to 2011 (the first year of the time series) since only adults were assigned as offspring. Similarly, sires and dams that had their first reproductive event after 2017 (the 2nd last year of the time series) were not included as these would only include offspring of 0+ and 1+ age classes and therefore offer an incomplete estimate of reproductive fitness.

To investigate whether *vgll3* genotype influenced reproductive fitness of iteroparous compared to semelparous individuals, negative binomial GLMs were constructed with reproductive success as a response variable, *vgll3* genotype and respawner as fixed effects and sea age as a cofactor. Models were run for sires and dams and total reproductive success and reproductive success for the first reproductive event separately using the restricted dataset.

To test whether *vgll3* genotype conformed to additive and/or dominance effects, a negative binomial GLM was fitted with additive (i.e. *vgll3**EE = 1, *vgll3**EL = 0 and *vgll3**LL = −1; continuous covariate) and dominance (*i.e.*, *vgll3**EE & *vgll3**LL = 0 and *vgll3**EL = 1; continuous covariate) effects on reproductive success with sea age as a covariate (Niemelä et al. 2022; Xiang et al. 2018) using the restricted dataset. We included both additive and dominance fit to estimate whether there are dominance effects on top of additive effects (Xiang et al. 2018).

All statistical models were performed in R (R Core Team 2022). Interactions were first tested and removed if non-significant ($\alpha = 0.05$). Model fits were assessed by inspecting residual plots (i.e., normal Q-Q plots, standardized vs fitted residuals, and histograms) using the DHARMa version 0.4.5 package (Hartig 2022). All means are reported ± one standard error of the mean.

Results

A total of 685 adult Atlantic salmon (93 females, 592 males, Fig. S2), and 18,265 juvenile parr (Fig. S3) were sampled over the eight years. All adults and 18,172 parr were genotyped using the SNP panel. After filtering and removal of duplicate genotypes, 632 adult (86 females, 546 males) and 16,175 parr were used for parentage analysis. Using the conservative parentage dataset, 8176 offspring (50.5%) were assigned to at least one parent. A total of 4276 offspring were assigned to dams, 5954 offspring were assigned to sires, and 2054 offspring were assigned to both dams and sires. A total of 97 females were assigned as dams, 77 sampled as adults and 20 sampled as parr. A total of 337 males were assigned as sires, 301 sampled as adults and 36 sampled as parr (Table S2). The distribution of offspring in age classes did not significantly differ between those assigned to dams and sires (n = 10,230, χ^2 = 1.435, df = 3, p = 0.697, Fig. S4).

Sampled adults

On average, freshwater age was similar for adult females and males based on scale readings (females = 3.52 ± 0.06, range 3-5 years; males = 3.53 ± 0.03, range 3-6 years). However, females spent considerably more years at sea before returning to spawn for the first time (females = 2.34 ± 0.08, range 1-4SW; males = 1.29 ± 0.03, range 1-4SW).

Twenty-six sampled individuals (13 females, 13 males) were identified as iteroparous by scale aging analysis. The mean sea age at first maturity of these iteroparous females was 2.38 ± 0.24 SE (range 1-3 SW) at the time of first spawning. The mean sea age at first maturity of iteroparous males was 1.08 ± 0.08 SE (range 1-2 SW) at the time of first spawning. All iteroparous sampled individuals had a two year gap between the first and second reproductive events.

Mature male parr

A total of 41 mature male parr were collected in 2014-2019 accounting for 9.3% (± 2.3) of male parr in the 2-3+ class and 1.7% (± 0.1) of all parr per year. Mature male parr ranged from 2-3 years with a mean of 2.25 ± 0.08 based on scale data (n = 28) (Fig. S5). Mature male parr were significantly older and larger than immature male and female parr in the 2-3+ age class (female versus immature male parr scale age (years): estimate= 0.002 ± 0.018, t = 0.097, P = 0.923; female versus mature male parr scale age (years): estimate = 0.207 ± 0.043, t =4.790, P <0.001; female versus immature male parr length (cm): estimate = 0.022 ± 0.107, t = 0.203, P = 0.839; female versus mature male parr length (cm): estimate = 1.025 ± 0.262, t =4.289, P <0.001; Fig. S5).

Parentage analysis

Assigning offspring to discrete reproductive events based on back-calculated hatch dates demonstrated that 1.4% (62/4276) of offspring assigned to dams and 1.3% (77/5954) of offspring assigned to sires were not assigned to a major spawning year. These offspring were assigned to the proceeding spawning year.

The most common life history strategy for semelparous females was 3-2 and 4-2 (Figure S6). Parentage analysis identified 26% of adult females spawned in multiple years, with at least one dam spawning in up to four reproductive events and two dams that spawned in three reproductive events (Fig. 1). The reproductive events of iteroparous females were commonly two years apart. Other iteroparous dam spawning patterns included a pause between spawning that were three (3 dams) and five (1 dam) years between reproductive events.

The most common life history strategy for semelparous sires was 3-1 and 4-1 (Fig. S6). A smaller percentage of sires (9%) spawned multiply compared to females (Fig. 1). Repeat spawning patterns for iteroparous sires also showed a common two-year period between reproductive events. Additionally, the reproductive events of three iteroparous sires

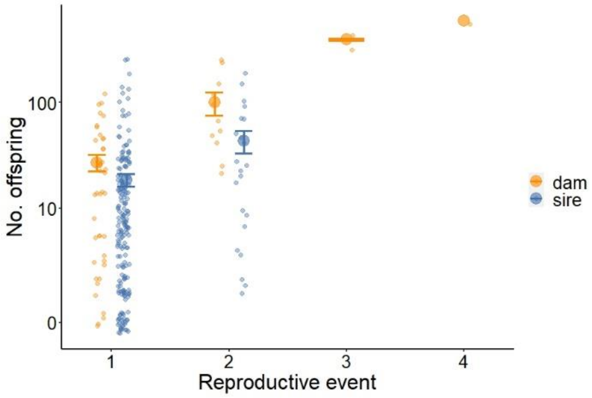

Figure 1. The relationship of total reproductive success (No. offspring) and the maximum number of reproductive events for individual sires and dams. Sires and dams that have 1 reproductive event are semelparous while sires and dams that had > 1 reproductive event were iteroparous. Large circles with error bars represent the mean ± SE, while small circles show individual data points. For clarity, individual points are jittered on the x- and y-axis and the y-axis for reproductive fitness is $\log_{10}$-transformed.

	EST.	SE	Z	PR(>|Z|)
(INTERCEPT)	2.0127	1.1125	2.809	0.0704
SEX	-0.4544	0.1840	-2.470	**0.0135**
RESPAWNER	0.2144	0.5983	0.358	0.7201
REPRODUCTIVE EVENT	1.1986	0.5072	2.363	**0.0181**

Table 1. Results of negative binomial GLM showing the effect of sex, whether the parent was iteroparous (respawner), and the maximum number of reproductive events on total reproductive success. Reference sex is female, reference respawner is iteroparous.

were three (3 sires), four (1 sire), or remarkably, seven (1 sire) years apart. Seven adult males lacking repeat spawning information from scale data had offspring > 1 year before they were sampled based on parentage analysis. Two of these males were 3SW males with many offspring consistent with a repeat spawning pattern of 1S1. One additional 3SW male sampled in 2011 had one adult offspring consistent with a 1S1 repeat spawn pattern. These three males were therefore considered 1S1. The remaining four adult males that lacked scale data had a repeat spawning pattern of 0S1, or maturation < 1SW and may represent offspring sired as mature male parr. However, these four males were assumed to have a freshwater age of four years and may only have spent three years in fresh water. Offspring sired by these males amounted to five offspring.

A negative binomial GLM investigating the relationship between sex, iteroparity, and the maximum number of reproductive events on total reproductive success demonstrated that dams had significantly more offspring than sires and that total reproductive success increased with the number of reproductive events. However, whether a parent was semelparous or iteroparous did not significantly influence this relationship (Fig. 1, Table 1).

The contributions of mature male parr to reproductive success was low, ranging from 0.05-2.56% of offspring sired, depending on the parentage analysis method (Table S3). Using the conservative parentage analysis method, only three offspring were potentially sired by sampled adult males that may have spawned as mature male parr. No offspring were assigned to sampled male parr (Table S3).

Reproductive fitness and the vgll3 locus

Sea age at first reproduction was strongly influenced by sex and the *vgll3* locus. A GLM showed significantly higher sea age at first reproduction in females and in *vgll3**LL genotypes (Table 2, Fig. 2). These results were similar in both sampled adults and in adult sires and dams identified through parentage analysis (Table 2, Fig. 2).

Results of GLMs on reproductive fitness demonstrated that dams possessing the *vgll3**EL genotype had three times more total offspring than either *vgll3**EE or *vgll3**LL dams (mean offspring *vgll3**EE: 33.1 ± 12.5; *vgll3**EL: 95.3 ± 24.8; *vgll3**LL: 27.1 ± 5.9, Table 3, Fig. 3). However, the relationship of sea age on total reproductive success was not significant (Table 3, Fig. 3). Sires, on the other hand, showed no significant relationship between total reproductive success and *vgll3* genotype after controlling for sea age. Despite this, sires

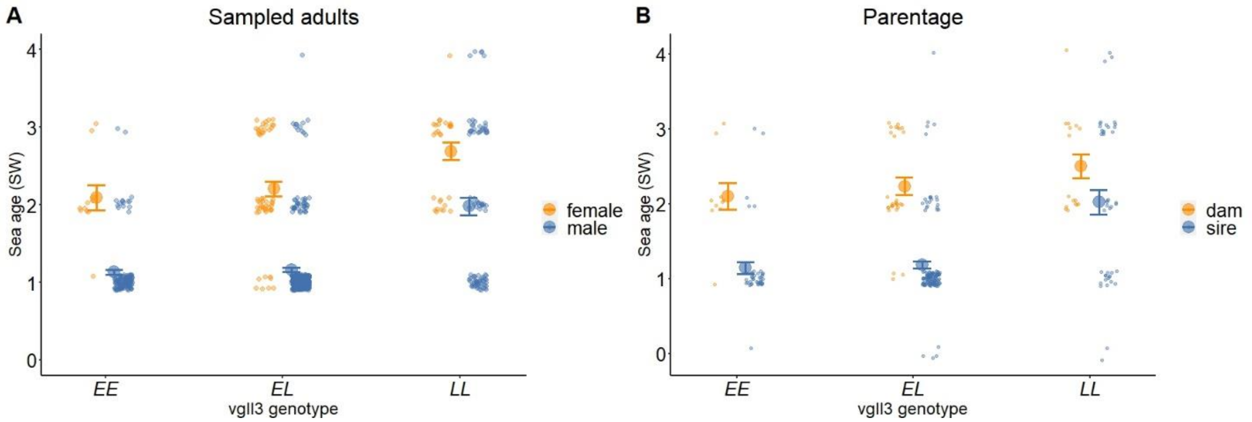

Figure 2. The relationship between sea age at first reproduction (sea winters, SW) and *vgll3*$_{\text{TOP}}$ genotype for A) sampled adults and B) dams and sires assigned offspring by parentage analysis. Large circles with error bars represent the mean ± SE, while small circles show individual data points. For clarity, individual points are jittered on the x- and y-axis.

possessing the *vgll3**LL genotype had two times more total offspring than either *vgll3**EE or *vgll3**EL sires after controlling for sea age (mean offspring *vgll3**EE: 14.1 ± 2.6; *vgll3**EL: 19.2 ± 5.5; *vgll3**LL: 40.3 ± 10.0, Table 3, Fig. 3). Sires showed a significant relationship of total reproductive success with sea age demonstrating that older and larger sires had more offspring (Table 3, Fig. 3). When linear models were restricted to offspring from just the first reproductive event, no effect of *vgll3* genotype on reproductive success was found in either dams or sires after controlling for sea age (Table 3, Fig. 3). Rather, a significant positive effect of sea age on reproductive fitness was found in dams and sires (Table 3, Fig. 3).

Table 2. Results of GLM showing the effects of sex and *vgll3* genotype on sea age at first reproduction. Separate models were run on adults sampled dataset and dams and sires using the conservative parentage dataset. Reference sex is female, reference *vgll3* genotype is *vgll3**EE.

	EST.	SE	T VALUE	PR(>|T|)
ADULTS SAMPLED				
INTERCEPT	2.0727	0.0799	25.940	**<0.0001**
SEX	-0.9394	0.0673	-13.960	**<0.0001**
***VGLL3**EL**	0.0431	0.0584	0.738	0.4610
***VGLL3**LL**	0.7915	0.0744	10.633	**<0.0001**
PARENTAGE				
INTERCEPT	2.0390	0.1258	16.213	**<0.0001**
SEX	-0.8817	0.1026	-8.590	**<0.0001**
***VGLL3**EL**	0.0572	0.1088	0.526	0.5990
***VGLL3**LL**	0.7561	0.1300	5.717	**<0.0001**

Comparing the first reproductive event between semelparous and iteroparous dams and sires revealed no effect of *vgll3* genotype after controlling for sea age (Table 4, Fig. 4). However, a significant positive effect of sea age on reproductive success was apparent in sires but not in dams (Table 4, Fig. 4).

Dominance effects of the *vgll3* genotype on total reproductive success were significant in dams but not in sires after controlling for sea age (Table 5). In these models of total reproductive success, sires, but not dams, showed a significant relationship to sea age and total reproductive success (Tables 5). When just limiting these models to the first reproductive event, dominance in the *vgll3* genotype was still apparent among dams but at a marginal level after controlling for sea age (Table 5). Males, on the other hand, showed no additive or dominance effects but show a significant effect of sea age on reproductive success (Table 5).

Discussion

The goal of this study was to study sex-specific selection on a gene linked to a life-history trait in

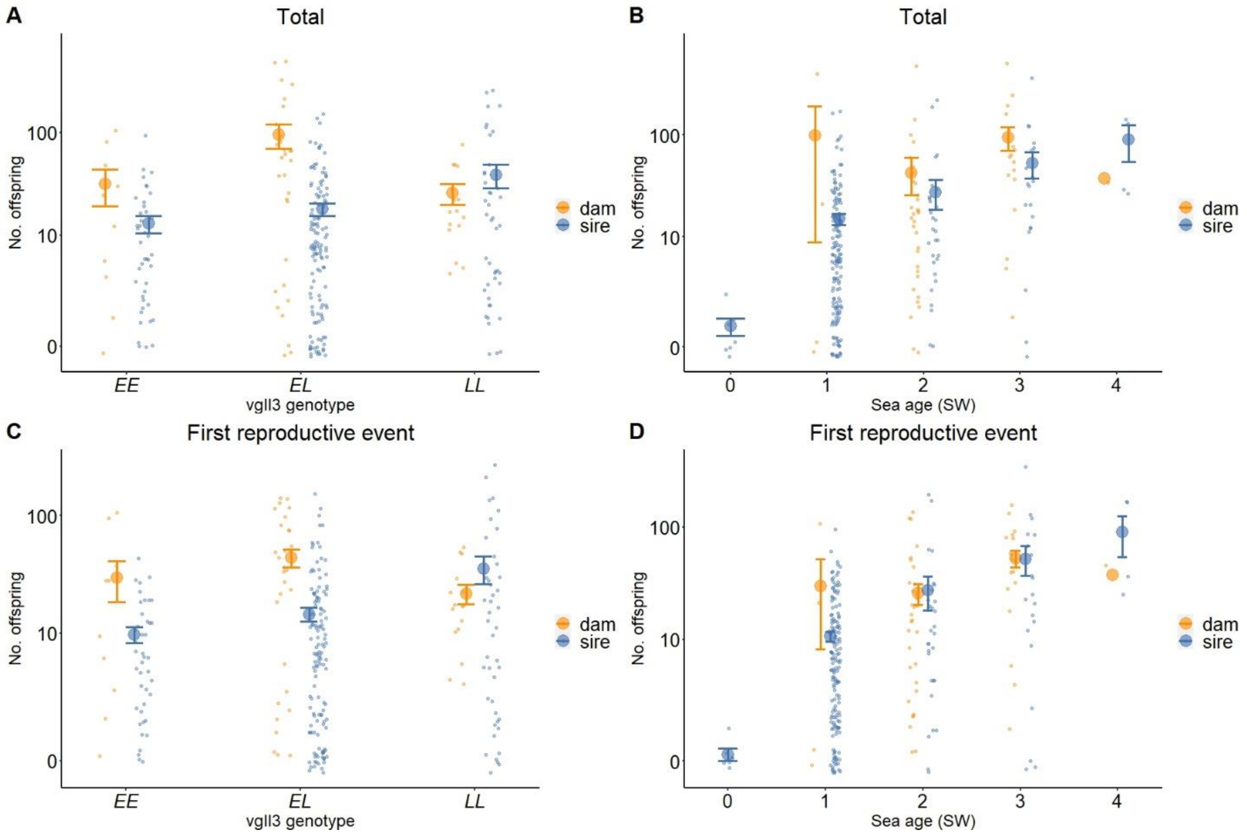

Figure 3. The relationship between reproductive fitness (number of assigned offspring) and *vgll3*$_{\text{TOP}}$ genotype and sea age at first reproduction (sea winters, SW) for dams and sires. A) Total reproductive success and *vgll3* genotype, B) total reproductive success and sea age, C) reproductive success of the first reproductive event and *vgll3* genotype, D) reproductive success of the first reproductive event and sea age (SW) using the restricted dataset. Large circles with error bars represent the mean ± *SE*, while small circles show individual data points. For clarity, individual points are jittered on the *x*- and *y*-axis and the *y*-axis for reproductive fitness is log$_{10}$-transformed.

a natural population. Reproductive fitness data of over 8000 offspring assigned to >600 parents over eight cohort years revealed that selection on female reproductive fitness is consistent with a pattern of overdominance, or heterozygote advantage at the *vgll3* locus, among female Atlantic salmon. This pattern is driven by a higher proportion of *vgll3**EL iteroparous females contributing to reproductive fitness compared to homozygous females. Males, on the other hand, show a pattern consistent with directional selection on sea age mediated by *vgll3,* as sea age and *vgll3* genotype covary in Atlantic salmon (Aykanat et al. 2019; Barson et al. 2015; Czorlich et al. 2018; Sinclair-Waters et al. 2020). Males with *vgll3**LL genotypes mature at older ages and larger sizes and show a two-fold higher reproductive fitness than male *vgll3**EE and *vgll3**EL genotypes which have similar sea age and reproductive fitness. Previous studies have shown that sex-specific selection and patterns of overdominance maintains polymorphism in traits associated with reproduction (Johnston et al. 2013; Mérot et al. 2020; Wright et al. 2018) and it has been previously suggested for the sea age-*vgll3* association in salmon also (Barson et al. 2015). Our results indicated it is indeed highly likely that sex-specific selection on reproductive fitness at the *vgll3* locus maintains variation at this locus. Interestingly, *vgll3* variation tended to explain little of the variation in reproductive fitness once sea-age variation was accounted for, suggesting that the influence of *vgll3* on age at maturity appears to be the main route by which *vgll3* is expected to influence reproductive fitness.

Table 3. Results of negative binomial GLMs showing the effect of *vgll3* genotype and sea age on total reproductive success and reproductive success of the first reproductive event. Separate models were run for dams and sires using the restricted dataset. Reference *vgll3* genotype is *vgll3**EE.

	TOTAL				FIRST REPRODUCTIVE EVENT			
	EST.	SE	Z value	Pr(>|z|)	EST.	SE	Z value	Pr(>|z|)
DAMS								
(INTERCEPT)	2.7173	0.6818	3.985	**<0.0001**	2.4222	0.5708	4.243	**<0.0001**
***VGLL3**EL**	1.0557	0.4432	2.382	**0.0172**	0.3131	0.3705	0.845	0.3980
***VGLL3**LL**	-0.3245	0.5008	-0.648	0.5169	-0.4610	0.4189	-1.101	0.2711
SEA AGE	0.3470	0.2682	1.293	0.1958	0.4628	0.2246	2.061	**0.0393**
SIRES								
(INTERCEPT)	1.9360	0.2235	8.664	**<0.0001**	1.3977	0.2113	6.614	**<0.0001**
***VGLL3**EL**	0.2174	0.2072	1.049	0.2940	0.2444	0.1957	1.249	0.2120
***VGLL3**LL**	0.2618	0.2721	0.962	0.3360	0.0965	0.2572	0.375	0.7070
SEA AGE	0.6250	0.1146	5.454	**<0.0001**	0.8510	0.1079	7.891	**<0.0001**

Previous studies have provided evidence that the strength of sex-specific effects of the *vgll3* locus may vary between populations, even within the same river catchment. For example, Czorlich et al. (2018) demonstrated that selection at sea against *vgll3**LL genotype individuals acts primarily on females in the same genetic population studied here (Tenojoki), but on males in another genetically-distinct population (Inarijoki) within the Teno River catchment. In our study, a highly male-biased sex ratio in adults which is a significant departure from an even sex ratio signifies that females are the limiting sex and limits male reproduction (Andersson 1994; Emlen and Oring 1977). In addition, a large proportion of sampled adult females reproduced (83%) and a relatively high proportion of females (26%) were iteroparous compared to males (50.5% males reproduced, 9% iteroparous) indicating that females are indeed limiting at this location.

Iteroparity in salmonids has been proposed as an evolutionary trade-off for reproductive fitness in their first reproduction for future reproductive benefits (Bordeleau et al. 2020; Christie et al. 2018; Persson et al. 2023). In general, iteroparous individuals have higher lifetime reproductive fitness than semelparous individuals (Christie et al. 2018). However, semelparous spawners in some salmonid species may have higher reproduction in the first reproduction than iteroparous individuals (Christie et al. 2018). Based on the comparison of reproductive fitness within the first reproductive event, there appears to be no reproductive fitness advantage to iteroparity compared to semelparity mediated by the *vgll3* locus in either dams or sires after sea age at maturity is taken into account. Rather, the increased likelihood of survival of *vgll3**EL females to iteroparity may help to explain the advantage of iteroparity Atlantic salmon.

Atlantic salmon have remarkable diversity in life history patterns (Erkinaro et al. 2019; Harvey et al. 2022; Mobley et al. 2021; Persson et al. 2023). Previous studies in the Teno River have uncovered 120 different combinations of freshwater age, sea age, and repeat spawning

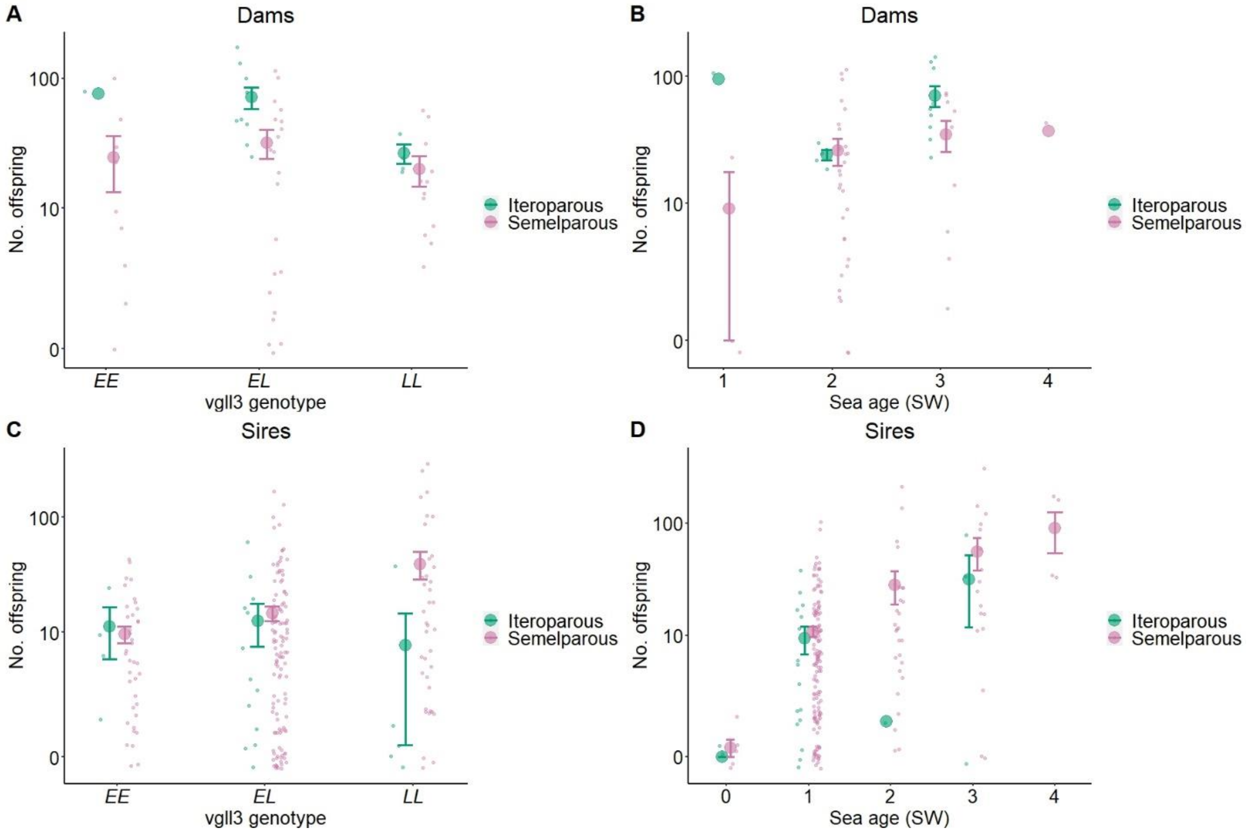

Figure 4. The relationship between reproductive success (number of assigned offspring) and *vgll3*$_{\text{TOP}}$ genotype and for dams and sires for the first reproductive event using the restricted dataset. Iteroparous (respawner) individuals are shown in green, semelparous (single spawner) individuals are shown in purple. Large circles with error bars represent the mean ± *SE*, while small circles show individual data points. For clarity, individual points are jittered on the *x*- and *y*-axis and the *y*-axis for reproductive fitness is $\log_{10}$-transformed.

patterns based on scale information (Erkinaro et al. 2019). In this study, 27 unique life history strategies were uncovered using combined information from scale data and parentage analysis from a single spawning ground in the Teno River. Sixteen of the unique life history strategies were based on scale data alone, while the remainder were only revealed following reconstructing patterns of multiple mating based on genetic parentage analysis. Thus, we demonstrate how scale analysis combined with genetic parentage analysis uncovered complex repeat spawning behavior of Atlantic salmon that would otherwise go unrecognized based on scale data alone. For example, several iteroparous individuals identified through parentage analysis did not have signatures of repeat spawning in scale readings indicating that scale-readings alone likely underestimate iteroparity and thus the overall life-history diversity in this system may be even higher than previously thought. Additionally, several individuals had unusually long gaps between spawning events as deduced from parentage analysis. It is possible that individuals that had greater than four years between spawning events may have skipped spawning entirely during this period, spawned in different locations in the interim, or returned to spawn on their natal spawning ground but were either unsuccessful or no offspring were sampled. Extending the time frame of the study, increasing sampling locations and/or increasing sampling effort may help to clarify genetic relationships between offspring and iteroparous adults.

Mature male parr occur commonly in Atlantic salmon populations although their contribution to reproductive fitness is unknown in most cases (Mobley et al. 2021). It is estimated that between 0-25% of ≥ 1 year old parr may be mature males in the Teno River system (Heinimaa and Erkinaro 2004). Previous investigations using microsatellite markers on four cohort years identified limited potential for mature male parr to contribute to reproductive fitness in the Utsjoki river mouth population (Mobley et al. 2020; Mobley et al. 2019). In this study, we found a small (9.3) percentage of mature male parr among 2-3 year old parr sampled in the field. These mature male parr had an age at maturity between 2-3 years and were significantly older and larger than immature males of the same age classes. Because no mature male parr were resampled as adults, we used a minimum of three years of age at maturation as a cutoff for mature male parr. Using this method, we reduced the potential for ascribing parentage to males that may have matured after 3 years in fresh water which was the second most common life history pattern in males (i.e., 3-1). Our analysis identified no offspring sired by sampled parr including those that were identified as mature male parr in the field, and only three offspring potentially sired as parr from adults collected in the field. These results strongly suggest that the contribution of mature male parr to reproductive fitness is negligible in this system. Because the mature male parr were larger than immature male parr of the same age class, it is possible that additional mature male parr may have been present but were either not sampled because they escaped electrofishing, were in deeper inaccessible sections of the river, or had migrated to other parts of the river system (Erkinaro and Niemelä 1995; Erkinaro et al. 1998). Therefore, there remains some uncertainty with estimating the contributions to mature male parr based on current available data, but it is unlikely to be significant.

In this study, we used a targeted SNP panel to construct a pedigree used for parentage analysis. Previous studies in the same sampling location identified patterns of local adaptation and sex-specific fitness effects using microsatellite markers in conjunction with parentage analysis (Mobley et al. 2020; Mobley et al. 2019). The previous studies were conducted over four cohort years with only the largest class of potential offspring (0+) sampled within each cohort year separately. The advantage to using a targeted SNP panel was that it allowed sex typing and *vgll3* genotyping as well as provided neutral markers for parentage. Additionally, all year classes of juveniles and adults were incorporated into the multigenerational pedigree presented here. As a result, a higher proportion of offspring

	EST.	**SE**	**Z VALUE**	**PR(>|Z|)**
DAMS				
INTERCEPT	3.0555	0.6928	4.411	**<0.0001**
RESPAWNER	-0.5663	0.3185	-1.778	0.0754
***VGLL3**EL**	0.1823	0.3654	0.499	0.6178
***VGLL3**LL**	-0.5000	0.4089	-1.223	0.2215
SEA AGE	0.3938	0.2269	1.736	0.0826
SIRES				
INTERCEPT	1.1444	0.3040	3.764	**<0.0001**
RESPAWNER	0.2938	0.2495	1.178	0.2390
***VGLL3**EL**	0.2490	0.1953	1.275	0.2023
***VGLL3**LL**	0.1235	0.2570	0.480	0.6310
SEA AGE	0.8344	0.1079	7.733	**<0.0001**

Table 4. Results of negative binomial GLMs showing the effect of *vgll3* genotype, sea age and whether the parent was iteroparous (respawner) on reproductive success of the first reproductive event. Separate models were run for dams and sires using the restricted dataset. Reference respawner is iteroparous, reference *vgll3* genotype is *vgll3**EE.

	TOTAL				**FIRST REPRODUCTIVE EVENT**			
	Estimate	SE	Z value	Pr(>|z|)	Estimate	SE	Z value	Pr(>|z|)
DAMS								
(INTERCEPT)	2.5550	0.6643	3.846	**0.0001**	2.1917	0.5567	3.937	**<0.0001**
***VGLL3* ADDITIVE**	0.1623	0.2504	0.648	0.5169	0.2305	0.2094	1.101	0.2711
***VGLL3* DOMINANCE**	1.2180	0.3292	3.700	**0.0003**	0.5436	0.2755	1.973	**0.0485**
SEA AGE	0.3470	0.2682	1.293	0.1958	0.4628	0.2246	2.061	**0.0393**
SIRES								
(INTERCEPT)	2.0669	0.2221	9.306	**<0.0001**	1.4460	0.2104	6.872	**<0.0001**
***VGLL3* ADDITIVE**	-0.1309	0.1361	-0.962	0.3360	-0.0483	0.1286	-0.375	0.7070
***VGLL3* DOMINANCE**	0.0865	0.1685	0.513	0.6080	0.1962	0.1591	1.233	0.2180
SEA AGE	0.6250	0.1146	5.454	**<0.0001**	0.8510	0.1079	7.891	**<0.0001**

Table 5. Results of negative binomial GLM models showing the effect of additive and dominance effects of *vgll3* genotype and sea age on total reproductive success and reproductive success of the first reproductive event using the restricted dataset. Additive effects were coded as *vgll3**EE = 1, *vgll3**EL = 0, *vgll3**LL = −1, Dominance effects were coded as *vgll3**EE = 0, *vgll3**EL = 1, *vgll3**LL = 0.

were assigned to parents when using all information. It is important to note that we used three different criteria for parentage assignment and our results were sensitive to priors used to construct the pedigree. For example, the three methods used in parentage assignment gave identical results with respect to offspring assigned to sampled females and nearly identical results to sampled males. However, these methods differed dramatically in the number of juvenile parr assigned as parents. One potential source of discrepancy between these methods may be that parentage analysis is assigning related individuals (parents, siblings) rather than true parents. Because this study samples in an open population of migrating individuals, it is near impossible to verify these relationships. However, refinement of priors to eliminate the most improbable relationships with the conservative data set led to the most biologically realistic interpretations of parentage.

Understanding the link between life history traits, reproductive fitness, and the genetic underpinnings of such relationships is a fundamental goal of evolutionary biology. This study reveals sex-specific selection on a gene responsible for an important life-history trait. Balancing selection on females through overdominance likely maintains polymorphism at the *vgll3* locus. Future studies that can investigate selection on adults in the marine environment may help to clarify internal and external sources for selection on this important life history gene. This study also demonstrates the utility of multigenerational pedigrees in investigating how sex-specific selection influences reproductive fitness and how genetic variation is maintained in natural populations.

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Author contributions: C.R.P, J.E. and P.O. conceived the original idea; K.B.M., C.R.P. and H.J.B. conceived the study. M.E., K.B.M., P.O., O.G., H.P., and J.E. coordinated and/or participated in sample collection. C.R.P., A.R., and K.B.M. coordinated molecular data generation. H.J.B. performed parentage analysis. K.B.M. analyzed the data. K.B.M. drafted the manuscript, with input from all other authors.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data will be uploaded to the Zenodo digital repository upon acceptance of the article. All code will be deposited in github.

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Supplemental information

Sex-specific overdominance at the maturation *vgll3* gene for reproductive fitness in wild Atlantic salmon

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Supplementary text: Comparison of different parentage assignment methods
Tables: S1-S3
Figures: S1-S6
References

Comparison of different parentage assignment methods:

Using the default method in Sequoia (Huisman 2017), female parr assigned as dams had an age of first reproduction just one year after hatching (Fig. S1) which is highly improbable as the age at first reproduction among adult females in this population is four years or older (Mobley et al. 2020; Mobley et al. 2019). Moreover, most male parr assigned as sires had an age of reproduction <2 years in the default and informed method (Fig. S1). Reproduction at one year after hatching is highly unlikely as all mature male parr were a minimum of two years at the age of first reproduction (2-3+, this study). Therefore, all models were carried out using the conservative method of parentage analysis as the distribution of parr age at first reproduction was most realistic for that expected in dams and sires.

Table S1. SNP loci with high linkage (r > 0.5). Loci in bold were retained in the analyses, and the rest removed.

Locus 1	**Locus 2**	**r**
AKAP11_4	c25_684F_713R_SACa	0.6717
c25_684F_713R_SACa	c25_684F_713R_SACb	0.9997
c25_1441_SAC	UtagF_SS_147a	0.6867
c25_1441_SAC	UtagF_SS_148c	0.8377
UtagF_SS_147a	UtagF_SS_148c	0.8592
X21_1	TN_301	0.6287

Table S2. Parentage assignment results comparing the three parentage methods implemented in Sequoia (default, informed, conservative). The total number of parents and the total number of offspring assigned as either adults or parr are listed for sires and dams.

	default		**informed**		**conservative**	
	adult	parr	adult	parr	adult	parr
Parents						
dams	77	90	77	25	77	20
sires	309	138	305	134	301	36
Offspring						
dams	4253	107	4257	26	4255	21
sires	5915	167	5912	165	5912	42

Table S3. Parentage assignment estimates for adult males and mature male parr (MMP) using the three parentage analysis methods implemented in Sequoia (default, informed, conservative). The total number of sires and offspring assigned to either adults or mature male parr are based on estimates from cohort analyses. Age at maturity was estimated by subtracting the back-calculated hatch dates from the cohort year calculated by cohort analysis. Sampled males were assumed to be spawning as adults if the hatch year of offspring at the first reproductive event – the hatch year of male was > the freshwater age; otherwise, they were assumed to be spawning as mature male parr. Sampled male parr were assumed to be spawning as adults if the first reproductive event – hatch year > 3 years; otherwise they were assumed to be spawning as mature male parr. Offspring assigned to mature male parr is also given as a percentage of the total number of assigned offspring.

		default		**informed**		**conservative**	
		sires	offspring	sires	offspring	sires	offspring
Adult	Adults	301	5896	301	5900	301	5909
	Parr	31	33	31	33	36	42
MMP	Adults	14	19	7	11	3	3
	Parr	108	134	104	132	0	0
% offspring MMP			2.5		2.3		0.05

Figure S1. Ridgeline density plot of the distribution of age at maturity (years since hatching) of different age classes of sires and dams as parr for default, informed, and conserved parentage assignment methods.

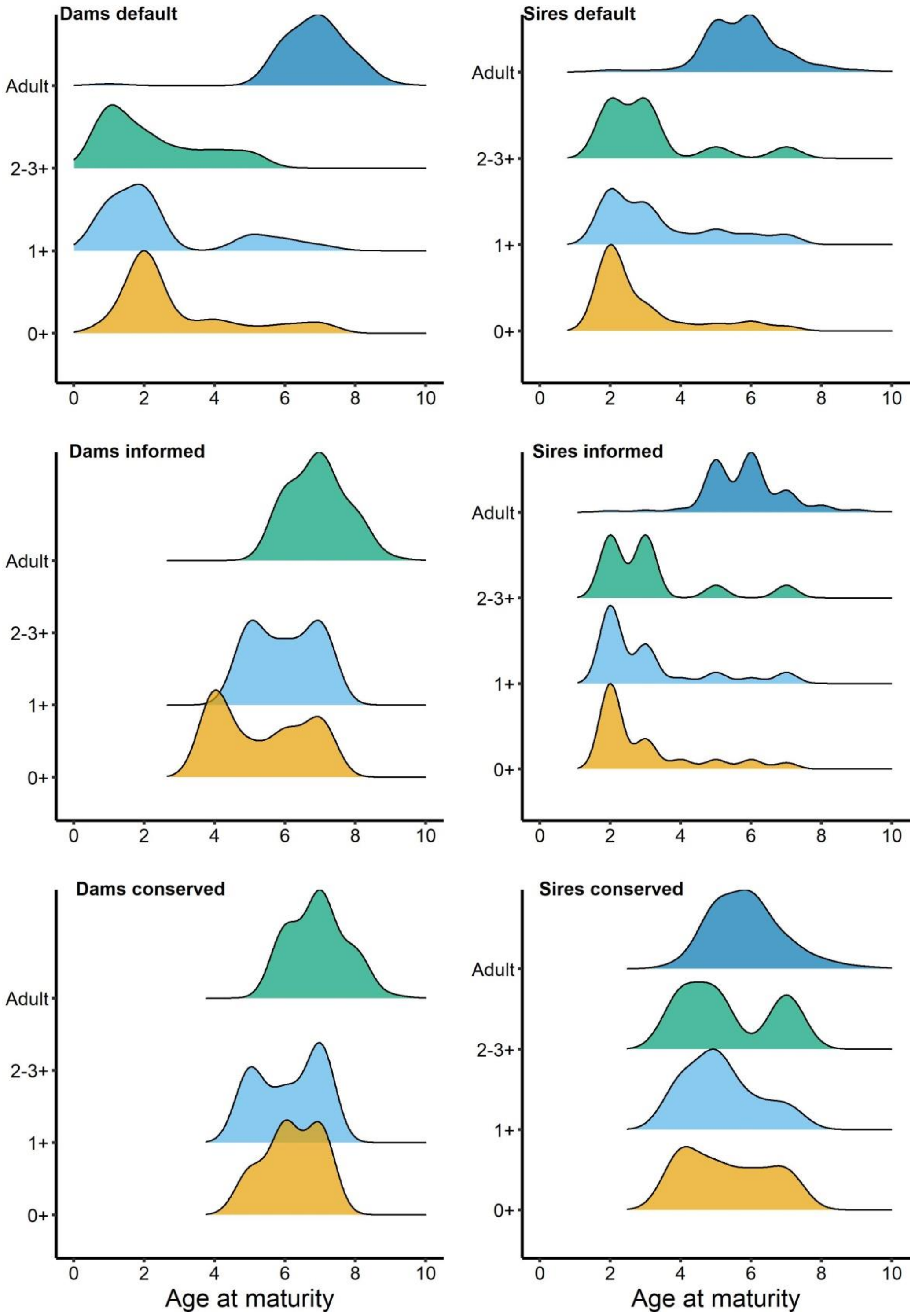


Figure S2. Total number of adults (n = 685, 93 female, 592 male) collected from the lower Utsjoki sampling site each year.

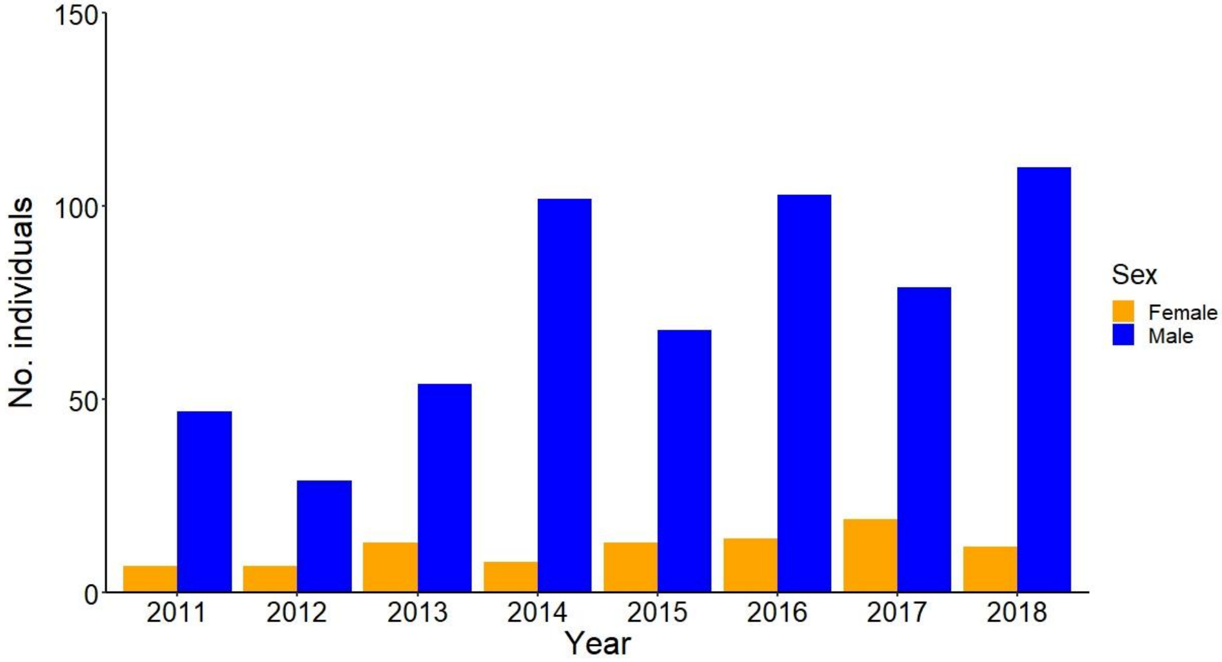


Figure S3. Total number of parr (n = 18,265) collected from the lower Utsjoki sampling site each year and for each age class.

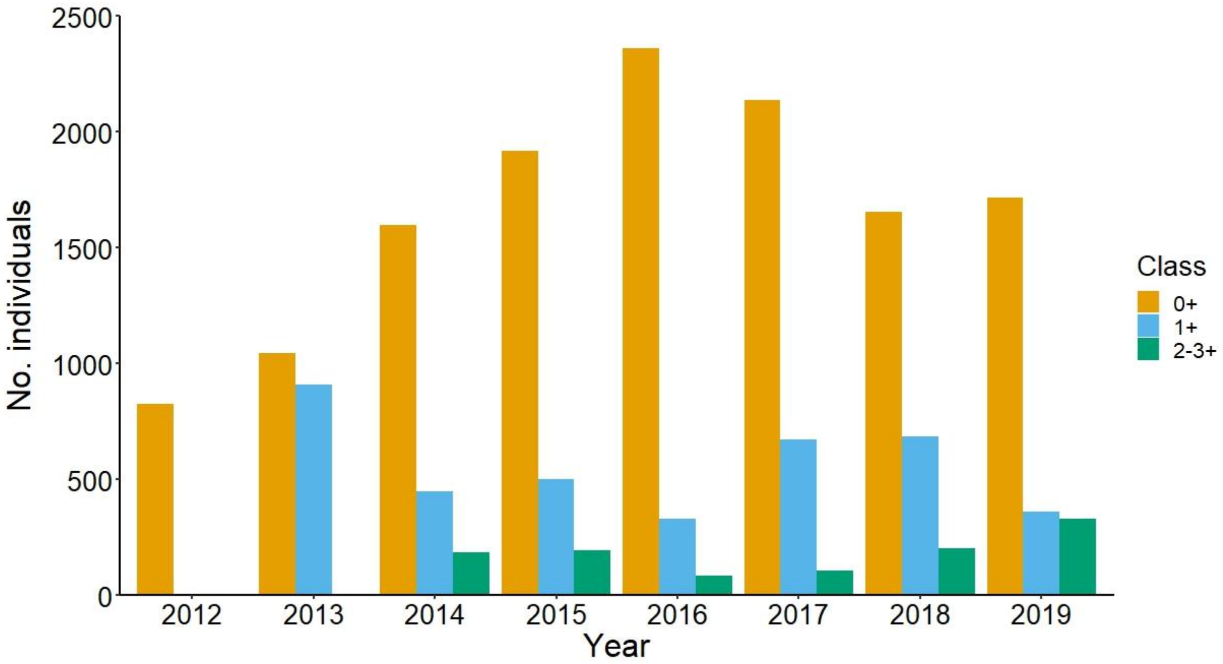


Figure S4. Number of individuals of different parr age classes or adults assigned to dams and sires using the conservative parentage analysis dataset. The distribution did not significantly differ between dams and sires (*n* = 10,230, χ^2 = 1.435, df = 3, p = 0.697).

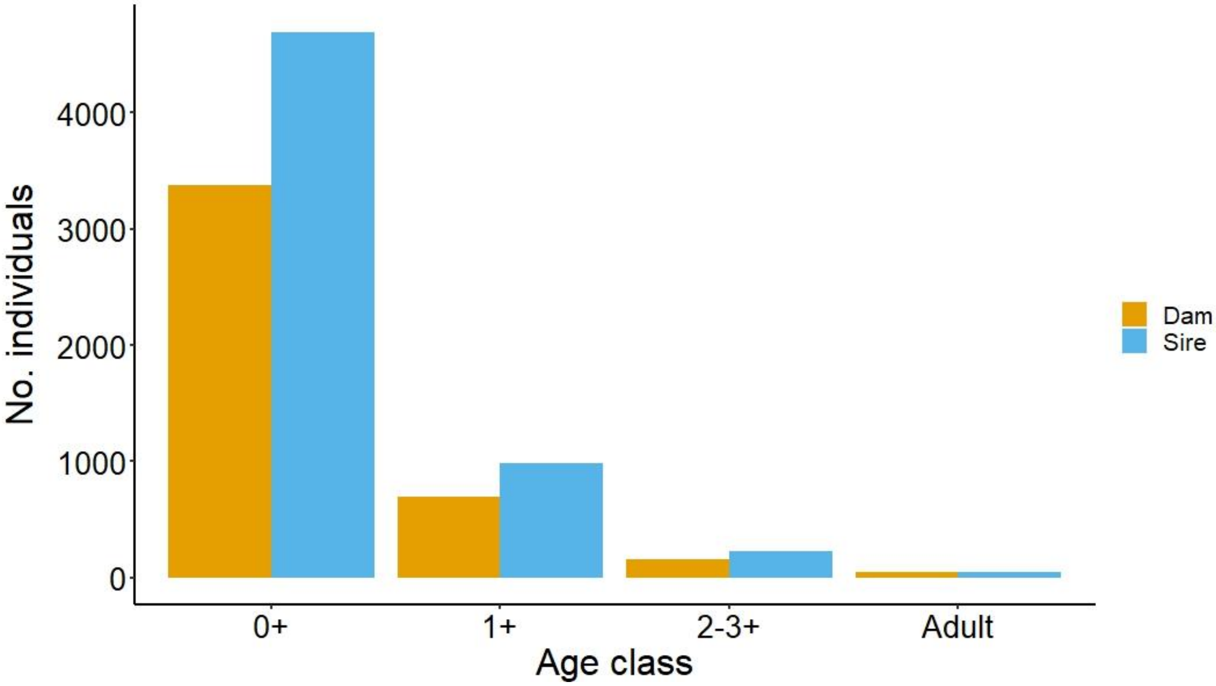


Figure S5. Ridgeline density plot of A) the distribution of 2-3+ parr scale age (years) and B) total length (cm) for female (n = 327, immature male (n = 292) and mature male (n = 28) parr in age class (2014-2019).

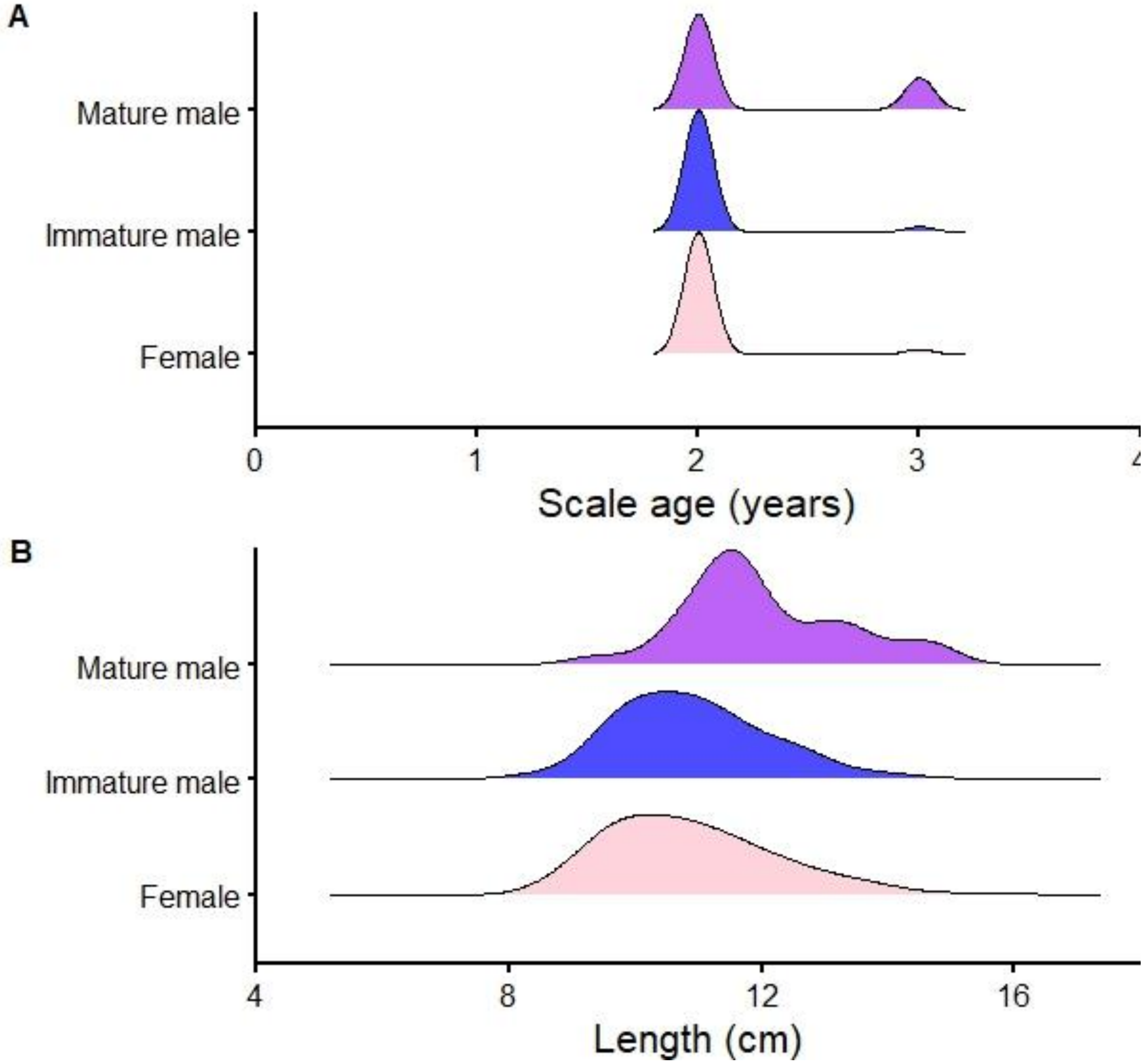
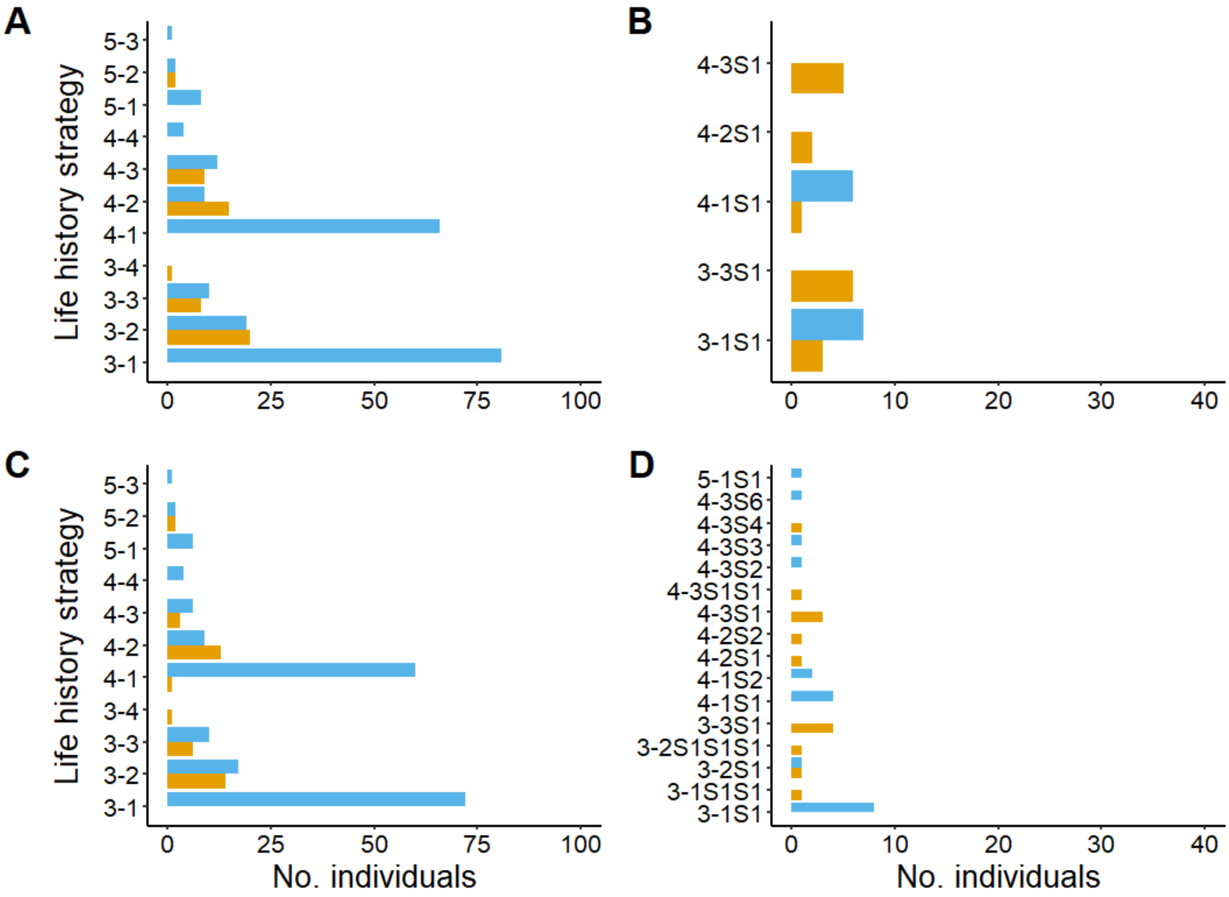


Figure S6. Adult life history strategy frequencies for dams (orange) and sires (blue). A) semelparous scale data only, B) iteroparous scale data only, C) semelparous scale and parentage analysis combined, D) iteroparous scale and parentage analysis combined. Notation: freshwater age – sea age, S pause between multiple mating events followed by number of years between reproductive events. In total, 27 different life history strategies were observed.



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