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DNA parentage analysis reveals inter-annual variation in selection: results from 19 consecutive brood years in steelhead trout

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ABSTRACT

Questions: Given costs and trade-offs, can selection be consistent or strong in animal populations? Is selection on body size and arrival timing, and opportunities for selection, related to indicators of breeding competition (breeding density and sex ratio)?

Methods: Our data set consisted of genetic, phenotypic, and demographic data taken from adults of a small wild population of steelhead trout (*Oncorhynchus mykiss*). We measured the lifetime reproductive success of adults by matching breeding adult offspring to their parents using genetic data and exclusion-based parentage assignment. We calculated the opportunity for selection and estimated selection coefficients using regression analysis.

Results: Selection more often favoured large males and females than small ones, although the strength of selection operating on body size varied among years. Selection on arrival date varied widely in shape, direction, and strength among years. Male-biased sex ratios and greater male breeding density increased the opportunity for selection on males. Higher female breeding density increased the opportunity for selection on females. Sex ratio and breeding density were unrelated to the strength or direction of selection on male or female body size and arrival timing. Selection always favoured large male body size in years when the sex ratio was male biased, but varied greatly in direction among years when the sex ratio was female biased.

Keywords: breeding density, genetic parentage analysis, lifetime reproductive success, *Oncorhynchus mykiss*, selection, sex ratio.

INTRODUCTION

Patterns of lifetime reproductive success are determined by sexual and natural selection during breeding, combined with natural selection on offspring during their lifetime (Clutton-Brock, 1988). Although females and males have fundamentally different strategies for maximizing lifetime reproductive success, both body size and the timing of migration and breeding affect reproductive success for both sexes of many species (Andersson, 1994; Shuster and Wade, 2003).

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Although advantages may exist for smaller individuals, 'bigger' has commonly been thought to be better (Blanckenhorn, 2000; Kingsolver and Pfennig, 2004) in terms of reproductive success. Larger females of many species tend to produce more eggs, if only because their larger body cavity can hold more eggs (Andersson, 1994), and they may also produce larger and hence more viable offspring. Larger males commonly have an advantage in physical competition for mates, or they may be chosen as mates by females (Andersson, 1994), although alternative male morphs occur in many species (Gross, 1996). Kingsolver and Pfennig (2004) reviewed published studies of selection on size and found a preponderance of positive selection coefficients, indicating selection for larger size. However, their data were unable to resolve whether selection consistently favours large size at the species or population level.

Some studies have demonstrated that selection for size may vary in direction among generations (Grant and Grant, 2002), or that bigger is not always better (Quinn *et al.*, 2001). Directional selection must be offset by trade-offs or negatively correlated selection on other traits (Stearns, 1992). For selection on body size, the variability in direction of selection is likely influenced by trade-offs and costs associated with growth or growth rates (Arendt, 1997; Blanckenhorn, 2000; Arnott *et al.*, 2006). Thus we might not expect selection within populations or species to be consistent in direction.

Breeding migration timing is another important trait clearly under some sort of selection pressure. However, expectations of fitness consequences of variation in the timing of arrival on the breeding grounds for migratory animals are more complicated than that for body size. In contrast to body size, no general trend in fitness of individual arrival date (e.g. earlier is better) exists, although some relative patterns exist, such as protandrous arrival timing at breeding sites (Morbey and Ydenberg, 2001). However, substantially less is known about how variation in the timing of arrival on breeding grounds influences individual reproductive success. Consistent directional selection for either earlier or later arrival would have the effect of shifting the migration patterns of the population earlier or later. Since major shifts in migration timing are rare, the timing of migration is generally thought to be under stabilizing selection, with costs and benefits associated with early or late arrival. For example, in the American redstart (*Setophaga ruticilla*), early arrival at breeding grounds benefited both males and females through increased offspring quality, but early arriving individuals suffered in terms of food availability and environmental conditions (see Smith and Moore, 2005, and references therein). However, whether or not selection for migration timing is consistently stabilizing in nature is unknown.

Selection in Pacific salmonids

Pacific salmon (*Oncorhynchus* spp.) are an ideal system for testing the importance of body size and timing of arrival on the breeding grounds to fitness because considerable intra-population variation exists in both of these traits. In particular, previous research has revealed that selection favours large size at the time of breeding. Larger females tend to produce more eggs than smaller females (Beacham and Murray, 1993; Hendry *et al.*, 2001). Large females can bury their eggs deeper in the gravel than smaller females, offering their offspring some extra protection from the scouring effects of high flows and nest site re-use (Steen and Quinn, 1999; Essington *et al.*, 2000; Lapointe *et al.*, 2000). Females compete for access to spawning habitat and large females tend to defeat smaller females (Fleming and Gross, 1994). Large size confers fitness advantages for males as well because larger males exclude smaller males from

physical proximity to females in dominance contests (Keenleyside and Dupuis, 1988; Quinn and Foote, 1994; Dickerson *et al.*, 2002).

Furthermore, selection on maternal body size and breeding date may extend beyond mating due to maternal effects. Large females produce larger eggs (Beacham and Murray, 1993), which produce larger fry (Beacham and Murray, 1990). Progeny of early-spawning females also tend to be larger at a given date due to their earlier emergence and onset of feeding (Beacham and Murray, 1990; Einum and Fleming, 2000), although the strength of this relationship will likely vary extensively in species with extended freshwater residence, due to territoriality (Chandler and Bjornn, 1988), predation (Brännäs, 1995), and food availability (Keeley and McPhail, 1998). Freshwater mortality tends to be size-selective (Smith and Griffith, 1994; Quinn and Peterson, 1996; Einum and Fleming, 1999, 2000) and generally, but not always (Good *et al.*, 2001; Connolly and Petersen, 2003), favours larger individuals. If food is abundant, the maternal effects of egg size and spawning date diminish over time and fry size is eventually uncoupled from these maternal traits (Kinnison *et al.*, 1998; Heath *et al.*, 1999). Survival during seaward migration may be size-related (Collis *et al.*, 2001), and larger juveniles within a given cohort are more likely to survive at sea than smaller ones (Ward and Slaney, 1988; Ward *et al.*, 1989; Holtby *et al.*, 1990; Henderson and Cass, 1991; Yamamoto *et al.*, 1999; Moss *et al.*, 2005).

Trade-offs related to body size and arrival timing have been indicated in salmonids (Hendry *et al.*, 2003; Quinn *et al.*, 2005). For example, Holtby and Healey (1986) found that, in some years, offspring of smaller coho salmon (*O. kisutch*) survived better than those of larger parents. Carlson *et al.* (2004) found that large juveniles had lower survival than smaller individuals. The ability of large male pink salmon to exclude smaller males varied intra-seasonally, depending on the number of competitors (Dickerson *et al.*, 2002), which in turn depended on the timing of spawning (Quinn *et al.*, 1996). The ability of larger females to provide high-quality habitat for their offspring is to some extent a function of breeding timing (Fukushima *et al.*, 1998), but also of the predictability of environmental conditions (Seiler *et al.*, 2002). Long-term studies of selection in salmonids are lacking, however, making any inference of consistency of the nature of selection impossible.

Given, then, the apparent variation in selection and costs and benefits to growth or migration timing, can selection be consistent in direction, shape, and strength? Using 19 years (or about five generations) of data on the lifetime reproductive success of individuals from a small, wild, naturally spawning and unexploited population of steelhead trout (*O. mykiss*), our goals were: (1) to estimate selection on parental body size and timing of arrival on breeding grounds using numbers of returning adult offspring; (2) to determine patterns of selection in terms of direction, shape, and strength across 19 brood years; (3) to determine how the opportunities for selection on males and females are related to the sex ratio and density during reproduction; and (4) to determine how the direction and strength of selection on parental body size and timing of arrival are related to the sex ratio and density during reproduction.

MATERIALS AND METHODS

Sampling site and sample collection

Adult steelhead (anadromous rainbow trout) were sampled from Snow Creek, Washington, by employees of the Washington Department of Fish and Wildlife (WDFW). Most fish were captured in a trap at a permanent weir located 0.95 km from the mouth of the creek (see map in Seamons *et al.*, 2004b). Returning adults have been captured every year since the weir was

installed in 1977, although a few adults spawn below the weir. For all adults captured, body length from snout to the fork of the tail (fork length), sex, date, and origin (hatchery/wild) were recorded. Scales removed for age analysis were dried in a folded piece of paper, and later mounted on gummed cards for examination. These and other scales were made available for DNA extraction and analysis. Scales were available for some fish before 1982 but the collections were incomplete, so scales from adults from only 1982 through 1996 were used. From 1997 to 2004, fin tissue samples were also taken and stored in 95% ethanol for DNA analysis. All captured arriving adults were marked just before being released upstream of the weir. Any unmarked adults captured during downstream migration were sampled as described above. As a matter of current WDFW policy, all stray hatchery steelhead, identified by a missing adipose fin, are killed at the weir. However, in the years covered by our data set, a few hatchery adults bypassed the weir during high flow events or were purposely released (upstream or downstream) after capture (8.5% of all adults). Cutthroat trout (*O. clarki clarki*) were also sampled and included in parentage analysis (see below) because hybridization with steelhead can occur (Young *et al.*, 2001) and visual identification of adult hybrids is difficult (Baumstieger *et al.*, 2005). Finally, the Snow Creek steelhead population is maintained for research and monitoring purposes and so all directed fishing is prohibited. The timing of migration by adults makes it very unlikely that they are intercepted to any significant extent in any distant fisheries.

Molecular analyses

DNA was extracted from scales and fin clips using Qiagen DNeasy Tissue 96-well extraction kits (Qiagen Inc., Valencia, CA, USA) following the manufacturer's guidelines. Polymerase chain reactions to amplify 12 microsatellite loci were carried out as described in Seamons *et al.* (2004b). For scale samples, the total number of cycles in the thermalcycler profile was raised from 25 to 30. Alleles were visualized and size fractionated using methods described in Seamons *et al.* (2004b).

Genetic data were tested for deviations from expected Hardy-Weinberg proportions (HWE) with a two-tailed test using the Markov Chain method implemented in Genepop v3.4 (Raymond and Rousset, 1995). The significance of the probability values was adjusted using the sequential Bonferroni approach of Rice (1989). Observed and expected values of heterozygosity and F_{IS} were also calculated using Genepop v3.4. Global and locus specific exclusion probabilities were calculated per brood year using Cervus 2.0 (Marshall *et al.*, 1998).

Parentage assignment

Most steelhead in this population were 4 years old at maturity, so complete data were obtained for parental brood years 1982–2000 as adult offspring returning in 1986–2004. Some spawning occurs in Snow Creek below the weir, and some males and females may spawn both below and above the weir (Thom Johnson, WDFW, personal communication). Therefore, all adults that were captured and sampled in Snow Creek were used as potential parents. This included putative stray hatchery fish, which might have spawned below the weir even if they were subsequently killed. Adult cutthroat trout (37 total) and large (> 250 mm fork length) juvenile steelhead of questionable maturity (~60 total) were also included as potential parents. These latter fish were possibly *O. mykiss*/*O. clarki* hybrids or steelhead exhibiting an atypical life history.

Parents were assigned to individual adult offspring using the principles of exclusion. That is, only adults that shared at least one allele at each locus with the putative offspring were called the true parents. When two adults genetically matched a putative offspring, they had to match the opposite allele at each locus. All non-matching adults were considered genetically excluded from parentage. If all adults were excluded from parentage, it was assumed that the true parents were not sampled. Since individuals may spawn in more than one year, all adults returning in the years before the brood year of the adult offspring were considered possible parents when performing parentage analysis. Because we wished to minimize the error of assigning adults as parents that were not actually parents, no accommodation was made for genotyping errors. [See Seamons *et al.* (2004a) for more information on these methods.]

In some cases, all adults except one were excluded as parents. The chance of finding a single genetically matching adult is higher than finding two genetically matching adults simultaneously (Marshall *et al.*, 1998). We calculated the largest probability of a random match of a single adult using equation (1) in Seamons *et al.* (2004a). This value puts an upper bound on the probability of matching a single adult by chance.

Statistical analyses

The number of adult parents used in selection analysis was different than the actual number that returned in some brood years due to missing data on length or arrival date. If the arrival date could be estimated to within a day the estimated date was used (for example, if the trap was not fishing for 3 days, the middle date was the estimate), but if the date was more ambiguous it was left blank. Our data set included all adults regardless of missing data, but the analyses were restricted to those fish for which the necessary data were available. In addition, there was no variation in reproductive success for one or the other sex in some brood years (e.g. no adult offspring were detected for any of the anadromous males in two brood years). These data were included in the pooled analysis, but could not be analysed by individual brood year.

Males and females were expected to have different strategies to maximize reproductive success, so their data were analysed separately. Nineteen males (4.7%) and 54 females (12.4%) spawned in more than one year. These reproductive events were not independent because the same parent was involved. However, the suite of competitors and potential mates differed among years, as did the physical environment (e.g. temperature and flow). Therefore, for statistical purposes we treated repeat spawning individuals as different individuals in each of their return years.

The opportunity for sexual selection ($I_{\mathbf{males}}$ or $I_{\mathbf{females}}$) was calculated as the variance in male or female reproductive success divided by the squared mean male or female reproductive success within a brood year (Shuster and Wade, 2003). The relationship between the sex ratio or spawning density and I was determined by least squares regression. $I_{\mathbf{males}}$ was natural log transformed to correct for heteroscedasticity.

Selection on parental traits was investigated using least squares linear regression analysis as outlined in Lande and Arnold (1983) and summarized by Brodie *et al.* (1995). The measure of fitness was the number of returning adult offspring per parent (lifetime reproductive success). We included two traits in our analysis: fork length and arrival date. Both traits were standardized by subtracting the within-sex, within-brood year mean trait value and dividing by the within-sex, within-brood year standard deviation. Quadratic and

cross-product terms were calculated using the trait value relative to the within-sex, within-brood year mean as the raw trait value. Squared terms and the cross-product term were both calculated and then standardized to a mean of zero and a standard deviation of unity. We analysed the pooled data for adults returning in 1982–2000, including brood year as a fixed effect using generalized linear model (GLM) analysis. The brood year term was non-significant ($P > 0.10$) in all tests in which it was included, so it was dropped from the analyses and the data were analysed using least squares linear regression.

Selection coefficients were generated from four sets of regressions. First, total selection on a trait (selection differential), including direct selection on that trait plus any indirect selection on correlated traits, was calculated as the regression coefficient from a simple linear regression. Second, direct selection (selection gradient) acting on each of the focal traits was calculated as the partial regression coefficient for each trait from a multiple regression including both traits. Third, quadratic (non-linear) selection differentials were calculated as two times the partial regression coefficient for the squared term from a multiple regression including each trait and its squared value. Fourth, quadratic selection gradients and the non-linear, bivariate selection gradient were calculated as two times the partial regression coefficient for the squared term and the cross-product term from a multiple regression including each trait, its squared value, and the cross-product term. The estimates of direct selection included any indirect selection from unmeasured phenotypic traits that were correlated with body size and arrival date.

To visualize shapes of selection surfaces, we calculated univariate cubic splines (Schluter, 1988) for length and arrival date for males and females. Values of lambda, a smoothing factor, were calculated by running simulations with all years of data for each trait of each sex. The lambda value used to generate all cubic splines for a particular trait of a particular sex was the average of the minimum lambda generated by all simulations among years of that trait in that sex.

To facilitate comparison with other published data sets, we also calculated mean-standardized selection coefficients [elasticities (Hereford *et al.*, 2004)]. Mean-standardized coefficients are interpreted as the proportional change in mean fitness with change in the mean trait value; however, only elasticities for length have this straightforward interpretation. Traits with an arbitrary origin, such as our measure of arrival date, do not represent true ratios (Hereford *et al.*, 2004). Elasticities calculated for length were unrealistic (much larger than unity), so the results of this analysis are not shown.

We used one-tailed chi-squared goodness-of-fit tests to test the null hypothesis that selection among brood years was random with respect to direction. Specifically, the number of positive or negative selection differentials or gradients for a parental trait, across brood years, was no different than half positive and half negative.

We also examined trends in the strength of selection across brood years using Spearman's rank correlation. The strength of sexual selection for males was expected to be higher in brood years when the sex ratio was male-biased and lower in brood years when females were more numerous, relative to males. For the purposes of charting the relationship between the sex ratio and strength of selection, the sex ratio was calculated as the bias in sex ratio, measured as the number of females minus the number of males, divided by whichever was smaller, number of males or number of females. In this case, a year with twice as many males as females would have a value of −1 and a brood year with twice as many females as males would have a value of +1.

Since the number of days spent upstream was unknown for adults of all brood years used

in this study, we used data from previous (1977–1989) and subsequent (2001–2004) brood years to estimate a population average. All adults returning in 1977–1989 were physically tagged by WDFW employees; number of days spent upstream was known because tagged fish were observed leaving on a particular date. All adults returning in 2001–2004 were sampled for tissue as they arrived and again as they left; arrival and exit dates of adults were determined by matching genotypes. The number of days spent upstream was simply the departure date minus the arrival date. We then took the average of the yearly average number of days spent upstream of the weir by adults in 1977–1989 and 2001–2004 to obtain the population average number of days spent upstream. This population average was then added to the arrival date for adults in brood years 1982–2000 to estimate outmigration date. Next, we calculated the number of adults upstream of the weir on any one day of the spawning period by subtracting the number of adults that left from the number that arrived on any one day. Finally, we used the value from the date with maximum number of adults upstream of the weir as our estimate of spawning density. Given that the total spawning area is fixed, and assuming that the quantity and quality of suitable spawning habitat was similar among years, the strength of selection for females was expected to be higher in years when many females returned (higher spawning density). Since competition for females was expected to be more intense in years with higher male spawning density, the strength of selection for males was expected to be higher in years with higher spawning density. Using Spearman's rank correlation, we tested the null hypothesis of no relationship between either sex ratio or estimated spawning density and the strength of selection on females and males.

RESULTS

Demography

A total of 921 anadromous, wild and hatchery steelhead returned and were sampled at Snow Creek in brood years 1982 through 2000 (Table 1), and this small population was characterized by great variation among years in many attributes. The number of adults returning in any one year varied from 6 (brood year 1990) to 143 (brood year 2000) (Table 1) with an average of 49 adults per brood year. Overall, more females than males returned to Snow Creek (1.15 females per male), but the sex ratio varied considerably among brood years. In the extreme cases, nearly twice as many males as females returned in 1998, whereas in 1986 nearly three times as many females as males returned.

Body length also varied in Snow Creek steelhead (from 330 to 885 mm in males, and from 302 to 860 mm in females). The annual average male length ranged from 574 mm (brood year 1993) to 676 mm (brood year 1996), and female length ranged from 619 mm (brood year 2003) to 693 mm (brood year 1990) (Table 1). Finally, a large amount of natural variation was apparent in arrival timing. Individuals arrived at the breeding grounds as early as November and as late as the end of April. Neither males nor females consistently arrived before the other sex within and among brood years. The peak arrival date for both sexes was generally near the second week of March (Table 1).

Population genetics

Forty-one of 228 individual tests (~18%) of loci showed significant ($\alpha = 0.05$) departures from HWE (Table A1; see http://www.evolutionary-ecology.com/data/2134Appendix.pdf).

Table 1. For each brood year, the number of adult male and female steelhead returning to Snow Creek, Washington (with the number of hatchery origin adults, #HO), mean fork length (L_{mean}) with one standard deviation (rounded to the nearest whole millimetre or day), and mean arrival date (AD_A) with one standard deviation for 19 parental brood years

	Males			Females		
Brood year	*N* (#HO)	L_{mean} (1SD)	AD_A (1SD)	*N* (#HO)	L_{mean} (1SD)	AD_A (1SD)
1982	28 (6)	606 (93)	27 Feb (19)	37 (5)	642 (74)	10 Mar (18)
1983	21 (3)	614 (125)	24 Feb (26)	18 (2)	643 (48)	14 Feb (20)
1984	76 (23)	618 (84)	12 Mar (29)	57 (10)	640 (58)	8 Mar (27)
1985	48 (4)	639 (75)	4 Apr (11)	35 (1)	674 (52)	2 Apr (12)
1986	8 (3)	581 (116)	22 Mar (16)	21 (1)	650 (61)	9 Mar (10)
1987	28 (6)	634 (87)	27 Feb (27)	34 (2)	639 (42)	21 Feb (24)
1988	10 (3)	640 (57)	19 Mar (11)	16 (0)	684 (52)	23 Mar (11)
1989	12 (0)	634 (44)	2 Mar (5)	10 (0)	634 (106)	4 Mar (5)
1990	2 (1)	623 (10)	15-Feb (17)	4 (1)	693 (106)	28 Feb (33)
1991	7 (0)	603 (34)	2 Mar (15)	11 (0)	619 (49)	10 Mar (17)
1992	14 (1)	653 (51)	8 Mar (12)	23 (1)	636 (42)	14 Mar (13)
1993	6 (1)	574 (80)	18 Mar (15)	12 (0)	655 (66)	13 Mar (6)
1994	14 (6)	662 (59)	12 Mar (18)	15 (2)	646 (66)	14 Mar (23)
1995	12 (0)	650 (28)	24 Mar (22)	14 (0)	639 (26)	13 Mar (10)
1996	17 (1)	676 (57)	NA	24 (1)	645 (45)	NA
1997	11 (1)	623 (103)	19 Mar (4)	20 (0)	692 (62)	19 Mar (4)
1998	35 (0)	650 (32)	12 Mar (16)	18 (0)	638 (68)	17 Mar (15)
1999	30 (5)	647 (105)	8 Mar (40)	30 (2)	658 (41)	5 Mar (33)
2000	65 (1)	645 (56)	12 Mar (22)	78 (0)	641 (48)	17 Mar (20)
Total	444 (65)			477 (28)		

NA: In brood year 1996, the upstream trap at Snow Creek weir was not fishing through the 8th of April. All adults sampled this year were sampled as outmigrants (kelts).

Of the 41 significant locus tests, 37 had positive F_{IS} values, indicating a deficiency of heterozygotes. Of these 37, 18 tests had F_{IS} values greater than 0.10. Twelve of these 18 tests were locus *Omy77*, a locus whose pattern of allelic inheritance was consistent with a relatively high frequency of null alleles. *Omy77* alone accounted for 68% of the locus tests significantly out of HWE. The remaining significant tests were spread among all other loci and years except *Oki23*, which had no significant tests in any year. Six of 228 tests remained significant after sequential Bonferroni corrections. These six were for *Omy77*, *Ots107*, and *Ots108*. Since we were amplifying DNA from scale samples, it is likely that many of the deviations were due to upper allele dropout, although a qualitative scan of parentage assignments suggested that *Ots108* and *One108* had low-frequency null alleles (several matching potential parents that were homozygous, as was the offspring). We did not calculate allelic genotyping error with this data set, but we did not expect it to be substantially different from 0.6% per allele, as reported for a subset of samples (Seamons *et al.*, 2004a).

Average observed heterozygosity across all brood years and all loci was 0.88, ranging from a low of 0.52 in brood year 1995 (*Omy77*) to a high of 1.00 (several brood years and loci) (Table A1; see http://www.evolutionary-ecology.com/data/2134Appendix.pdf). The global exclusion probability across all brood years and all loci was 1.00 for both parents.

Across loci, the exclusion probability for all brood years combined ranged from a low of 0.641 in *Omy1004UW* to a high of 0.936 in *Ots107*. Within brood years across loci, the exclusion probabilities ranged from a low of 0.502 in *Omy77* in brood year 1990 to a high of 0.926 in *Ots107* in brood year 2000.

Parentage

Null alleles at *Omy77* were expected and taken into account in parentage assignment. When the offspring in question was homozygous at *Omy77* and one potential parent mismatched, but was also homozygous, while the other putative parent matched, it was assumed that the mismatching adult was heterozygous with a null allele. There were also a few cases where *Omy77* would not amplify in an individual, suggesting a double null genotype. This hypothesis was further supported by parentage data; putative parents of these individuals appeared to be heterozygous with a null allele or putative offspring appeared to have inherited a null allele. Null alleles in *Ots108* and *One108* were taken into account in parentage assignments as described for *Omy77*.

We did not expect to be able to assign parents to cutthroat, hatchery origin steelhead (strays, by definition) or to steelhead spawned before 1982. After considering species, origin, and parental brood year (i.e. offspring age at return), we successfully assigned at least one parent to 65.7% (494) of all returning adults. Of these fish, 36.8% (182) were assigned both parents, 53.2% (263) were assigned only a mother, and 9.9% (49) were assigned only a father. We had the highest probability of randomly matching single parents to offspring in brood year 1990 (0.013), the year when only six fish returned to spawn. The next lowest probability of a random match was 0.006 (brood year 1988), which was more representative of the probabilities seen in all other brood years (mean = 0.002). Successful parental assignment to known-age adult offspring varied by brood year from 48% to 95% (mean = 73%) across all 19 parental brood years (1982–2000). As expected, assignment success was lower for adults from brood years when the trap was compromised by unusually high stream-flows.

Reproductive success was skewed for both males and females; most adults produced no adult offspring (Fig. 1a, b). Two females produced the highest absolute number of offspring ($n = 8$; Fig. 1a), but a male produced the highest relative number of offspring (38 times the mean of that brood year; Fig. 1b).

Selection analysis

The opportunity for sexual selection in males (I_{males}) increased with an increasingly male-skewed total observed sex ratio ($r^2 = 0.31$, $P = 0.001$ – ln transformed data, Fig. 2a – untransformed data), and with an increase in the total number of males that returned ($r^2 = 0.74$, $P < 0.001$; Fig. 2c). The opportunity for sexual selection for females (I_{females}) was unrelated to the observed total sex ratio ($P = 0.95$, Fig. 2b), but was higher in years when more females returned (linear regression, $r^2 = 0.30$, $P = 0.009$; Fig. 2d).

Overall, larger and earlier-arriving males had higher reproductive success (i.e. produced more adult offspring) than did smaller and later-arriving males in terms of total and direct linear selection ($P < 0.05$, pooled data; Table 2). However, these relationships explained very little of the variation ($r^2 < 0.03$, all tests of pooled data). Larger females also had more offspring than did smaller females overall ($P < 0.05$, total and direct linear selection – pooled data; Table 3), but, as with males, length explained very little of the variation in

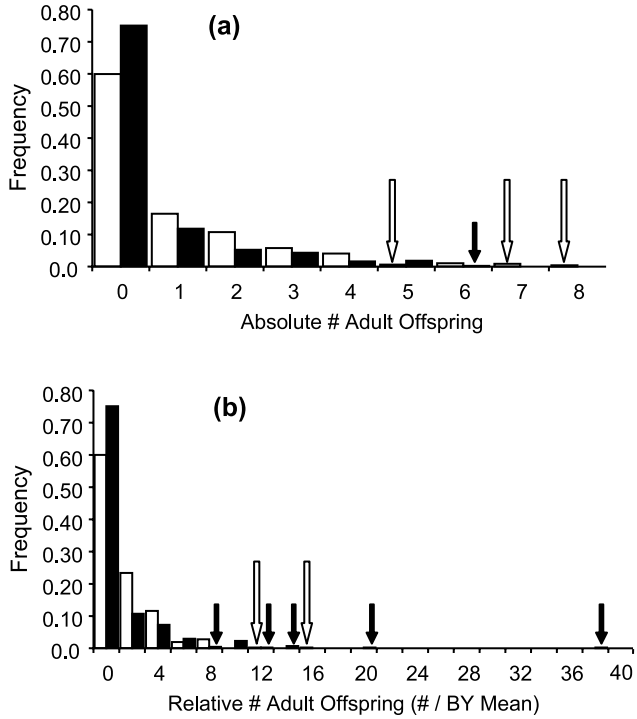


Fig. 1. Histograms showing the frequency of maternal (□) and paternal (■) half-sib families in terms of both the absolute number of adult offspring per family (a) and relative to the within-brood year average number of adult offspring per family (b). Arrows point to non-zero values that are difficult to distinguish from the axis (below 0.01).

offspring number ($r^2 < 0.02$, tests of pooled data). Female arrival date was unrelated to the number of offspring produced ($P > 0.10$).

The shape, strength, and direction of estimates of selection varied widely among brood years for both males and females (Fig. 3; Tables 2 and 3). However, patterns of selection on female length were remarkably similar among years for a particular shape of selection (Fig. 3, plots g–i). For example, the disruptive selection that was evident for brood years 1985, 1987, 1994, and 1996 (Fig. 3, plot i) was similar in intensity and shape among those years. The shape of selection on male length and arrival date and selection on female arrival date appeared more variable (Fig. 3, plots a–f, j–m). Selection for male length was usually positive among brood years, favouring larger individuals (χ^2 test, $P < 0.05$, total and direct linear selection; Fig. 4), as was selection for female length ($P < 0.05$, total and direct linear selection; Fig. 4). No consistent pattern was found for all other selection coefficients – that is, they showed no difference from expectations of half positive and half negative. Selection appeared stronger on length in males than females (Mann-Whitney test, absolute values: $P < 0.05$, differentials; $P < 0.10$, gradients), and selection on male arrival date appeared to be stronger than that on female arrival date (Mann-Whitney test, absolute values of coefficients: $P < 0.10$, gradients only).

There was no correlation between the observed sex ratio and the strength of total or direct selection on male length; however, selection differentials and gradients appeared to

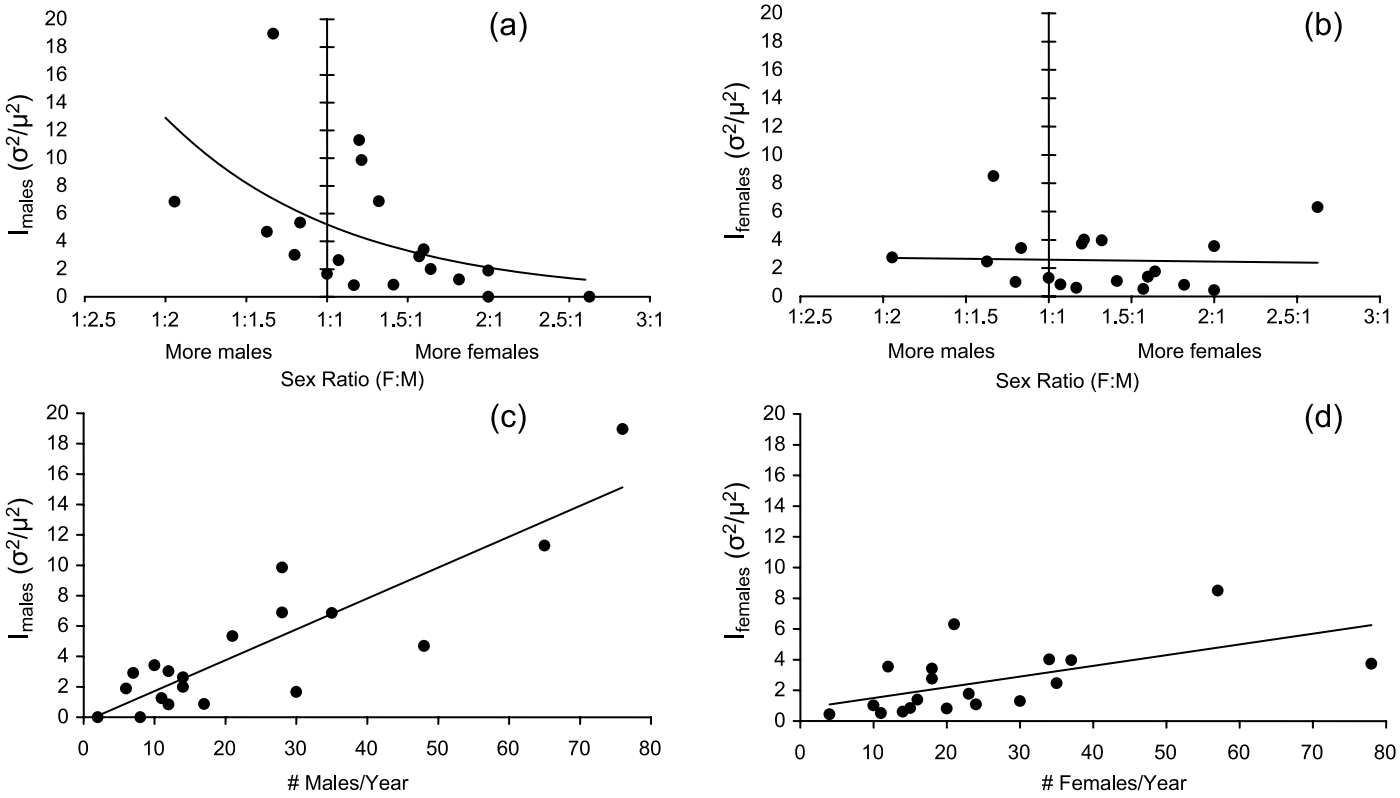


Fig. 2. The relationship between the annual observed sex ratio or observed number of adults and the opportunity for sexual selection (σ^2/μ^2) for adult male (a and c) and female (b and d) steelhead from Snow Creek, Washington. Lines are predicted from least squares regression analysis. The response variable (I_{males}) for male sex ratio analysis was natural log transformed to correct for heteroscedasticity. Data shown are untransformed.

Table 2. Directional, univariate quadratic, and bivariate quadratic selection differentials and gradients (1 standard error) for fork length and arrival date of adult male Snow Creek steelhead in 19 brood years

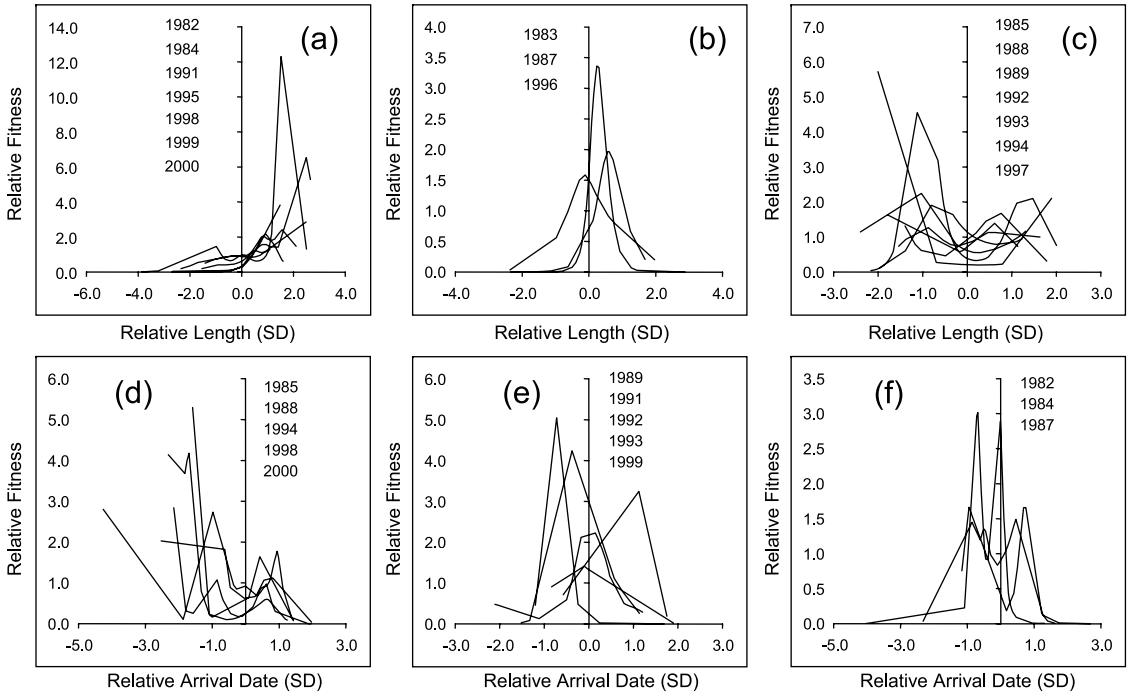
Brood year	Differentials				Gradients				
	Length	Date	Length^2	Date^2	Length	Date	Length^2	Date^2	Length*Date
1982	0.193	−0.071	0.316	−0.282	0.181	−0.077	0.137	−0.354	−0.159
	(0.132)	(0.142)	(0.256)	(0.155)	(0.141)	(0.140)	(0.262)	(0.162)	(0.182)
1983[a]	0.181	NA	−0.379	NA	NA	NA	NA	NA	NA
	(0.159)		(0.184)						
1984	0.062	−0.065	0.125	−0.265	0.080	−0.082	0.262	−0.270	−0.136
	(0.078)	(0.078)	(0.092)	(0.097)	(0.080)	(0.080)	(0.105)	(0.107)	(0.099)
1985	0.126	−0.159	0.054	0.020	0.098	−0.140	0.133	0.143	0.177
	(0.101)	(0.100)	(0.106)	(0.126)	(0.102)	(0.102)	(0.115)	(0.182)	(0.167)
1986[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1987	0.039	−0.115	−0.248	−0.043	0.062	−0.126	−0.218	−0.101	−0.106
	(0.143)	(0.141)	(0.144)	(0.160)	(0.146)	(0.146)	(0.176)	(0.175)	(0.172)
1988	−0.176	−0.263	1.121*	0.239	−0.048	−0.238	0.933	−0.345	0.363
	(0.242)	(0.232)	(0.150)	(0.244)	(0.293)	(0.293)	(0.415)	(0.266)	(0.501)
1989	0.011	0.148	−0.186	−1.221***	0.020	0.150	−0.345	−1.246	−0.132
	(0.223)	(0.218)	(0.238)	(0.294)	(0.231)	(0.231)	(0.406)	(0.395)	(0.419)
1990[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1991	0.528***	0.138	0.476	−2.761	0.552***	0.202	1.163	NA	−0.363
	(0.228)	(0.322)	(0.225)	(0.812)	(0.236)	(0.236)	(1.101)		(0.592)
1992[c]	−0.080	−0.282	0.200	0.071	−1.101**	0.589	NA	NA	NA
	(0.170)	(0.371)	(0.209)	(0.488)	(0.292)	(0.292)			
1993[c]	−0.189	−0.200	0.222	−0.633	−0.128	−0.147	NA	NA	NA
	(0.301)	(0.299)	(0.447)	(0.564)	(0.372)	(0.372)			
1994	0.032	−0.187	0.148	0.052	0.055	−0.193	0.096	0.515	−0.731
	(0.174)	(0.165)	(0.182)	(0.183)	(0.173)	(0.173)	(0.199)	(0.293)	(0.380)
1995[a]	0.226	NA	0.185	NA	NA	NA	NA	NA	NA
	(0.135)		(0.174)						
1996[a]	0.002	NA	−0.479**	NA	NA	NA	NA	NA	NA
	(0.124)		(0.112)						
1997[d]	0.094	0.174	−0.380	NA	0.173	0.233	−0.262	NA	0.375
	(0.172)	(0.166)	(0.195)		(0.176)	(0.176)	(0.202)		(0.179)
1998	0.243**	−0.266**	0.156	0.398	0.225***	−0.249**	−0.182	0.406	−0.469***
	(0.118)	(0.117)	(0.140)	(0.159)	(0.112)	(0.112)	(0.149)	(0.148)	(0.129)
1999	0.132	0.025	−0.190	−0.496	0.131	0.018	−0.238	−0.529	0.092
	(0.110)	(0.113)	(0.141)	(0.157)	(0.113)	(0.113)	(0.173)	(0.166)	(0.211)
2000	0.067	−0.056	0.044	0.073	0.062	−0.050	0.104	0.092	0.224
	(0.091)	(0.091)	(0.114)	(0.107)	(0.092)	(0.092)	(0.132)	(0.119)	(0.149)
Pooled	0.096*	−0.088**	0.043	−0.081	0.090*	−0.091*	0.109	−0.090	−0.008
	(0.032)	(0.035)	(0.034)	(0.038)	(0.035)	(0.035)	(0.038)	(0.038)	(0.036)

NA: data not available for the following reasons: [a] Little or no arrival date data available. [b] No variation in relative fitness. [c] No variation in relative fitness in fathers with date data. [d] Date^2 and Date values exceeded the collinearity threshold in the statistical analysis.
* $P < 0.01$, ** $P < 0.05$, *** $P < 0.10$.

Table 3. Directional, univariate quadratic, and bivariate quadratic selection differentials and gradients (1 standard error) for fork length and arrival date of adult female Snow Creek steelhead in 19 brood years

Brood year	Differentials				Gradients				
	Length	Date	Length^2	Date^2	Length	Date	Length^2	Date^2	Length*Date
1982	−0.028	0.061	−0.220	0.324	−0.082	0.053	0.134	0.273	−0.509**
	(0.118)	(0.122)	(0.136)	(0.137)	(0.123)	(0.123)	(0.159)	(0.139)	(0.120)
1983	0.009	0.411***	−0.766**	0.330	−0.090	0.405	−0.132	0.301	−0.203
	(0.183)	(0.215)	(0.161)	(0.227)	(0.230)	(0.230)	(0.493)	(0.362)	(0.446)
1984	0.150	−0.008	0.149	−0.126	0.152	−0.021	0.164	0.087	−0.258
	(0.095)	(0.098)	(0.099)	(0.115)	(0.097)	(0.097)	(0.102)	(0.157)	(0.143)
1985	0.211**	−0.009	0.596**	−0.080	0.211***	0.008	0.675**	0.151	0.144
	(0.103)	(0.109)	(0.132)	(0.136)	(0.105)	(0.105)	(0.144)	(0.129)	(0.124)
1986	0.067	−0.072	−0.584	−0.966	0.096	0.006	−0.783	−1.556	0.543
	(0.186)	(0.192)	(0.264)	(0.317)	(0.253)	(0.253)	(0.314)	(0.875)	(0.859)
1987	0.168	0.015	0.402	−0.162	0.174	0.040	0.492	−0.213	0.056
	(0.122)	(0.126)	(0.129)	(0.163)	(0.125)	(0.125)	(0.154)	(0.161)	(0.163)
1988	−0.005	0.064	−0.377	−0.058	0.038	0.083	1.503***	0.590	2.136**
	(0.141)	(0.140)	(0.169)	(0.154)	(0.169)	(0.169)	(0.390)	(0.171)	(0.400)
1989	0.346**	−0.176	0.265	0.068	0.336**	−0.154	0.127	−0.141	−0.144
	(0.106)	(0.149)	(0.119)	(0.220)	(0.097)	(0.097)	(0.145)	(0.168)	(0.141)
1990[a]	0.259	0.423*	−0.373	−0.426**	0.030	0.406**	NA	NA	NA
	(0.237)	(0.018)	(0.318)	(0.004)	(0.007)	(0.007)			
1991	0.056	0.056	−0.094	−0.256	0.064	0.064	0.184	−0.531	−0.407
	(0.128)	(0.128)	(0.187)	(0.132)	(0.135)	(0.135)	(0.297)	(0.272)	(0.389)
1992	0.110	−0.071	−0.069	0.134	0.034	−0.080	−0.215	0.064	−0.323
	(0.122)	(0.198)	(0.131)	(0.249)	(0.214)	(0.214)	(0.447)	(0.296)	(0.478)
1993	−0.013	−0.074	−0.338	−0.552	−0.083	−0.122	−0.175	−0.378	0.649
	(0.194)	(0.193)	(0.339)	(0.198)	(0.247)	(0.247)	(0.341)	(0.222)	(0.240)
1994	0.040	0.132	0.468	−0.292	0.033	0.128	0.174	0.505	0.572
	(0.123)	(0.127)	(0.152)	(0.261)	(0.133)	(0.133)	(0.205)	(0.452)	(0.274)
1995	0.094	−0.222	0.075	−1.568	0.268	−0.129	−0.993**	−11.899***	−9.991***
	(0.125)	(0.185)	(0.131)	(1.066)	(0.180)	(0.180)	(0.108)	(1.482)	(1.668)
1996[a]	−0.023	NA	0.526**	NA	NA	NA	NA	NA	NA
	(0.120)		(0.122)						
1997	0.032	0.058	−0.062	1.084	0.022	0.054	−0.059	1.196	0.362
	(0.110)	(0.110)	(0.116)	(0.339)	(0.114)	(0.114)	(0.113)	(0.363)	(0.117)
1998	0.036	−0.165	−0.340	−0.126	−0.096	−0.223	−0.575	−0.326	−0.391
	(0.168)	(0.163)	(0.316)	(0.186)	(0.209)	(0.209)	(0.468)	(0.405)	(0.536)
1999	−0.013	0.241*	−0.167	0.026	0.058	0.257*	−0.097	0.053	−0.013
	(0.099)	(0.087)	(0.102)	(0.113)	(0.092)	(0.092)	(0.120)	(0.132)	(0.128)
2000	0.039	−0.128	−0.068	0.442**	0.010	−0.126	−0.131	0.427***	−0.089
	(0.082)	(0.081)	(0.128)	(0.106)	(0.083)	(0.083)	(0.129)	(0.120)	(0.093)
Pooled	0.074**	−0.002	0.022	0.014	0.076**	0.010	−0.012	0.021	−0.085
	(0.030)	(0.032)	(0.032)	(0.034)	(0.032)	(0.032)	(0.037)	(0.034)	(0.033)

NA: data not available for the following reason: [a] Little or no arrival date data available.
* $P < 0.01$, ** $P < 0.05$, *** $P < 0.10$.


(a)
1982
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Relative Fitness
Relative Length (SD)
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(c)
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(d)
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Relative Fitness
Relative Arrival Date (SD)
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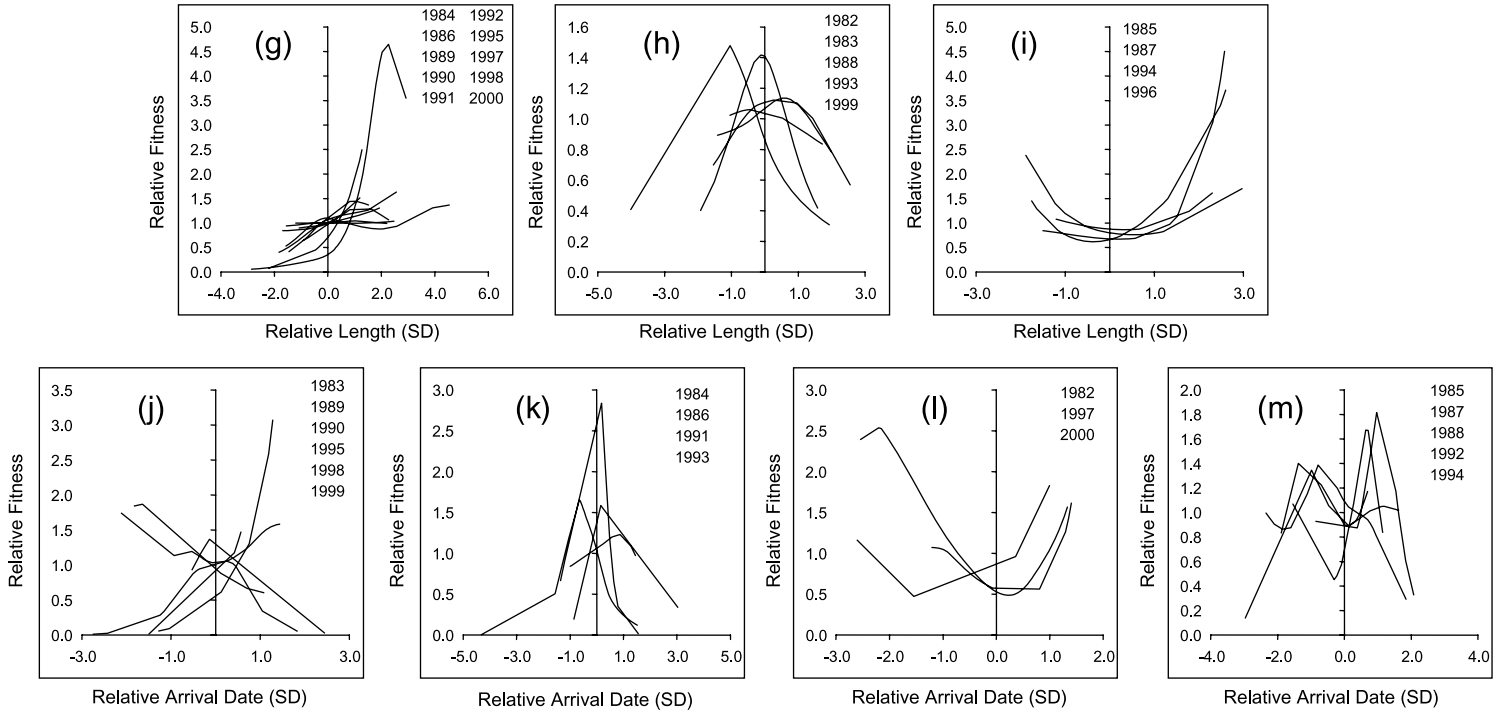

Fig. 3. Relationships between relative fork length or relative arrival date and relative fitness (lifetime reproductive success) for 19 brood years of adult steelhead. Each row of plots is the complete set of univariate cubic splines (Schluter, 1988) for one trait of a particular sex among brood years. To facilitate visualization of the data, yearly data were roughly grouped by apparent pattern of selection. Row 1, plots (a)–(c) show male relative length, $\lambda = -3.5$; row 2, plots (d)–(f) show male relative arrival date, $\lambda = -5.6$; row 3, plots (g)–(i) show female relative length, $\lambda = -0.3$; row 4, plots (j)–(m) show female relative arrival date, $\lambda = -2.7$. See 'Materials and methods' for how lambda was calculated.

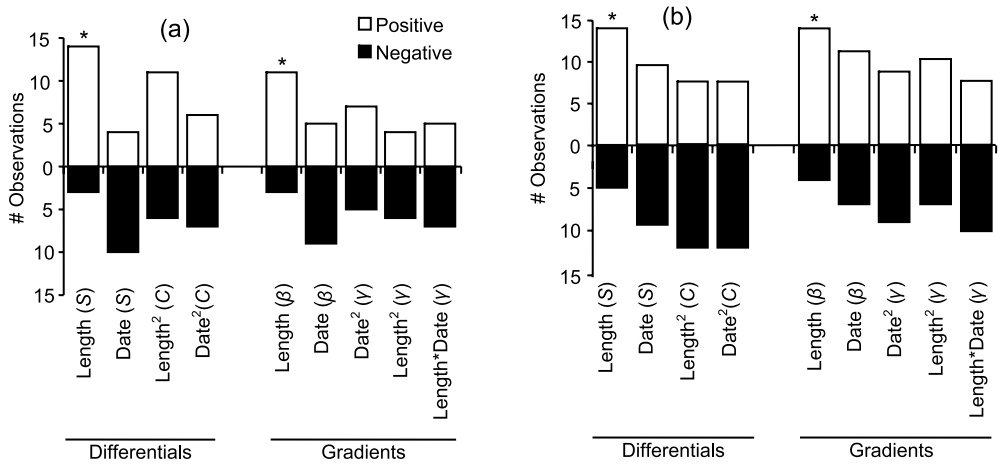

Fig. 4. Trends in the sign (positive or negative) of the linear and quadratic selection coefficients on fork length and arrival date of adult male (a) and female (b) Snow Creek steelhead in 19 brood years. Significant differences in the number of positive and negative coefficients are marked with an asterisk (χ^2 test, expectations of 1:1, $\alpha = 0.05$).

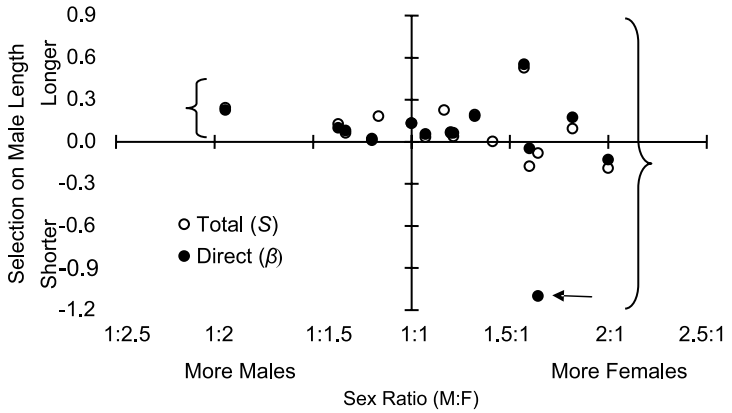

Fig. 5. The relationship between the observed annual sex ratio and total (S) and direct (β) selection on male length. Braces emphasize the range in values of differentials and gradients for years when the sex ratio was male (left) or female (right) biased. Differences in the variance of differentials and gradients among years grouped by sex ratio (male biased vs. female biased) were significant (F-test, $P = 0.03$), even when the apparent outlier (marked with an arrow) was excluded (F-test, $P = 0.03$).

vary more when the sex ratio was female biased (Fig. 5). We used an F-test (one-tailed) to test the null hypothesis that there was no difference in the variance of male length selection coefficients grouped by sex ratio (male biased vs. female biased). Total and direct selection on male length varied more when the sex ratio was female biased ($S: P = 0.03$; $\beta: P = 0.001$), even when the apparent outlier (large negative selection coefficient) was excluded ($S: P = 0.03$; $\beta: P = 0.03$). Total and direct selection on male arrival date was unrelated to the observed sex ratio. Total and direct selection on female length and arrival date

was unrelated to the observed sex ratio. Spawning adult (male or female) density was unrelated to the total and direct selection on either male or female length or arrival date ($P > 0.05$).

DISCUSSION

Body size

We examined the patterns of selection on size at breeding and timing of migration on a small population of steelhead, over 19 brood years, or about five generations. Large body size was expected to be favoured during breeding in salmonids based on the information collected on returning (i.e. surviving) adult progeny and this was what we documented. However, opposing selection occurs during the life of offspring (Blanckenhorn, 2000) due to costs associated with growth rates needed to attain large size (Healey, 1986; Holtby and Healey, 1986; Arendt, 1997), possibly preventing this and other salmonid populations from evolving increasingly large size and increased age at maturation. There is a significant risk of mortality for each additional year at sea (Matthews, 1968; Ricker, 1976) and the primary way to increase adult size is to remain at sea for an additional year. Using the data of Grant and Grant (2002: Figures 1 and 2), we compared the trends in direction of selection with changes in phenotypic traits. We performed the same chi-squared analysis on their selection differentials for body size (Grant and Grant, 2002: Figure 2), beak size, and beak shape for both species of finch, *Geospiza fortis* and *G. scandens*. Two tests, for body size and beak size of *G. scandens*, produced results nearly significant at $\alpha = 0.05$ ($P = 0.059$), suggesting that selection favoured larger size in both traits more often than by chance (all other tests were not significant, $P > 0.25$). Interestingly, both body size and beak size became smaller rather than larger over the same period (Grant and Grant, 2002: Figure 1). Actual evolution of traits is obviously more complicated than simply having consistent directional selection. Consistent selection on body size at time of breeding (as seems apparent in salmonids) over many generations would decrease the amount of genetic variation underlying body size, decreasing the genetic substrate upon which selection operates. What is more likely, however, in spite of frequent positive directional selection for large size, weak and non-significant selection for large size would be overcome by selection on correlated traits, or random effects. Correlations were much weaker, both in terms of the selection gradients and the amount of variation explained, than might be expected given the relationships between these traits and correlated fitness traits in salmonids, but were similar to our earlier studies examining numbers of juvenile offspring in Snow Creek (Seamons *et al.*, 2004a) and smolts migrating to sea (Seamons, 2005), and to those of other similar studies [Atlantic salmon, *Salmo salar* (Garant *et al.*, 2001); pink salmon, *O. gorbuscha* (Dickerson *et al.*, 2002, 2005)].

The intensity of male–male competition in many animals including salmonids has led to the evolution of a variety of life-history strategies involving age (and body size) at maturity (Gross, 1985), which could complicate interpretation of our results. Males that matured as parr [before or instead of migrating to the ocean (Seamons *et al.*, 2004b)] were not sampled in our study, which could affect our results in two ways. First, if we were able to include the mature parr lengths in the analysis we might see significant positive quadratic coefficients indicating that small male mature parr and large males were more successful than those of intermediate sizes (smaller anadromous males). Second, our results might be biased if smaller anadromous adults (male and female) were more likely to produce mature parr because

many of their viable, mature offspring would not be sampled at the weir. This is apparently true of some other salmonid species [coho salmon jacks, *O. kisutch* (Appleby *et al.*, 2003); Atlantic salmon (Duston *et al.*, 2005)], but not others [chinook salmon, *O. tshawytscha* (Unwin *et al.*, 1999)] and it is unknown for steelhead.

Timing

Patterns of arrival timing were expected to display characteristics of stabilizing selection, either within or among years. Selection in some years had the convex pattern expected under stabilizing selection (Fig. 3, plots k, m), but the shape of selection in other years was that of directional selection for early or late arriving or the concave pattern of disruptive selection (Fig. 3, plots j, l). Coefficients for selection for arrival date were equally likely to be positive or negative, suggesting that, unlike in some birds (Smith and Moore, 2005), the early arrival of steelhead females was neither consistently advantageous nor disadvantageous. In a comprehensive study of pink salmon, early-arriving females lived longer than their later-arriving counterparts (Dickerson *et al.*, 2002), which is believed to have evolved so that early-arriving females can prevent their nest sites from being re-used by later-arriving females. However, no relationship was found between arrival date and the number of adult offspring produced (Dickerson, *et al.*, 2005). In steelhead, early-arriving females produced larger juvenile offspring when measured at a fixed date later in the season (Seamons *et al.*, 2004a), and this is thought to give their offspring a survival advantage during freshwater residency (Sogard, 1997). However, the present results indicate that juvenile size advantage did not translate into higher survival to adulthood. The benefits, then, of arrival timing for females were probably more closely tied to environmental conditions experienced during spawning. The nests of early-spawning females may be susceptible to disturbance from later-arriving females (Essington *et al.*, 2000). However, the nests of all females, regardless of timing, were, to some extent, susceptible to disturbance from floods (Lapointe *et al.*, 2000), the frequency, duration, and timing of which were unpredictable from year to year (Seiler *et al.*, 2002). Although the half positive, half negative regression coefficients observed here might be due to non-random effects (e.g. environmentally determined direction of selection may appear random), we would expect to see the same pattern if the direction of selection was randomly determined or if there was no selection.

Pacific salmon males tend to arrive on the breeding grounds before females (Morbey, 2000). Morbey and Abrams (2004) hypothesized that this pattern of arrival could be an evolutionarily stable strategy connected with the evolution of senescence. Snow Creek steelhead males did not arrive before females (Seamons *et al.*, 2004b), possibly because they, unlike Pacific salmon, do not necessarily die after spawning. Interestingly, however, individual males nearly always arrived on the breeding grounds before the female with whom they mated (Seamons *et al.*, 2004b). Later-arriving males could also achieve reproductive success if they arrived before a female (Seamons *et al.*, 2004b). This may, in part, explain why there was no overall advantage or disadvantage for early arrival in male steelhead in terms of numbers of juvenile (Seamons *et al.*, 2004a) or adult offspring (this paper). Similarly, in pink salmon, a dynamic and complex relationship existed with arrival timing and body size and dominance such that large fish were favoured early in the breeding season, but small fish were favoured later (Dickerson *et al.*, 2002, 2005). Taken together, these results suggest that early arrival to the breeding grounds cannot be assumed to confer an advantage in salmonids.

Trends in strength and direction of selection

Selection on male and female steelhead traits was marginally stronger than that reported for organisms in general, based on the review by Kingsolver *et al.* (2001), although interpretation of the strength of selection must be cautious since sample sizes were generally small in our data set (Hersch and Phillips, 2004). When compared with the data set of Kingsolver and colleagues (2001), our median differentials for male length and arrival date were between the 60th and 70th percentiles. While female selection differentials were closer to or below the median reported by Kingsolver *et al.* (length = 43rd percentile, arrival date = 51st percentile), selection gradients also fell between the 60th and 70th percentiles. The greater observed strength of selection may be due in part to our allocation of some selection on offspring to selection on parents (Wolf and Wade, 2001); we were unable to partition selection between parents and offspring.

Opportunity for selection

Opportunity for selection for male steelhead was higher in years with male-biased sex ratios and more males overall, as Fairbairn and Wilby (2001) found in their experiments with insects. This presumably reflected increased competition for mates when there were more males than females (Fleming and Gross, 1994; Quinn *et al.*, 1996; Clutton-Brock *et al.*, 1997; Røed *et al.*, 2002), and suggests that the absolute number of male competitors is more important than the number relative to females in determining the opportunity for selection. We also found an increase in opportunity for selection on females with an increase in the absolute number of females. This may reflect density dependence inherent in this species where production exceeds the carrying capacity of limited freshwater rearing habitat (Johnson and Cooper, 1992).

The opportunity for selection did not correlate with selection coefficients and gradients in either males or females; others have found the same lack of relationship (Ferguson and Fairbairn, 2001). Given the correlation between opportunity for selection on males with sex ratio and male numbers, we also tested whether selection gradients were correlated with the sex ratio or total numbers of males. Although no correlation was observed for either variable, the strength and direction of selection on male body size appeared to vary less when the sex ratio was male biased and when more males returned to spawn. Perhaps when competition for access to females is strong large males are consistently more successful, but when competition is diminished (female-biased sex ratio, fewer males in total) all or nearly all males are able to mate and produce offspring. When this happens the reproductive success is randomly determined (at least with respect to body size), so that the direction and strength of selection are more variable.

Conclusion

We have shown that selection on body size and arrival timing, both of which are important fitness traits in salmonids and other animals, varied significantly among years in a small, natural population of steelhead. However, in spite of this variation, large adults were favoured over small ones more often than one would expect by chance. This result may be reconciled with the apparently stable size distribution by considering the costs in terms of survival needed for offspring to achieve large size. The opportunity for selection on males

was correlated with measures of breeding competition, sex ratio and spawner density, and the opportunity for selection on females was correlated with spawning density. Patterns of selection were uncorrelated with opportunity for selection, and to a great extent with measures of breeding competition; however, selection on male size was more predictable when the sex ratio was male biased – large males were always favoured. Whether this directional selection produces evolutionary change in body size remains to be seen.

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