The mating system of steelhead and the effect of length and arrival date on steelhead reproductive success

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Abstract

The mating system of steelhead and the effects of length and arrival date on steelhead reproductive success

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Mating systems reflect the degree of sexual selection in the past and present. Sexual and natural selection operating before and during spawning and natural selection on offspring combine to determine patterns of individual reproductive success. The direction and strength of selection may change over the lifetime of parents and their offspring or over the course of years or seasons. In order to first, describe the genetic mating system of steelhead, and second, test hypotheses concerning the effects of body size and arrival date on individual reproductive success parents were genetically matched to young-of-the-year (YOY), yearling and smolt offspring in 4 brood years, and to returning adult offspring in 19 brood years in a small population of steelhead (*Oncorhynchus mykiss*). Male and female steelhead mated multiply in all brood years. No evidence was found of size-assortative mating, however males tended to arrive before the females with whom they mated. Mature parr were inferred from the excess of parental assignments of mothers over that of fathers. YOY offspring of early arriving females were larger than the offspring of later arriving females; large females had large YOY offspring in only one brood year. Large and early arriving males produced more smolt and adult offspring than small or late arriving males, and large females produced more smolt and adult offspring than small females. Large males and females had more adult offspring consistently across brood years, while selection for arrival date for both sexes varied in direction among years. Standardized variance in reproductive success was positively correlated with both the sex ratio and the spawning density for males and with only the spawning density for females. The strength and direction of selection on male length varied more when the sex ratio was female biased. In females, the strength of selection was unrelated to either the sex ratio or the spawning density. Freshwater survival of maternal and paternal half-sibling families appeared unrelated to young-of-the-year length, except for maternal half-sibling families in one brood year when families which were smaller survived in higher numbers. Marine survival of maternal and paternal half-sibling families appeared unrelated to either smolt length or date of migration, and instead appeared to be random or equal among families in the three years for which we had data. Extensive yearly variation in the direction and shape of selection on both traits in concert with overlapping generations will confound response to selection on both traits, thus making predictions of evolutionary change in response to changing environments difficult.

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Rock and Roll Cleveland!

Introduction

Male and female steelhead (*Oncorhynchus mykiss*), like all sexual organisms, have different strategies to maximize the number of offspring they produce (i.e. their reproductive success). Females maximize the number and quality of their eggs while males maximize the number of eggs they fertilize. For both sexes, reproductive success has long been related to body size (or morphology associated with size), with the common paradigm that "bigger is better". In salmonids, large females produce more and larger eggs than small females. Males compete for access to spawning females and all other things equal, the large males win. More recently, arrival and spawning dates have also been recognized as being important determinants, though their relationship to reproductive success is complicated enough that the working hypothesis for phenological traits might be "it depends". Offspring number and size may depend on the spawning date of the parents, which is mainly determined by females – males may spawn throughout the season, but have to wait for a receptive, ripe female. The ability of a male to exclude other males from spawning females depends on the prevailing operational sex ratio, which may depend on the male's arrival date. Lifetime reproductive success also depends on natural selection on offspring, which may also have morphological and phenological components. Survival of juvenile salmonids may be size selective in both the freshwater and marine environments, and anadromous salmonids have a period of migration from freshwater to saltwater, the timing of which may affect survival. This natural selection may augment or diminish the sexual and natural selection during spawning.

Most of what we know about these processes has been gleaned (necessarily) piecemeal from controlled experiments; the overall effects are assumed or inferred. The interactions among these processes under natural conditions, over entire life cycles, are not well understood. Using a small population of wild steelhead in Snow Creek on the Olympic Peninsula in Washington State, USA I determined the effects of parental body size and arrival date on directly measured individual reproductive success.

Little is known about the mating systems of steelhead, the anadromous form of rainbow trout (*O. mykiss*). Steelhead differ significantly from other anadromous Pacific salmonids in some characteristics of their life history (e.g., protracted spawning season, iteroparity), so differences in mating system may be expected. Assortative mating may amplify or dampen responses to selection. The strength of sexual selection depends on the variation in mating success of individuals. The mating system of steelhead (i.e., who mated with whom, numbers of mates for males and females, etc.) was assessed by reconstructing the parentage of juveniles sampled in the creek prior to seaward migration (Chapter 1).

Most mortality in anadromous salmonids occurs in freshwater. Mortality may be substantial; mortality from egg to smolt (i.e., all freshwater) in Snow Creek has been as high as 99.84% and as low as 88%. In freshwater, larger juveniles typically have a survival advantage over smaller ones because they can evade predation due to gape limitations and because their large size may benefit them during the period of limited energy during the winter. The lifetime reproductive success of parents results from the processes of natural and sexual selection during spawning and natural selection throughout the lifetime of their offspring. For anadromous salmonids, the natural selection operating on offspring involves both freshwater and saltwater components that are likely to be quite different. The patterns of selection on parental traits in steelhead families through the first year and a half of life in freshwater are reported in Chapter 2.

Survival may also be size-related during seaward migration, however very little research has been done on this topic. Regardless, selection pressures are likely to be different from those experienced during freshwater residency. Size based selection in freshwater may amplify or dampen patterns of selection seen during spawning. Patterns of selection based on numbers of smolt offspring are composed of selection both during spawning and afterward through all freshwater life history; these data are reported in Chapter 3.

Are any effects of natural and sexual selection during spawning left after freshwater *and* marine mortality has taken its toll? Which life history stage (spawning, YOY, yearling, smolt, adult) sets the overall patterns of selection and reproductive success? Are trends in reproductive success measured by the number of returning adult offspring common across brood years? Are there patterns in variance in reproductive success related to spawning conditions (sex ratio, density)? Is there family specific marine mortality related to the size and timing of smolt half-sib families? Answers to these question, and more, are found in Chapter 4.

Chapter 1: The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny

Synopsis

The development of molecular markers has allowed behavioral ecologists to link parents to specific offspring, providing insights into breeding systems that were not apparent from direct observations of the social system. Studies of this type in fishes have focused on species with male parental care such as centrarchids, and on salmonids, a family with little parental care. In order to gain further insight into the mating system of steelhead trout (*Oncorhynchus mykiss*), a winter-spawning species whose reproductive system is poorly known, adults returning to spawn were captured in four consecutive years in a small, unfished, wild population. Juvenile offspring were sampled by electrofishing and parentage was determined by exclusion based on a 12 locus microsatellite genotype. Both males and females mated with multiple individuals, though single pair matings were also inferred. Females and males tended to have the same number of mates (median = 1), but males were more likely to have no apparent partner (43% vs. 23% for females) and the maximum number of mates were obtained by males (range 0-10 vs. 0-5 for females). There was no difference in median arrival date by sex, but 80% of the females mated with males that had already arrived rather than males arriving with or after the females (median = 7.5, range=1-63 days difference). Contrary to expectations, there was no evidence of size-assortative mating; larger males and larger females did not tend to mate with each other more often than would have occurred by chance. Of the juveniles with only one identified parent, most had a known mother and an unknown father rather than the reverse (88% vs. 11%). We interpret this as indirect

evidence that non-anadromous males achieved a significant number of fertilizations. Thus the steelhead mating system was complex, being more strongly structured by arrival date than fish size, and including a significant genetic contribution by mature male parr.

Introduction

The relatively recent development of molecular markers suitable for discriminating genetic relationships among individuals has brought a wealth of comparisons between the observed reproductive behavior (social mating system) and genetic mating system of many organisms (Hughes, 1998). Early work focused on the mating systems of birds, comparing observed patterns of paternity and offspring care with genetic patterns, often documenting extra-pair fertilizations in putatively monogamous species (e.g., Westneat, 1987). The inferences about mating systems drawn from observational and genetic studies often differed dramatically (e.g., Birkhead *et al.*, 1990; Sillero-Zubiri *et al.*, 1996), leading to many new theories of sexual selection, sperm competition and mate choice (Birkhead, Møller, 1998).

There are now many examples of this type of comparison in fishes, mostly investigating patterns of male reproductive success in species with male parental care (e.g., DeWoody, Avise, 2001; Jones, Avise, 2001). Many of the other studies of genetic mating systems in fishes involve patterns of paternity, number of mates and spawning location in salmonid species (Bentzen *et al.*, 2001; Garant *et al.*, 2001; Hutchings, Myers, 1988; Taggart *et al.*, 2001).

The social mating system and reproductive behavior of salmonids (*Oncorhynchus*, *Salvelinus* and *Salmo* spp.) is well known for most species. Males tend to arrive on the spawning grounds before females (Morbey, 2000). The ability of individual males to dominate access to spawning females (and thereby maximize reproductive success) is influenced by body size, prior residence (Foote, 1990), and by the operational sex ratio (OSR: ratio of sexually active females to sexually active males at any given time; Emlen & Oring 1977) which is a function of date within the spawning period (Quinn *et al.*, 1996). Males arriving early may have first access to early arriving ripe females, whereas later in the spawning season, when OSRs may be more strongly male biased, no single male may be able to control access to females (Dickerson *et al.*, 2002). Additionally, males that establish dominance early in the season may, regardless of size, maintain their position in the dominance hierarchy (Healey, Prince, 1998).

After females arrive they immediately dig one or (less frequently) more nests, termed redds, in streambed gravel, and are courted by one or more males. They then deposit eggs fertilized by males in several discrete pockets within the redd over a period of a few hours to a few days. The female covers the fertilized eggs in each pocket with gravel before preparing the next pocket. In semelparous species, the female protects the embryos in her redd from disturbance by other females until she dies. In iteroparous species, females do not guard the completed nest; adults of both sexes migrate to feeding grounds in the ocean or fresh water habitats after spawning and may survive to return and spawn again in subsequent years.

Males may choose their mates based on size (Foote, 1988), and large males may exclude smaller males from access to females through aggressive interactions (Hanson, Smith, 1967; Keenleyside, Dupuis, 1988; Quinn, Foote, 1994). Females may also exhibit preference by delaying spawning when courted by a small or otherwise undesirable male (Berejikian *et al.*, 2000; de Gaudemar *et al.*, 2000; Foote, 1989). One might infer that large males should be observed in dominant positions courting large females but this is not always the case (Quinn, Foote, 1994).

The outcome of aggressive competition among males for access to females is generally determined by size and shape (Fleming, Gross, 1994; Quinn, Foote, 1994). However, many populations of anadromous salmonids have males that mature at a much smaller size than the smallest females. These males either spent a shorter period of time at sea than most males (jacks; e.g., Gross 1985) or matured without migrating to sea (precocious parr; e.g., Fleming 1998).

In contrast to other salmonids, little is known about the social or genetic mating systems of steelhead, the anadromous form of rainbow trout (*O. mykiss*) other than basic behaviors (Needham, Taft, 1934; Shapovalov, Taft, 1954; Tautz, Groot, 1975). Steelhead differ significantly from other Pacific salmon in life history, so differences in mating system may be expected. Compared to most Pacific salmon, steelhead have a protracted migration and spawning season (3 months or more; Busby et al.), and are generally found at much lower densities than salmon (Busby *et al.*, 1996). Steelhead are also iteroparous and seem to produce a significant number of mature male parr (Shapovalov, Taft, 1954), though the phenomenon has not been well-studied. Indeed, the breeding system of steelhead more closely resembles that of Atlantic salmon than their closer relatives, the Pacific salmon. In addition, steelhead may spawn at night, and spawn in the winter and spring when river levels are high in coastal streams, making direct observations of spawning behavior difficult. Molecular genetic tools may be especially suited to a study of steelhead mating system and reproductive behavior providing a basis for contrasts with the behavior of better known Pacific and Atlantic salmon.

The purpose of this paper is to describe the genetic mating system of steelhead, in particular the number of mates of each sex and the extent to which the breeding system is structured around the size or arrival timing of the mates in a wild population. First, we expected that both males and females would have multiple mates, but that males would be more variable in the number of mates than females. Second, we predicted that male steelhead would tend to return before females and that females would mate with early arriving males. Finally, we expected that pairs of steelhead would be matched by size but that mature parr might fertilize some eggs.

Methods

Study site, tissue collection, and genetic analysis

A permanent fish weir, approximately 0.95 km upstream of the mouth at Discovery Bay (Figure 1.1), has been operated continuously since 1977 on Snow Creek, Washington, by the Washington Department of Fish and Wildlife (WDFW). Fish are unable to pass up- or downstream without being sampled except during the most extreme floods. All adult steelhead reaching the weir were sampled each spring from 1997 to 2000. Length, date of arrival, and sex were recorded for each fish, and several scales were removed for age estimation. Small pieces of caudal fin were removed for DNA analysis from adults and from approximately 300 juvenile steelhead that were sampled annually in October 1997-2000 by single-pass electrofishing in four different sections of Snow Creek (Figure 1.1). All tissue samples were stored in 95% ethanol in 1.5-mL microcentrifuge tubes at room temperature.

DNA from adults was isolated using a modified CTAB/phenol/chloroform extraction protocol (Fields *et al.*, 1989). The DNA of juvenile fish was extracted using the DNeasy Tissue Kit (Qiagen Inc. Valencia, CA, USA) following recommended protocols. Twelve microsatellite loci (Table 1.1) were amplified for each individual using the following recipe: 10 µL reaction volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1-2 mM MgCl2 (see Table 1), 0.04 mM each dNTP, 1 U *Taq* DNA polymerase (Promega, Madison, WI), 0.5 µM each primer, and approximately 100 ng DNA template. An MJ Research PTC-200 thermal cycler (Watertown, Massachusetts) was used for all PCR amplifications. The thermal cycler profile consisted of the following conditions: 1) three cycles of 95°C (1 min) + X°C (30 s) + 70°C (1 min); 2) 22 cycles of 95°C (10 s) + X°C (30 s) + 70°C (1 min); 3) one cycle of 70°C (45 min), where X was an annealing temperature that varied among primer pairs (Table 1.1). Microsatellite DNA was visualized using the MegaBACE 1000 capillary electrophoresis system (Amersham Pharmacia Biotech Limited, Piscataway, NJ). One primer of each primer pair was labeled with one of three fluorescent chemical labels (FAM, NED or HEX) and electrophoresed with a 900 base pair internal size standard similarly labeled (ET900-R, Amersham Biosciences).

Locus characteristics, parentage assignment and data analysis

A two-tailed exact test of Hardy-Weinberg equilibrium (HWE) was performed for each locus in each brood year (BY) combination using the Markov Chain method implemented in Genepop 3.1 (dememorization number 1000, batches 100, 1000 iterations per batch; Raymond & Rousset 1995). Significance of probability values was adjusted for multiple tests using sequential Bonferroni correction (Rice, 1989). F_{IS}, a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation, was calculated according to Weir and Cockerham (1984) using Genepop 3.1 software (Raymond, Rousset, 1995). Observed and expected heterozygosity were calculated using GENETIX 4.02 software (Belkhir *et al.*, 2001).

Parentage was assigned through exclusion using the program WHICHPARENTS 1.0 (Will Eichert, Bodega Bay Marine Lab, California) using all twelve loci, but allowing three mismatches. The genotypes of adults identified by WHICHPARENTS as potential parents were then directly compared to juvenile genotypes; only adults that matched one allele at each locus were finally called parents. If no adults matched one allele at each locus with a juvenile it was assumed that the actual parents were not in the adult sample. Juveniles not assigned any parents were dropped from subsequent analyses. As a conservative measure, because of repeat spawning and the possibility of incomplete adult sampling in some years, all four years of adults were treated as potential parents of all juveniles.

In many cases only one parent was assigned to a juvenile. We calculated the probability of finding a single matching parent at random from the parental pool by using the equation: probability of a match at a single locus = $1-[1-(pA + pB)]^2$, where pA is the probability of matching allele A and pB is the probability of matching allele B based on the parental population allele frequencies, multiplied across all loci. The chance of a single parent matching at random the most common possible offspring genotype (i.e. homozygous for the most frequent allele at each locus in the parent pool) for each year was between 1 and 2 in 1000 and no juvenile offspring actually had that genotype. Thus we concluded that the single assigned adult was indeed one of the parents of that juvenile.

The numbers of mates for individuals of each sex was determined by counting the number of different mates from genetically determined steelhead matings from yearly parentage data. In cases where only one parent was known it was assumed that all offspring of that parent were the offspring of only one other adult though this may underestimate the actual number of mates. Yearly male and female distributions of numbers of mates were compared using a Kolmogorov-Smirnov test (Zar, 1999). To assess the patterns of arrival timing of adults we calculated an arrival score for each year of adult data using the 'protandry score' of Morbey (2000). We then calculated the frequency of males or females that arrived before their mate. We calculated the median and mean number of days between each pair of mates for those pairings where the male arrived first. To determine whether mating was size-assortative, we used Spearman's rank correlation (Zar, 1999) for correlation of male and female lengths in inferred mated pairs in each year.

Results

Adult characteristics

A total of 275 adult steelhead were collected over the four years of this study (Table 1.2) ranging from 31 in 1997 to 139 in 2000. The overall sex ratio was close to 1:1 (135 males and 140 females) but varied considerably among years. In 1999 and 2000 the sex ratios were nearly 1:1 but in 1997 there were almost twice as many females as males, and in 1998 the reverse ratio was observed. Body lengths varied considerably, from 420 - 815 mm in males to 565 - 860 mm in females. The date of first arrival to Snow Creek varied from December to March among the four years, but most steelhead returned in March and April, and the median arrival date was in the last week of March in all four years.

Locus characteristics, parentage and familial relationships

Only 2 tests out of 48 were out of HWE after sequential Bonferroni corrections (Table 1.3). *Omy77*, a locus known to have null alleles in Snow Creek steelhead (Ardren *et al.*, 1999), showed the expected deviation from HWE ($P<0.0001$) in one of four brood years (2000), and had a significant positive F_{IS} value associated with null allele segregation ($F_{IS}=0.209$). Locus *Ots108* was also out of HWE for BY 2000 adults ($P=0.001$) and also showed a positive F_{IS} value (0.028).

Average heterozygosity across all 12 loci in our sample was 90% (Table 1.1), and the probability of exclusion using all 12 loci was 0.9999999 (CERVUS 2.0; Marshall et al. 1998). We were able to assign at least one parent to 73% of all juvenile samples, and two parents to approximately 40%. Of those juveniles assigned only one parent, nearly

90% were assigned only a female parent (Figure 1.2), significantly more than expected by chance (Chi-square goodness-of-fit, P<0.001, expected 1:1). Four percent (12) of all adult steelhead returned to spawn in Snow Creek in more than one year. Fifty-four juveniles appeared to be the offspring of these adults. These juveniles were assigned to a brood year by one of two methods: if a male and female adult from one BY and only a single adult from another BY (the repeat year of one of the assigned adults) were assigned to a juvenile it was assumed to have been spawned in the BY of the two parents assigned (31; no juveniles appeared to be offspring of two repeat spawning adults); if only one parent was assigned (the same individual, but two brood years) the juvenile was assigned a brood year by its length using juvenile age/length relationships from known age juveniles (23).

At least some male and female steelhead mated with multiple partners in all years (Figure 1.3). Males mated with up to 10 different females, but the median was one female (33%). Females mated with up to five different males (median = 1) with two mates being the mode. More males than females had no mates over all four years (43 vs. 23%). The male and female distribution of number of mates was significantly different for the pooled data (Kolmogorov-Smirnov test, P<0.001).

The multiple matings resulted in complex patterns of relatedness. For example, in 2000 we inferred that there were eight single pair mating groups (i.e., a male and a female were each other's only mates that year; Figure 1.4). However, there were also two cases where one female's eggs were fertilized by two different males and three cases where one male mated with two different females. A series of progressively more complex mating groups were inferred, including one group of seven males and nine females that interbred (Figure 1.4). Similarly diverse patterns were inferred in the other three years of the study (Table 1.4).

There was a significant correlation between male and female size in mated pairs of steelhead only in 2000 when a slight negative correlation was found (Spearman's rank correlation, R=-0.26 P=0.046). Jack males (i.e., those spending only one year at sea) may use a different mating strategy so we repeated the analysis after excluding them but there was still no relationship between the sizes of the parents.

In contrast to the lack of size-based structure in the apparent mating system, there was considerable temporal structure. Males arrived only slightly before females, with a difference in median date of 0-3 days (Table 1.2). The 'protandry score' (Morbey, 2000), an alternative method to estimate which sex arrives first, indicated that in 1998 and 2000 males arrived before females by about four days, and in 1997 and 1999 females arrived before males by about four days.

Despite the similarity in arrival patterns, the male almost invariably arrived before the female with whom he mated (Figure 1.5). On average males arrived about 15 days before the females they mated with, and the yearly median difference in days ranged from 3 -19.5 days before females. This result was not the consequence of a lack of later arriving males; an average of five males arrived on the same day or within two weeks after the arrival of each female, but they seldom mated with such females.

Discussion

Males were expected to have multiple mates because in other salmonids sperm production does not limit reproduction and females tend to spawn all their eggs within a few days (e.g., chum salmon - Schroder, 1981). Thus when a female has spawned all of her eggs, the dominant male would be expected to seek breeding opportunities with other females. Females may mate with multiple males as a form of mate choice, choosing better mates with each successive mating or they might benefit from diversity among their offspring in a variable environment (see Garant *et al.*, 2001, for an in-depth discussion for Atlantic salmon). While a female might exercise some choice in mates, eggs may be fertilized by males with whom the female never intended to mate, such as satellite males, jacks or mature parr. We were unable to observe mating directly so we cannot determine whether the multiple sires resulted from female choice or male competition. Observations of salmon (e.g., Quinn *et al.*, 1996) and paternity analysis under controlled conditions (e.g., Chebanov *et al.*, 1983; Foote *et al.*, 1997; Schroder, 1981) suggest that male competition is probably the dominant process.

Our data showed that male and female steelhead mated with multiple partners, but the numbers of mates for each sex should be viewed as conservative estimates for several reasons. First, these numbers are biased by the numbers of offspring per parent; a male with only one offspring can only be inferred to have mated with one female. We sampled a small but unknown fraction of the offspring in the creek, and any matings that produced offspring that were not sampled or offspring that failed to survive incubation and their first summer would also be undetected. Thus fish may have had mates that we did not detect. Our conclusion that both males and females mated with multiple mates would not change with complete data but the ranges would probably increase. Second, the differences between sexes may be biased by family size. Maternal half-sib families averaged ~1 offspring more than paternal half-sib families, possibly accounting for some of the difference in the number of mates between the two sexes.

Examination of the patterns of sibship among the juveniles revealed many different types of mating, including monogamy, polygamy, polyandry and polygynandry, resulting in complex patterns of relatedness among offspring. This complexity of mating may be one reason why size assortative mating was not revealed. Size assortative mating was expected because there are apparent advantages for each sex to mate with large individuals. Large females are more fecund (Beacham, Murray, 1993) and construct deeper redds which better protect embryos (Steen, Quinn, 1999). Large males are more often able to win in competition for access to females (Quinn, Foote, 1994) and may pass traits for size on to offspring (Gjerde, 1986). However, large males and females mated with multiple individuals of the opposite sex, including smaller individuals. In addition, it is possible that size assortative mating did take place, with each female mating with the largest available male at that moment, even though that male may have been smaller than ones available at other times during the season or other locations in the creek. More likely, however, a female was courted by one dominant male and other males participated in the spawning act despite his attempts to monopolize access to her. Males are also sensitive to the timing of female reproduction and will court a female about to deposit eggs rather than a larger one that is less likely to spawn shortly (Schroder, 1981).

Steelhead matings were structured by arrival timing. Females almost always mated with males that had arrived before them, despite the fact that several males arrived with or soon after each female. Early male arrival timing is common in Pacific salmon (Morbey, 2000), presumably to offset the skewed OSR that results from the fact that males remain sexually active until they die or leave the spawning grounds whereas females complete spawning in a few days and thereafter are not available for reproduction (Quinn *et al.*, 1996). Male steelhead did not consistently arrive before females, and in general the breeding period was protracted and characterized by relatively few ripe females at any time. Our data showed that males need not arrive early on an absolute basis but need to arrive a week or two before a female arrives to maximize their chances of breeding with her. We have several hypotheses for this apparent pattern. First, males may have established territories within the stream and maintained them by a prior residence advantage. Assuming that most males do not stay in fresh water for the entire spawning season, which may last for up to 4 months, territories are continuously established and vacated over the spawning season. Some degree of territoriality has been reported in male sockeye salmon but it is qualitatively different from the behavior shown by females (Foote, 1990). Females actively defend their redd site even in the absence of males whereas males are much less aggressive in the absence of females. Second, some amount of learning of the freshwater habitat may be necessary for males to be comfortable or established enough to spawn. Third, males may wait for females to arrive then intercept them on their upstream migration, follow them and establish a 'territory' near the female. In any of these scenarios, males arriving with or after females are less likely to mate with them than established males. However, males that arrived too early may have already left the stream or depleted their energy to the point where they cannot compete with more recent arrivals, hence the successful males tended to have arrived about two weeks prior to their mate.

Of all the juveniles, 39% were assigned only one parent and of those, 88% were assigned only a female parent. Several processes might prevent us from assigning parents to a juvenile trout, but we believe the contribution of mature male steelhead parr is the most likely explanation for many reasons. First, genotyping error may account for the inability to assign two parents to all juveniles, but it was unlikely to be biased toward assigning only a female parent because the DNA samples were not arranged by sex. Second, other types of laboratory or sample handling error could have caused us to be unable to assign two parents, but again this type of error was unlikely to be sex biased because tissue and DNA samples were arranged in the order that they arrived, not by sex. Third, although a few adults apparently passed the weir without being sampled, especially during floods that took place in two of the years, there was no systematic difference in timing of arrival between the sexes so this would not explain the surplus of males that were missed. Fourth, hybridization between male cutthroat or female steelhead, may have occurred. There is some evidence of steelhead hybridization with coastal cutthroat trout in Snow Creek (Young *et al.*, 2001). However, we tested our juvenile fish with a single powerful genetic marker (Growth hormone 2 intron D; Baker et al. 2002) and found very low hybridization rates (~2%).

Mature male parr occur in steelhead populations (Needham, Taft, 1934; Shapovalov, Taft, 1954) as well as in many other salmonids (Atlantic salmon, Salmo salar - Fleming, 1998; masu salmon, O. masou - Tsiger *et al.*, 1994; chinook salmon, O. tshawytscha - Unwin *et al.*, 1999) and in Atlantic salmon can have collectively high reproductive success as a group (Morán *et al.*, 1996). If we are correct in our deduction that mature male parr are the cause of the difference between missing male and female parents, then they are consistently achieving considerable reproductive success. In some years of low return the mature parr may collectively be more successful than anadromous male steelhead. Most importantly for conservation, mature parr may significantly increase the effective population size (Martinez *et al.*, 2000), thereby increasing the chances of persistence for small populations of steelhead. Ardren (2003) found that in Snow Creek the ratio of effective population size to the actual number of spawners was significantly higher in years with low spawner density. One explanation for this observed pattern may be a proportional increase in reproductive success of mature male parr when numbers of anadromous males are scarce. We currently have no estimate of their actual abundance in the population; mature parr have not been evident in our sampling operations in October.

Nearly 27% (294) of all juveniles were not assigned any parents. To account for potential errors we first assigned these juveniles to brood years using age/length histograms made from known age juveniles. Nearly 88% (258) were assigned to BY 1997 or 1999, both years in which there were floods. While it was known that high water compromised the weir in 1997 the extent to which the weir was compromised in 1999 was previously unknown. Only 23 and 13 juveniles from BY 1998 and 2000 respectively had no known parents. Tissue from these 36 juveniles and all the adults from BY 1998 and 2000 were re-extracted and re-genotyped at all loci resulting in six (two from BY 1998 and four from BY 2000) of the juveniles being assigned a single parent and one juvenile from BY 2000 that already had been assigned one parent having a second parent assigned.

The lack of parental assignment to the remaining 30 juveniles from BY 1998 or 2000 may have been due to several types of error. First, these juveniles may have been misassigned by size to BY 1998 or 2000. Some overlap in size distribution was observed, especially in 1998 YOY and 1997 yearling juveniles. Six juveniles without known parents sampled in 1998 fell within the size overlap range and could therefore be from BY 1997. Second, mutations at one or more loci could have occurred. Steinberg *et al.* (2002) recently calculated the mutation rate for microsatellite loci in pink salmon (*O. gorbushcha*) and found that two loci had mutation rates on the order of $4\text{-}9 \times 10^{-3}$ while the remaining six loci had no detected mutations. Therefore mutation does not explain a significant fraction of failed parental assignments. Third, null allele segregation could have occurred. Indeed three juvenile genotypes matched adult genotypes at all loci but *Omy77*, and at that locus both juvenile and adult were homozygous possibly indicating inheritance of a null allele. In addition, one adult individual would not amplify at locus *Omy77* despite repeated DNA extractions and PCR attempts. Since *Omy77* was known to have null alleles, it was assumed that this individual had two null alleles (or two copies of the same null allele) at this locus. Fourth, genotyping errors could have occurred.

Some electrophoretic variation at dinucleotide loci caused allelic size distributions to overlap. Rather than attempting to assign alleles to bins by probabilistic methods, overlapping alleles were combined into one size bin. This conservative method reduced the power of the single locus to assign parentage, but also reduced the genotype mismatch error at that locus. The loss of power in a single locus was offset by using many different loci to assign parentage. Fifth, some of the remaining juveniles may have been the offspring of unidentified rainbow trout, the non-anadromous form of *O. mykiss*, though this was unlikely since no known population of rainbow trout exists in the Snow Creek watershed (Thom Johnson, WDFW, 283236 Highway 101, Port Townsend, WA, 98368 – personal communication). Finally, some juveniles might have been cutthroat trout (*O. clarki*), which are nearly impossible to distinguish from steelhead at small sizes in the field without killing the fish. Snow Creek harbors a resident cutthroat population above a barrier waterfall as well as a very small anadromous population in the lower reach.

While not substantially different from the observed mating system of other Pacific salmon, the steelhead mating system did appear to be more similar to that of Atlantic salmon in that it included multiple mates and (apparently) male parr. The complexity and flexibility of steelhead mating behavior may be important to survival in a highly variable environment and may be one reason they have been able to persist at relatively low numbers for many generations.

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References

Ardren WR, Borer S, Thrower F, Joyce JE, Kapuscinski AR (1999) Inheritance of 12 microsatellite loci in *Oncorhynchus mykiss*. *Journal of Heredity* **90**, 529-536.

Ardren WR, Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (Ne) reveals genetic compensation in steelhead trout. *Molecular Ecology* **12**, 35-49.

Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* **42**, 485-508.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows ™ pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier (France).

Bentzen P, Olsen JB, McLean JE, Seamons TR, Quinn TP (2001) Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. *Journal of Heredity* **92**, 127-136.

Berejikian BA, Tezak EP, LaRae AL (2000) Female mate choice and spawning behaviour of chinook salmon under experimental conditions. *Journal of Fish Biology* **57**, 647-661.

Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP (1990) Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology* **27**, 315-324.

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection, p. 826. Academic Press, London.

Busby PJ, Wainwright TC, Bryant GJ, *et al.* (1996) Status review of west coast steelhead from Washington, Idaho, Oregon, and California, p. 261. U. S. Dep. Commer.

Chebanov NA, Varnavskaya NV, Varnavskiy VS (1983) Effectiveness of spawning of male sockeye salmon *Oncorhynchus nerka* (Salmonidae), of differing hierarchichal rank by means of genetic-biochemical markers. *Journal of Ichthyology* **23**, 51-55.

de Gaudemar B, Bonzom JM, Beall E (2000) Effects of courtship and relative mate size on sexual motivation in Atlantic salmon. *Journal of Fish Biology* **57**, 502-515.

DeWoody JA, Avise JC (2001) Genetic perspectives on the natural history of fish mating systems. *Journal of Heredity* **92**, 167-172.

Dickerson BR, Quinn TP, Willson MF (2002) Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethology Ecology and Evolution* **14**, 29-44.

Fields RD, Johnson KR, Thorgaard GH (1989) DNA fingerprints in rainbow trout detected by hybridization with DNA of bacteriophage M13. *Transactions of the American Fisheries Society* **118**, 78-81.

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 59-76.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Foote CJ (1988) Male mate choice dependent on male size in salmon. *Behaviour* **106**, 63-80.

Foote CJ (1989) Female mate preference in Pacific salmon. *Animal Behaviour* **38**, 721-722.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Foote CJ, Brown GS, Wood CC (1997) Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1785-1795.

Garant D, Dodson JJ, Bernatchez L (2001) A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* **92**, 137-145.

Gjerde B (1986) Growth and reproduction in fish and shellfish. *Aquaculture* **57**, 37-55.

Hanson AJ, Smith HD (1967) Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age-groups. *Journal of the Fisheries Research Board of Canada* **24**, 1955-1977.

Healey MC, Prince A (1998) Alternative tactics in the breeding behaviour of male coho salmon. *Behaviour* **135**, 1099-1124.

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology* **79**, 383-399.

Hutchings JA, Myers RA (1988) Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia* **75**, 169-174.

Jones AG, Avise JC (2001) Mating systems and sexual selection in male-pregnant pipefishes and seahorses: insights from microsatellite-based studies of maternity. *Journal of Heredity* **92**, 150-158.

Keenleyside MHA, Dupuis HMC (1988) Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **66**, 262-265.

Martinez JL, Moran P, Perez J, *et al.* (2000) Multiple paternity increases effective size of southern Atlantic salmon populations. *Molecular Ecology* **9**, 293-298.

Morán P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity* **77**, 655-660.

Morbey Y (2000) Protandry in Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1252-1257.

Needham PR, Taft AC (1934) Observations on the spawning of steelhead trout. *Transactions of the American Fisheries Society* **64**, 333-338.

Quinn TP, Adkison MD, Ward MB (1996) Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* **102**, 304-322.

Quinn TP, Foote CJ (1994) The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* **48**, 751-761.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Schroder SL (1981) *The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon* Ph.D., University of Washington.

Shapovalov L, Taft AC (1954) Life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. *California Department of Fish and Game, Fisheries Bulletin* **98**, 375.

Sillero-Zubiri C, Gottelli D, Macdonald DW (1996) Male philopatry, extra-pack copulations and inbreeding avoidance in Ethiopian wolves (*Canis simensis*). *Behavioral Ecology and Sociobiology* **38**, 331-340.

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Canadian Journal of Zoology* **77**, 836-841.

Steinberg EK, Lindner KR, Gallea J, *et al.* (2002) Rates and patterns of microsatellite mutations in pink salmon. *Molecular Biology and Evolution* **19**, 1198-1202.

Taggart JB, McLaren IS, Hay DW, Webb JH, Youngson AF (2001) Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Molecular Ecology* **10**, 1047-1060.

Tautz AF, Groot C (1975) Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **32**, 633-642.

Tsiger VV, Skirin VL, Krupyanko NI, Kashlin KA, Semenchenko AY (1994) Life history form of male masu salmon (*Oncorhynchus masou*) in South Primor'e, Russia. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 197-208.

Unwin MJ, Kinnison MT, Quinn TP (1999) Exceptions to semelparity: Postmaturation survival, morphology, and energetics of male chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1172-1181.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Westneat D (1987) Extra-pair fertilizations in a predominantly monogamous birds: genetic evidence. *Animal Behaviour* **35**, 877-886.

Young WP, Ostberg CO, Keim P, Thorgaard GH (2001) Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). *Molecular Ecology* **10**, 921-930.

Zar JH (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Upper Saddle River, New Jersey.

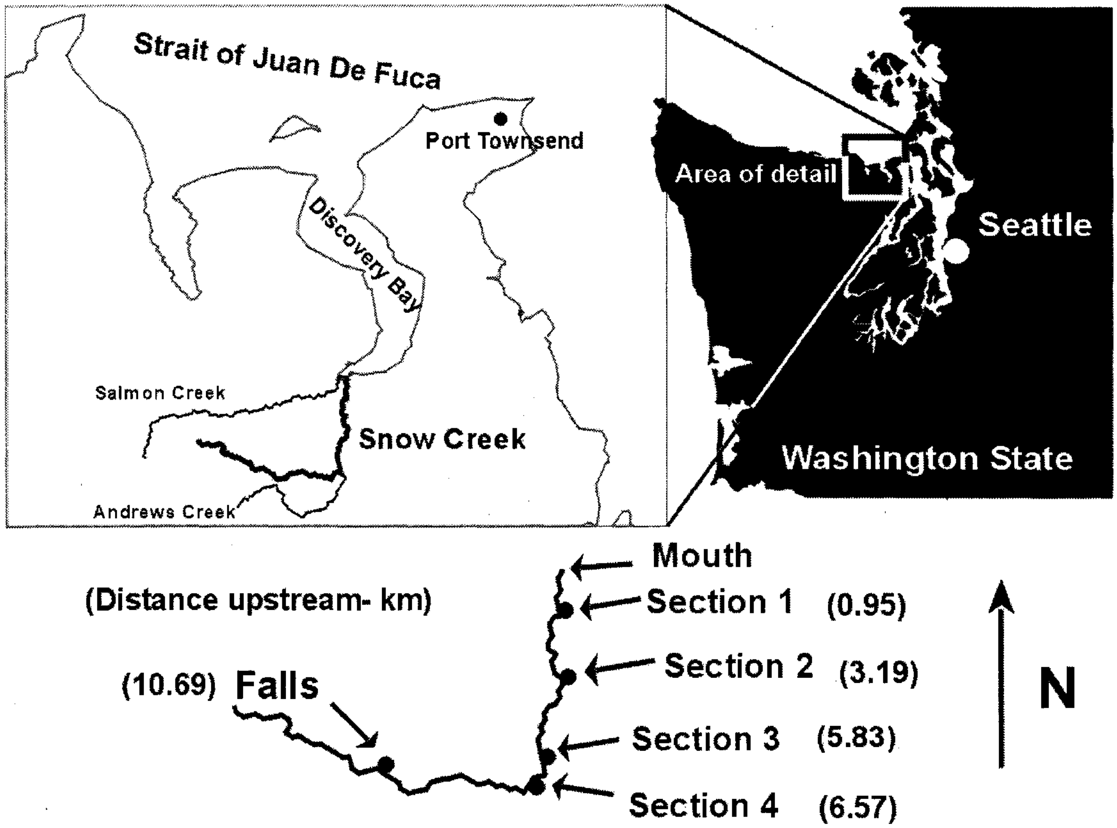

Figure 1.1. Map of western Washington State showing the location of Snow Creek, sampling sections within Snow Creek and their distance upstream from the mouth at Discovery Bay.

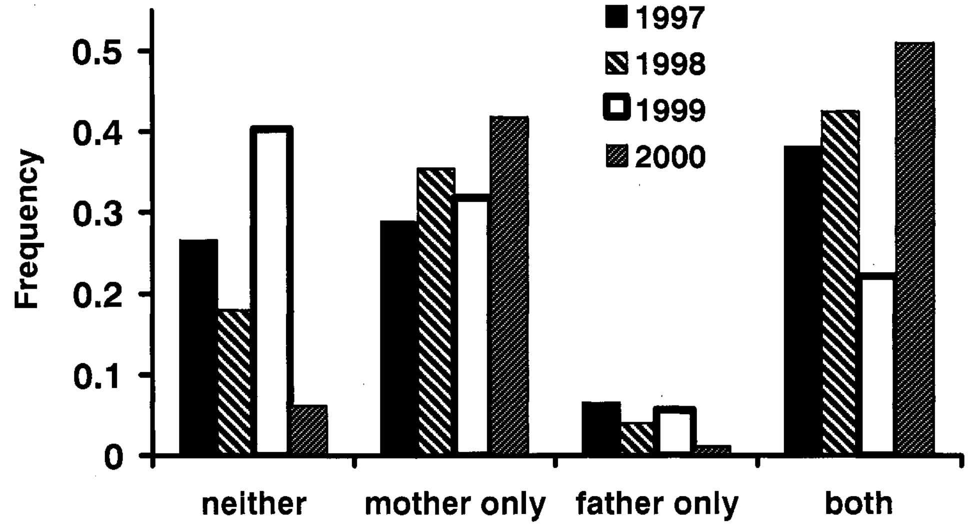

Figure 1.2. Frequency of the type of parental assignment - both parents, one parent (mother or father only) or neither parent - for all juvenile fish samples by brood year (1997-2000).

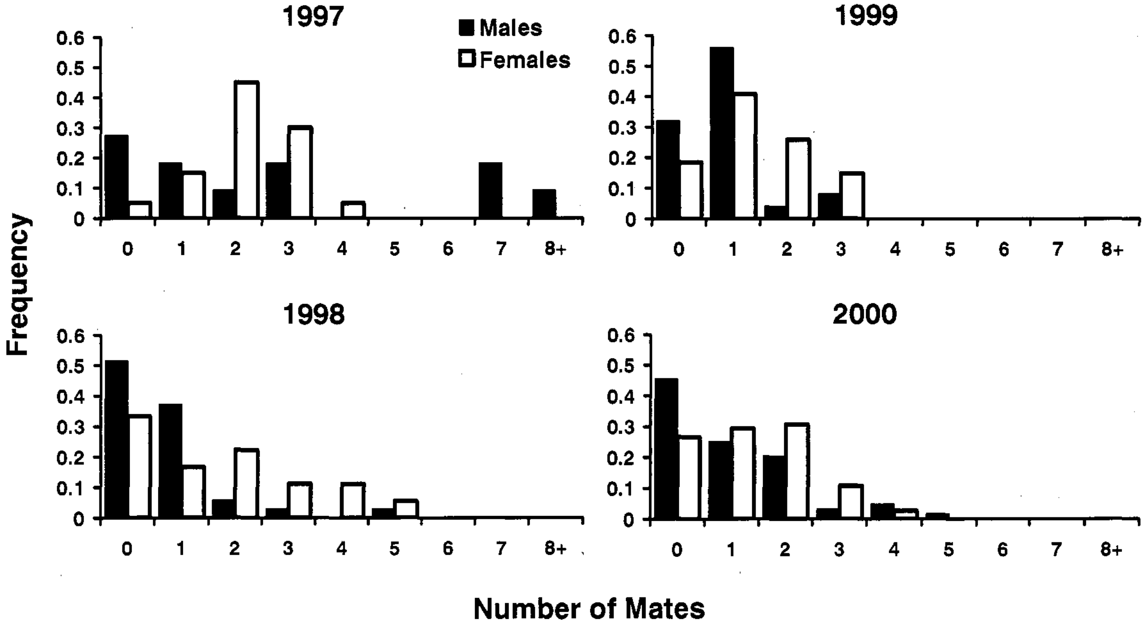

Figure 1.3. Estimated frequency of inferred numbers of different mates for males (black) and females (white) in four consecutive brood years (1997-2000).

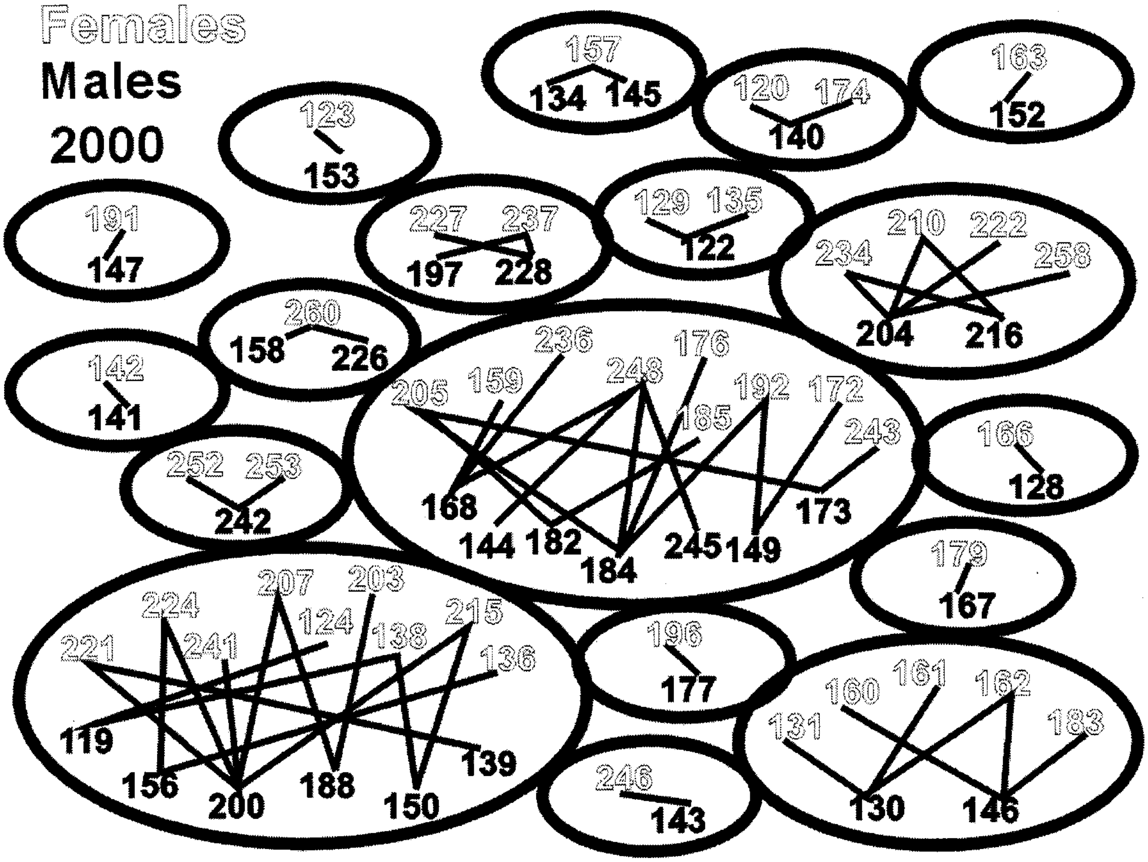

Figure 1.4. Inferred mating patterns of steelhead in Snow Creek from brood year 2000. Each oval encompasses the individual parents (shown as identification numbers) in a mating group inferred from an extended half-sibling family of offspring. Lines connect males (solid black numbers) and females (white with black outline numbers) with their inferred mate(s). Four types of mating were apparent: monogamy, polygyny, polyandry and polygynandry.

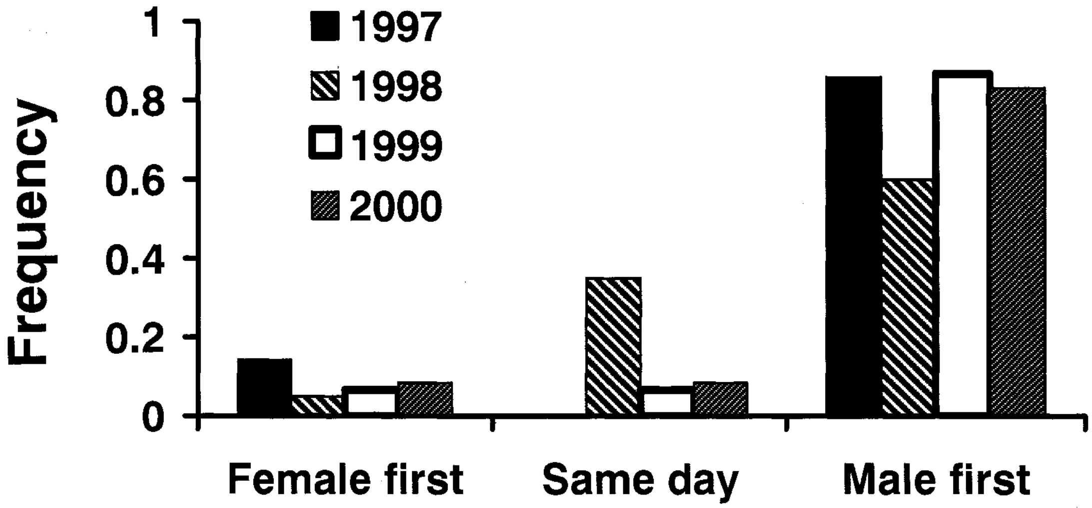

Figure 1.5. The order of arrival at the Snow Creek weir for inferred mated pairs of adult steelhead. Frequencies of each type of arrival order (female first, same day, male first) are shown for brood years 1997-2000.

Table 1.1. Microsatellite loci used for parentage assignment, references for each locus, annealing temperature (T_A), magnesium chloride concentration in mM and repeat unit in base pairs (bp).

Locus	Source	T_A	[$MgCl_2$]	Repeat Unit (bp)
Oki23	A. Spidle[a]	55	1	4
Omy1001UW	P. Bentzen[b]	55	1	4
Omy1004UW	P. Bentzen[b]	50	1	4
Omy1011UW	P. Bentzen[b]	55	1	4
Omy1191UW	P. Bentzen[b]	65	1	4
Omy1212UW	P. Bentzen[b]	65	1	4
Omy77	Morris et al. 1996	55	1	2
One108	Olsen et al. 2000	55	1	4
One2	Scribner et al. 1996	55	1	2
Ots107	Nelson & Beacham 1999	55	1.5	4
Ots108	Nelson & Beacham 1999	55	1.5	4
Ssa85	O'Reilly et al. 1996	60	2	2

[a] – Unpublished, GenBank accession # AF272822

[b] – Unpublished, Paul Bentzen

Table 1.2. Number of steelhead males and females (N_M/N_F), total number of returning adults (N_T), male length size range (M_{SR}), average male length (Avg_M), female length size range (F_{SR}), average female length (Avg_F), male median return date (M_{DATE}) and female median return date (F_{DATE}) for each brood year (1997-2000).

Brood Year	N_M/N_F	N_T	M_{SR}	Avg_M	F_{SR}	Avg_F	M_{DATE}	F_{DATE}
1997	11/20	31	420 – 750	612.7	595 – 830	692.3	25-Mar	28-Mar
1998	35/18	53	600 – 735	649.6	565 – 795	638.6	20-Mar	21-Mar
1999	25/27	52	400 – 815	655.6	600 – 740	658.3	23-Mar	23-Mar
2000	64/75	139	430 - 785	645.4	565 - 860	640	23-Mar	25-Mar
Total	135/140	275						

Table 1.3. Allelic variability at 12 microsatellite loci in four brood years of steelhead in Snow Creek. Number of alleles per locus (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability after test for goodness of fit to HWE (P_{HWE}), inbreeding coefficient (F_{IS}) and the total number of alleles across all brood years (Total N_A) are shown.

		Locus											
Brood Year		*Oki23*	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1997	N_A	17	12	12	14	18	19	9	11	24	24	16	12
	H_E	0.89	0.87	0.74	0.88	0.92	0.92	0.8	0.88	0.92	0.94	0.91	0.86
	H_O	0.87	0.96	0.84	0.9	1	0.9	0.73	0.87	1	0.87	0.87	0.9
	P_{HWE}	0.75	0.91	0.14	0.62	0.99	0.22	0.13	0.22	0.15	0.37	0.2	0.28
	F_{IS}	0.045	-0.094	-0.119	-0.014	-0.075	0.037	0.096	0.031	-0.073	0.093	0.064	-0.036
1998	N_A	16	12	9	15	18	24	11	14	24	38	19	16
	H_E	0.88	0.86	0.83	0.89	0.9	0.94	0.82	0.88	0.93	0.96	0.91	0.86
	H_O	0.92	0.86	0.85	0.94	0.91	0.96	0.77	0.89	0.98	0.94	0.92	0.89
	P_{HWE}	0.86	0.33	0.39	0.68	0.6	0.53	0.01	0.33	0.46	0.36	0.36	0.82
	F_{IS}	-0.037	0.006	-0.015	-0.055	0.007	-0.012	0.061	0.003	-0.043	0.025	0.003	-0.025
1999	N_A	15	15	12	20	19	11	14	17	29	41	19	18
	H_E	0.87	0.89	0.83	0.91	0.91	0.93	0.84	0.9	0.94	0.93	0.9	0.87
	H_O	0.9	0.96	0.79	0.9	0.88	0.94	0.73	0.85	0.94	0.96	0.87	0.85
	P_{HWE}	0.22	0.42	0.61	0.26	0.76	0.38	0.03	0.01	0.03	0.64	0.44	0.19
	F_{IS}	-0.029	-0.069	0.056	0.012	0.036	-0.001	0.137	0.066	0.011	0	0.052	0.037
2000	N_A	18	17	11	19	22	32	16	17	33	53	22	23
	H_E	0.9	0.88	0.8	0.89	0.9	0.95	0.79	0.9	0.94	0.96	0.91	0.83
	H_O	0.95	0.9	0.78	0.92	0.93	0.96	0.63	0.87	0.99	0.96	0.88	0.83
	P_{HWE}	0.14	0.15	0.75	0.9	0.49	0.18	0.0000*	0.02	0.004	0.06	0.001*	0.37
	F_{IS}	-0.054	-0.019	0.021	-0.033	-0.038	-0.016	0.209	0.037	-0.052	0.008	0.028	0.012
Total	N_A	20	18	15	20	25	35	17	19	39	58	25	27

* - significant at $\alpha = 0.05$

Table 1.4. Numbers of different types of mating groups (1 male (M)-1 female (F), 1 male-2 females, 2 males-1 female, multiple males-multiple females) inferred from extended half-sibling families of juvenile offspring for brood years 1997-2000. The numbers of adult males (N_M) and females (N_F) in the largest mating group with multiple individuals of both sexes are in parentheses.

Brood Year	**1M-1F**	**1M-2F**	**2M-1F**	**nM-nF (N_M/N_F)**
1997	2	0	0	1 (5/14)
1998	2	0	3	1 (5/5)
1999	3	2	2	1 (3/3)
2000	8	3	2	5 (7/9)

Chapter 2: The effects of adult length and arrival date on individual reproductive success in wild steelhead trout, *Oncorhynchus mykiss*

Synopsis

To determine the relative importance of adult size and arrival date on reproductive success in a natural setting, we first genetically determined relationships between all spawning adult steelhead trout (*Oncorhynchus mykiss*) and juvenile offspring in four brood years. We then tested hypothesized relationships between parents' phenotypes and the number and size of their juvenile offspring. Patterns of reproductive success varied among years, but in all cases a few adults of each sex produced most of the offspring. In general, offspring number was poorly explained by parental attributes and offspring size was primarily related to the mother's arrival date (early arrival = larger progeny). The number of offspring showed a weak, positive relationship to the mother's size, but not to her arrival date. Paternal reproductive success varied considerably, but was only weakly associated with size in 1998 and weakly related to arrival date in 2000. In 1997, 1998 and 1999, the offspring of early spawning females were larger at the end of their first and second summers in the stream, however in 2000 both arrival date and length were positively related to offspring size. There was no general trend in offspring size in relation to paternal size or arrival date, however paternal length affected offspring size in 1998 and male arrival date affected offspring size in 2000.

Introduction

In salmonid fishes, body size is widely believed to determine reproductive success. Larger females produce more numerous and larger eggs (e.g., Beacham, Murray, 1993), and are able to bury their eggs deeper (Steen, Quinn, 1999), reducing the likelihood of disturbance by the digging of other females (Essington *et al.*, 2000), and protecting them from streambed scour during high flow events. Large females may also be able to exclude smaller females from the best spawning sites, though prior residence effects may outweigh size (Foote, 1990). Larger males can exclude smaller males from access to females through aggressive interactions (Keenleyside, Dupuis, 1988; Quinn, Foote, 1994). However, dominance is also influenced by prior residence (Foote, 1990), and by the level of competition for ripe females, which is a function of date within the spawning period (Quinn *et al.*, 1996).

Salmonids breeding early risk having their redd disturbed by the digging of later-arriving females, and such density-dependent mortality can greatly affect the production of juveniles (Essington *et al.*, 2000; Fukushima *et al.*, 1998). Small males arriving early may gain access to early arriving ripe females while later in the spawning season even very large males may be unable to dominate access to females (Dickerson *et al.*, 2002).

The size of juveniles may be affected by both maternal size, through correlations with egg size (e.g., Beacham, Murray, 1990), and by parental spawning date (Einum, Fleming, 2000). The progeny of early-spawning adults tend to emerge from the gravel early, and may benefit by territory acquisition by prior claim (Chandler, Bjornn, 1988; Titus, Mosegaard, 1991) and advantages in territorial competition from greater size (Keeley, McPhail, 1998) when later fry emerge. Mortality of juveniles can be size-selective in fresh water (Quinn, Peterson, 1996; Smith, Griffith, 1994) and at sea (Healey, 1982; Ward *et al.*, 1989), though the strength of selection as well as the direction may vary with rearing environmental conditions (Good *et al.*, 2001). However, relative size within a population may not be maintained as time passes (Heath *et al.*, 1999; Herbinger *et al.*, 1995), depending on food resources (Einum, Fleming, 1999), and there may be survival costs to territorial behavior. In addition, early emergence may lead to poor growth if food is not available, and survival rates may be reduced if there is extended exposure to predation (Brännäs, 1995).

Almost all studies of selection on size and breeding date in salmonids have been based on behavioral observations, often in experimental settings, and most have measured a surrogate for adult or juvenile fitness such as social dominance or body size rather than actual reproductive success. Population level studies have produced equivocal findings, including evidence for (Garant *et al.*, 2001; Helle, 1989) and against (Holtby, Healey, 1986) the hypothesis that larger fish are more productive. Studies using genetic analysis of the parentage of progeny to directly determine reproductive success have almost invariably been conducted in artificial channels or arenas (e.g., Schroder, 1981), and progeny have generally been sampled as embryos (e.g., Taggart *et al.*, 2001), precluding consideration of the compensatory processes that operate during later life history stages. Moreover, most studies have neglected variation in female reproductive success and focused on paternity (Jordan, Youngson, 1992; Morán *et al.*, 1996; Schroder, 1981). Finally, it is important to determine whether possible interactions between adult size and reproductive timing are additive, multiplicative, or whether compensatory processes in natural populations minimize the effects of these factors.

The objective of this study was to determine the relative importance of adult body size and arrival date on reproductive success in salmonids, using a wild population of steelhead (*Oncorhynchus mykiss*) as the model system. We predicted that larger females would have larger and more numerous offspring than smaller females, and that larger males would have more offspring than smaller males. We further predicted that the progeny of early arriving adults (male or female) would be larger at the end of their first summer of life, and would remain larger in their second summer than the progeny of later breeders.

Methods

Sampling site, collection methods and genetic analysis

Each spring from 1997 to 2000 the length, sex and date of upstream migration were recorded for all adult steelhead captured at a permanent fish weir on Snow Creek, Washington. Scales were collected for age estimation, and small pieces of the caudal fin or other tissue were taken for DNA analysis. Our basic assumption was that the weir allowed us to sample the entire spawning population. However, flooding occurred in both 1997 and 1999, enabling adults to pass upstream unsampled. This problem was ameliorated to some extent because a hole was punched in the opercle of adults captured in the upstream trap. Post-spawning adults migrating downstream ("kelts") were examined and any that were unmarked we sampled at that time.

Caudal fin tissue from approximately 300 juvenile steelhead was collected annually the first week of October 1997-2000 by single-pass electrofishing in four different sections (each about 100 m long) of Snow Creek. Sampling sites were primarily chosen to cover the upper and lower ends of the range of steelhead rearing habitat with two sections in the middle (Seamons *et al.*, 2004), and secondarily for accessibility.

There were no obvious differences in habitat characteristics (e.g., gradient, substrate size) among sampling sites. Immediately above our uppermost sampling site the gradient appeared steeper with more exposed bedrock. Although juvenile steelhead were found in this habitat, there were substantially fewer compared to the lower reaches encompassed by our sampling sites. All tissue samples were stored in 95% ethanol in 1.5-mL microcentrifuge tubes at room temperature.

DNA extracted from adult and juvenile tissue was used to PCR (polymerase chain reaction) amplify 12 microsatellite loci (Table 2.1). All loci were visualized on the MegaBACE 1000 capillary electrophoresis system (Amersham Pharmacia Biotech Limited, Piscataway, New Jersey) using fluorescently labeled forward primers for each locus. DNA extraction protocols, PCR conditions and thermalcycler profiles can be found in (Seamons *et al.*, 2004).

Locus characteristics, parentage and statistical analysis

Adult genetic data from all loci in each sampling year was tested for conformation to Hardy-Weinberg proportions (HWE) with a two-tailed exact test using the Markov Chain method implemented in Genepop 3.1 (dememorization number 1000, batches 100, 1000 iterations per batch; Raymond and Rousset 1995). Significance of probability values was adjusted for multiple tests using sequential Bonferroni correction (Rice, 1989). Observed and expected heterozygosity were calculated using GENETIX 4.02 software (Belkhir *et al.*, 2001). F_{IS}, a measure of inbreeding, was calculated according to Weir and Cockerham (1984) using Genepop 3.1 software (Raymond, Rousset, 1995).

A list of potential parents was constructed for each juvenile using the program WHICHPARENTS 1.0 (Will Eichert, Bodega Bay Marine Lab, California), which compiled a list of all adults that matched one allele at each locus with each juvenile. When compiling the list we used all 12 loci, but allowed three mismatches. The genotypes of adults identified by WHICHPARENTS as potential parents were then directly compared to juvenile genotypes and both adult and juvenile genotypes were checked for errors. Only adults that matched one allele at each locus were assigned as parents. As a conservative measure, because of repeat spawning and the possibility of incomplete adult sampling in some years, all four years of adults were treated as potential parents of all juveniles. In many cases only one parent was assigned to a juvenile. To determine the veracity of our single parental assignment we calculated the probability of finding an unrelated adult that by chance matched one allele at each locus with a juvenile using the equation:

$$\text{p(random match)} = \prod_{i=1}^{n} 1 - \left[1 - \left(\text{pA}_i + \text{pB}_i\right)\right]^2 \tag{1}$$

where pA is the probability of matching allele A and pB is the probability of matching allele B based on the parental population allele frequencies, multiplied across n loci. If no adults matched one locus at each allele with a juvenile we assumed that the actual parent(s) had not been sampled. Juveniles not assigned any parents were dropped from subsequent analyses.

To rule out sample processing error as an explanation for our disability to assign at least one parent to all juveniles, we assigned juveniles that had no identified parents to brood years (BY, the year that they were spawned) using age/length histograms made from known age juveniles. DNA from juveniles and all adults from BY 1998 and 2000 was re-extracted and re-genotyped at all loci, and parentage assignment was performed a second time. These two BY were chosen because they had fewer juveniles with no assigned parents, allowing us to minimize the expense of re-processing.

To adjust for yearly differences in adult arrival timing and size, all such data were standardized by BY to facilitate combined analyses. Lengths of individual adults were subtracted from the yearly average adult length by sex. Return dates were analyzed as days before or after the median return date by sex within years. Average offspring lengths for each family were calculated and then standardized to the average length of juveniles of that age for that BY. Ages of juveniles were determined from parentage data. About 10% of the adults spawned in more than one year and these individuals were treated as if they were different individuals in the subsequent return year because there was no statistically valid way to compare their performance among years, given the small number of such fish and other confounding factors.

We were unable to record the date when individual fish actually spawned so we used the date of their arrival at the weir as a surrogate for breeding date. Of the 31 steelhead that returned in 1997, only four were captured in the upstream trap before the high water event; the rest were captured as downstream migrants after the water had subsided. The actual return date of these fish is unknown, but we assumed that it was during the week when the trap was disabled, so we assigned the mid-week date as their arrival date for analysis. Though flooding also occurred in 1999, all adult steelhead sampled in 1999 were caught in the upstream trap so no estimation of arrival dates was necessary.

Relationships between size and arrival date of parents and the number and size of offspring were first analyzed using a multicovariate weighted least squares general linear model (WLS-GLM; type III; SPSS Version 8.0). Covariates consisted of standardized parent length, return date and year as a fixed factor (for any unaccounted for interannual variation) against number of assigned offspring or average offspring length (within maternal or paternal half-sib families) standardized by the average juvenile length in each brood year. Selection was expected to be stabilizing on reproductive timing in salmonids; therefore the term return date squared was included for quadratic analysis of arrival date effects on numbers of offspring for females (Lande, Arnold, 1983). Analyses of number of offspring were weighted by yearly sample size because the number of juveniles sampled varied among years. Analyses of offspring size were weighted by the inverse of the family size variance since families consisted of different numbers of individuals. All possible interaction terms were included in all GLM analyses. If in any test the year or a year interaction term was significant, tests were run as weighted least squares linear regressions (SPSS Version 8.0) using individual yearly data. The response variable, "number of assigned offspring," was natural log transformed to correct for heteroscedasticity in the data. Whenever individual years were analyzed separately by regression analysis, sequential Bonferroni adjustments for multiple tests were made (Rice, 1989).

Undoubtedly, many families of steelhead were missed during our juvenile sampling. Adults not assigned any offspring may have actually produced no offspring or they may have produced offspring that were rearing between our sampling sites. Including adults that produced no offspring would bias analyses, as would excluding adults that produced some offspring. Though it likely affected adult females more than adult males, we chose to exclude all adults that had no assigned offspring from GLM and regression analyses.

Results

Characteristics of adults

From 31 (in 1997) to 139 (in 2000) adult steelhead were captured and sampled at the Snow Creek weir (Table 2.2). The overall sex ratio was close to 1:1, but varied considerably among years. In 1997 there were nearly twice as many females as males, in 1998 the reverse ratio was observed, and in 1999 and 2000 the sex ratios were nearly 1:1 (Table 2.2). Body lengths varied considerably: males from 420 - 815 mm, and females from 565 - 860 mm. Although the date of first return to Snow Creek varied from December to March, in all years, most steelhead returned in March and April, and the median date of return was in the last week of March in all four years.

Locus characteristics, parentage and familial relationships

Only 2 tests out of 48 were out of HWE after sequential Bonferroni corrections (Table 2.1). Both *Omy77* and *Ots108* showed deviation from HWE ($P < 0.0001$ and $P = 0.001$, respectively). Each was associated with a significant positive F_{IS} value associated with null allele segregation (*Omy77*, $F_{IS} = 0.209$; *Ots108*, $F_{IS} = 0.028$). Average heterozygosity across all 12 loci in our sample was 90% and the probability of exclusion per locus ranged from 0.93 (*Ots107*) to 0.64 (*Omy77* and *Omy1004*). The probability of exclusion using all 12 loci was 0.9999 (CERVUS 2.0; Marshall et al. 1998).

We were able to assign at least one parent to 73% of all juvenile samples, and two parents to approximately 40%. The chance of an unrelated adult matching at random the most common possible juvenile genotype (i.e., homozygous for the most frequent allele at each locus in the parent pool) for each year was between 1 and 2 in 1000. Thus in cases where only one adult was assigned we concluded that that adult was indeed one of the parents. Nearly 27% (294) of all juveniles were not assigned any parents. Based on their body length, 88% of them (258 fish) were apparently from BY 1997 or 1999, both years in which there were floods. Only 23 and 13 juveniles from BY 1998 and 2000, respectively, had no known parents. Re-processing of these 36 juveniles and all adults from BY 1998 and 2000 resulted in six (two from BY 1998 and four from BY 2000) of the juveniles being assigned a single parent and one juvenile from BY 2000 that already had been assigned one parent having a second parent assigned. The genotyping error rate (i.e., total number of incorrect alleles/total number of alleles) based on re-processed adult samples was 0.6% compared to 3% in re-processed juveniles.

Twelve adult steelhead returned to spawn in Snow Creek in more than one year (4% of the total), and they produced 54 juvenile offspring. These offspring were assigned to a BY by one of two methods. In 31 cases a male and female adult from one BY and only a single adult from another BY (the repeat year of one of the assigned adults) were assigned to a juvenile, thus we assumed that it was spawned in the BY for which two parents were assigned. In 23 cases only one parent was assigned (the same individual, but two BY), so the juveniles were assigned to a BY based on juvenile age/length relationships from known age juveniles.

At least one parent was assigned to 191 juveniles from BY 1997, 141 from BY 1998, 210 from BY 1999 and 245 from BY 2000 (Table 2.2). Not all adults contributed either young-of-the-year (YOY) or yearling offspring to our samples (Figure 2.1). Over 50% of the YOY were the offspring of just 7% (10) of the males and 14% (20) of the females. More than 50% of the yearlings were assigned to only 2% (3) of the total number of males and 6% (9) of the total number of females. Many families present in the YOY age-class were not present in the yearling age-class, consequently the number of adults that failed to produce offspring in our samples increased from 45% to 75% for males and from 28% to nearly 40% for females from YOY to yearlings, respectively. Males were more likely than females to have no offspring assigned to them, and the parents with the most offspring were also males. For both YOY and yearlings, the distributions of offspring among males and females were significantly different (pooled data, two-sample Kolmogorov-Smirnov test, $P = 0.002$ and $P < 0.001$ respectively).

Factors affecting the number of offspring

From 1 to 22 YOY offspring were assigned to individual adult female steelhead, with means ranging from 4.4 offspring per female in 2000 to 9.3 offspring per female in 1998 (Table 2.3). Standardized variation (σ^2/mean^2) ranged from 0.47 in 1998 to 0.89 in 2000. The range of and mean number of yearling offspring assigned to adult female steelhead also varied little among years (Table 2.3). Standardized variation ranged from 0.63 in 1999 to 1.06 in 1998.

The GLM model which best explained the variation in the number of YOY offspring assigned to females included only the year term ($P = 0.035$) and the length term ($P = 0.05$), however this model explained very little variation ($r^2 = 0.09$). Separate regression analysis for each year also failed to reveal any significant linear relationships (Table 2.4). However, females that produced five or more YOY offspring were the largest, followed by those that produced three or four offspring, and those that produced one or two were the smallest (Table 2.5, one-way ANOVA, $F_{3,136} = 5.38$, $P = 0.002$; categories binned to homogenize variance and sample size). Interestingly, the females that produced no detected offspring were intermediate in size, consistent with the idea that this group included both females producing no offspring and some that produced offspring that we did not sample. No GLM model successfully explained the number of yearling offspring assigned to adult females, nor did any regression analyses (Table 2.4). ANOVA comparisons among females categorized by number of yearling offspring revealed no significant differences in mean length among groups (Table 2.5).

Yearly ranges of YOY offspring assigned to adult male steelhead varied among years from 1-20 in 1997 to 1-29 in 1998 (Table 2.3), and mean values ranged from 3.9 offspring per male in 2000 to 10.0 in 1997. Standardized variation ranged from 0.40 in 1997 to 2.64 in 1998. Ranges in number of yearling offspring assigned to adult male steelhead also varied among years from 1-3 per male in 1999 to 1-15 in 1997, and means varied from 1.4 in 1999 to 5.1 in 1997. Standardized variance ranged from 0.41 in 1999 to 0.96 in 1997.

The GLM model that best explained variation in number of YOY offspring assigned to adult male steelhead included length and several year, length and timing interaction terms ($P < 0.02$, all terms), but explained little of the variation ($r^2 = 0.23$). Regression analysis of individual yearly data revealed a significant relationship between length and number of offspring assigned in 1998; no other regression analyses revealed significant relationships (Table 2.4). One-way ANOVA of mean lengths of males, categorized by number of YOY offspring assigned, was not significant (Table 2.5), unlike females. No GLM model explained any variation in the number of yearling offspring assigned to adult male steelhead. Linear regression of individual yearly data also yielded no significant results (Table 2.4), as did one-way ANOVA analysis (Table 2.5).

Factors affecting the size of offspring

The best GLM model for variation in YOY offspring size included adult female length, arrival date and the year*length interaction term ($P < 0.04$ all terms) and explained a large proportion of the variation ($r^2 = 0.64$). Linear regression analysis revealed that in 1997, 1998 and 1999 arrival date alone explained YOY offspring size (early arrival = large progeny on the sampling date), and in 2000, length and arrival date both explained YOY offspring size variation (Table 2.4). Plotted against maternal arrival date, a range of about 35 mm in mean length was observed between maternal half-sib families of YOY offspring (Figure 2.2).

Yearling offspring size variation was best explained by the GLM model which included arrival date and the year*length interaction term ($P < 0.04$ both terms, $r^2 = 0.57$). Linear regression analysis revealed a significant relationship between maternal length and yearling offspring size in 1997 and between maternal arrival date in 1998 and 1999 (Table 2.4). A range of about 27 mm was observed between maternal half-sib families of yearling offspring (Figure 2.2).

Only the year and year*arrival date interaction terms explained any variance in the size of YOY offspring attributable to the father (GLM; $P < 0.007$ both terms, $r^2 = 0.33$). Linear regression analysis of individual yearly data showed that length alone predicted offspring size in 1998 and timing alone predicted YOY size in 2000 (Table 2.4). Neither 1997 nor 1999 showed significant effects of either length or timing. The small number of paternal half-sib families of yearling juveniles (3 in 1998, 2 in 1999) precluded any statistical analysis of effects of male traits on yearling size.

Discussion

Factors affecting the number of offspring detected

Contrary to predictions, little of the variation in offspring number was explained by female size or arrival date, though smaller females tended to have fewer YOY offspring. As suggested by Holtby and Healey (1986), fitness may be equal among females of various sizes, or may be explained by variation in nest site characteristics. In a low density spawning environment, competition for redd sites may be low (van den Berghe, Gross, 1989), so all females may have been able to find suitable spawning sites with little competition or redd site re-use. In addition, scour may not have affected survival of embryos at all, or it may have done so in a manner that was not size-selective. Simple models of scour depth predict that redds of smaller females should be more vulnerable than those of larger females in years of high discharge (e.g., Steen, Quinn, 1999). However, there is considerable variation in the depth of gravel scour among habitats within even a small area of stream (e.g., Lapointe *et al.*, 2000; Schuett-Hames *et al.*, 2000), and the habitats apparently preferred such as the tailouts of pools are also most vulnerable to scour during floods. If small and large females use different types of habitats, then the differential vulnerability of the sites to scour would result in complex relationships between body size and reproductive success that would vary among years.

A similar study on Atlantic salmon (*Salmo salar*) also failed to find a significant linear relationship between female length or fecundity and number of assigned offspring except when eight large females, assumed to be repeat spawners, were removed from the analysis (Garant *et al.*, 2001). Exclusion of repeat spawners from analysis in our study did not change the significance or nature of the relationship, however. Comparison of the reproductive success of females in their first breeding season to that in subsequent years for iteroparous species such as steelhead and Atlantic salmon is important but the effort will be hindered by low and variable numbers of repeat spawners and other factors affecting success including but not limited to body size, scour, redd reuse, etc. Previous studies (e.g., Fleming, Gross, 1994; van den Berghe, Gross, 1989) have emphasized the strong positive effect of female size on many aspects of reproductive success. Despite the logic and empirical evidence for each of these processes, the net result that we observed was much weaker than the sum of all the individual relationships.

We also found no clear relationship between adult male body size and number of offspring. Large males are observed excluding smaller males from access to ripe females (Quinn, Foote, 1994) and it is widely assumed that this behavioral dominance translates to greater reproductive success. While many males had no assigned offspring, there was no relationship to male length in three of four BYs. However, in one BY, male length was positively correlated with the number of offspring. One of the two largest males seen that year arrived early in the season, stayed late (inferred from the fact that he mated with a female that arrived near the end of the season) and was quite successful (sired 40% of the YOY juveniles) suggesting that size and arrival timing, combined with residence time on the spawning grounds may influence male reproductive success. This is consistent with correlations with longevity in male pink salmon (*O. gorbuscha*; Dickerson 2003), though steelhead, unlike Pacific salmon (*Oncorhynchus* spp.), do not necessarily die on the spawning grounds. Instead, male and female steelhead may return to the ocean after spawning. Therefore, male steelhead are expected to stay on the spawning grounds as long as possible to maximize mating success. We were unable to measure residence times for steelhead except for inferences from parentage data in brood years 1997-2000. However, double-sampling of steelhead as they migrated up and down past the weir revealed considerable variation in male residence time in brood year 2001 (mean = 27 days, $\sigma^2 = 350.5$). Such variation in male residence time on the spawning grounds may have confounded any connection with size.

Clearly the key to increased reproductive success is not as simple as being big, returning early and staying late. The operational sex ratio experienced by males may affect their ability to realize their potential reproductive success. In 1998, when our large, early and steadfast male was successful, we were not able to estimate the number of males upstream of the weir at any point in time, but over the entire spawning season the sex ratio was highly skewed towards males, therefore it is likely that at any point in time there were several males per female. Size may have had a larger effect in this year, whereas in years when more females were available, even small males would be able to mate. This idea is supported by the fact that in 1997, when the sex ratio was highly skewed towards females, the standardized variation was the lowest, compared to the highest standardized variation in 1998.

In addition, timing of breeding is generally believed to have evolved to optimize rearing conditions of offspring. There is a high heritability ($h^2 > 0.8$) for the timing of migration and spawning in salmonids (e.g., Quinn *et al.*, 2000), but success likely varies among years, as affected by fish densities and density-independent factors such as flow and temperature. We likely did not sample enough brood years to detect the small amount of variation in reproductive success due to spawning date, which would have evolved over many generations.

Finally, there are two possible methodological explanations for the lack of a relationship between parental traits and numbers of offspring: the small fraction of juveniles sampled, and their localized spatial distributions. Power to detect significant variation in family size depended on the number of families sampled as well as the number of individuals sampled from each family. The possible number of families depended on the number of adults returning and the mating system (Seamons *et al.*, 2004). The number of sampled juveniles ideally would change with the number of spawning adults in order to have the same chances of sampling a certain number of individuals from a single family, but this was not possible in a natural system. In addition, a representative sample would include sampling a large proportion of rearing habitat. We sampled four sections of Snow Creek, representing a small proportion of the rearing habitat (about 5% by stream length). Undoubtedly some families were not represented because parents spawned and progeny reared between sampled sections (i.e., no juvenile dispersion). As a qualitative test of the magnitude of juvenile dispersion, we tabulated the sections where members of full-sib families were sampled. Over 30% of the YOY full-sib families were found in two sampling sections (about 1.5 km apart) and 70% were found in only one section. When yearling members of the same full-sib families were added, 5% of full-sib families were found in three sampling sections, ~ 35% were found in two sampling sections and 60% in one. These results suggest considerable post-emergence dispersal by juveniles, especially in their second year. In spite of this we likely missed many families since, due to differential survival, the number of families in existence decreased with time, becoming rare or disappearing altogether, and as yearlings dispersed, they also became much less numerous than the YOY.

In spite of this evidence of juvenile dispersion, we chose to exclude adults assigned no offspring from analyses. Doing so likely underestimated the variance and overestimated the mean reproductive success since it was likely that some of these adults may have actually reproduced but their offspring were unsampled. Given higher expected variance in reproductive success in males we would expect this to affect females more than males in our analyses. Indeed, the mean length of females with no assigned offspring was higher than the mean length of those females with one or two offspring and lower than that of females with three or more offspring while there was no difference among male lengths assigned various numbers of offspring. It is possible that some of the larger females that apparently failed to reproduce were actually successful, but their offspring were unsampled. In addition, including males with no assigned offspring improved statistical significance of some linear regressions while doing the same for females did not.

Factors affecting the size of offspring detected

As predicted, large females and those that arrived (and presumably spawned) early had larger YOY and yearling offspring. Indeed, offspring size was more strongly related to the arrival date than the lengths of their parents, suggesting selection for early arrival (and spawning) if offspring size determines survival. However, selection for early spawning will be offset if the redds of early arriving females are disturbed by later arriving females (Essington *et al.*, 2000; Fukushima *et al.*, 1998); the strength of this density-dependent selection may vary among species, populations and years. For example, since females of iteroparous species such as steelhead and Atlantic salmon do not guard their nest after completing spawning (Fleming, 1998) one might expect more redd re-use than in semelparous species. On the other hand, iteroparous species tend to spawn at lower densities than semelparous species, offsetting this effect. In addition, the link between arrival date and progeny size may be influenced by variation among years in spawning season duration. If all females arrive (and spawn) within a very short time period, as seems to have occurred in 1997, female size may be more important in determining offspring size. Indeed, a significant positive relationship between maternal length and yearling offspring length was seen in 1997.

Surprisingly, male length and arrival date were related to YOY offspring size in one year each: length in 1998 and arrival date in 2000. A correlation between male size and offspring size might occur if large males spawned with large females but no such correlation was detected (Seamons *et al.*, 2004). Given that offspring size is highly correlated with maternal arrival date, a significant correlation between paternal length and maternal arrival date might also explain the relationship between paternal length and offspring length, but there was no significant correlation between these traits. Additionally, a correlation may exist if there was a high sire effect in additive genetic variation in length. Estimates of sire effects on heritability (h^2) for body length are moderate and are generally less than dam effects in hatchery reared rainbow trout (0.18-0.35, Gjerde, Gjedrem, 1984; Gjerde, Schaeffer, 1989). However, naturally reared fish may have higher heritabilities than fish in captivity; sire effects on h^2 for length, reported by Smoker et al. (1994), were much higher than dam effects in pink salmon reared in their natural environment.

The correlation between male arrival date and offspring in 2000 was likely actually due to the maternal effect of arrival date. Since females determine spawning date and spawning date is correlated with arrival date (e.g., Quinn *et al.*, 2000), males that only spawn shortly after they arrived, as opposed to spawning over the course of the entire season, would acquire the effects of the arrival (and spawning) date of the female with whom they mate. Males in the first three BY generally spawned many times over the course of the spawning season, or spawned at a date not predicted by their arrival date (as inferred from parentage data). However, in 2000 males appeared to spawn only very close to their arrival date.

Parentage assignment

We expected to be able to assign two parents to a great majority of our juvenile samples, and indeed we were able to assign two adult anadromous steelhead parents to many juveniles. Surprisingly, many juveniles were assigned only one parent, due in part to fertilization of eggs by mature male steelhead parr (Seamons *et al.*, 2004). Even more surprising was the number of juveniles assigned no parents at all. However, the great majority of these juveniles were from years when flooding occurred, 1997 and 1999, suggesting that in these years many adults spawned upstream of the weir without being sampled. The lack of parental assignment in the few remaining juveniles from BY 1998 and 2000 may have resulted from one of several processes. These juveniles may have been misassigned by size to BY 1998 or 2000, particularly the 1998 YOY whose size distribution significantly overlapped the distribution of 1997 yearlings. Six juveniles without known parents sampled in 1998 fell within the size overlap range and could therefore actually be from BY 1997. In addition, mutations at one or more loci could have occurred. We were unable to estimate mutation rate, but the rates recently calculated for microsatellite loci in pink salmon by Steinberg et al. (2002) would be insufficient to account for a significant portion of the failure to assign parents. Null allele segregation may also have occurred, though a significant deviation from HWE was found in only two loci in one BY. In many river systems rainbow trout, the non-anadromous form of *O. mykiss*, and steelhead are sympatric, and naturally interbreed (Zimmerman, Reeves, 2000). While this would explain the lack of parent assignments of the remaining juveniles, no rainbow trout population exists in Snow Creek (Thom Johnson, WDFW, 283236 Highway 101, Port Townsend, WA, 98368 – personal communication). Finally, some juveniles might have been cutthroat trout (*O. clarki*) or interspecific hybrids, which are nearly impossible to distinguish from steelhead at small sizes in the field without killing the fish. Indeed, using a single, powerful genetic marker (GH2D; Baker et al. 2002) four of the remaining juveniles were genetically identified as hybrids, though their actual parentage cannot be verified.

In summary, size at age may determine later life history strategies (Metcalfe, Thorpe, 1992), which may in turn affect future patterns of survival and reproductive success (Ward *et al.*, 1989). The lack of a significant relationship between either parental size or reproductive timing and the number of offspring was surprising, given the significant relationships between these parental characteristics and size of offspring that we observed, and the apparent size-selective mortality in juvenile salmonids (e.g., Einum, Fleming, 2000). The family-specific survival rates were probably determined by physical attributes of the breeding sites, which we could not sample, and compensatory processes occurring prior to our first sampling period, at the end of the first summer. Future research on salmonids should relate parental traits to the breeding site conditions and to the number, size, and migration date of smolts and their subsequent survival at sea. In addition, patterns may be very strong in a given year but not represent general selective forces and population responses, therefore field studies need to be carried out over several years in order to be meaningful (see also Dickerson *et al.*, 2002).

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References

Beacham TD, Murray CB (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Transactions of the American Fisheries Society* **119**, 927-945.

Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* **42**, 485-508.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows ™ pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier (France).

Brännäs E (1995) First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* **9**, 411-420.

Chandler GL, Bjornn TC (1988) Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. *Transactions of the American Fisheries Society* **117**, 432-443.

Dickerson BR, Quinn TP, Willson MF (2002) Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethology Ecology and Evolution* **14**, 29-44.

Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society London Series B* **266**, 2095-2100.

Einum S, Fleming IA (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* **54**, 628-639.

Essington TE, Quinn TP, Ewert VE (2000) Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 205-213.

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 59-76.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Fukushima M, Quinn TJ, Smoker WW (1998) Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 618-625.

Garant D, Dodson JJ, Bernatchez L (2001) A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* **92**, 137-145.

Gjerde B, Gjedrem T (1984) Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* **36**, 97-110.

Gjerde B, Schaeffer LR (1989) Body traits in rainbow trout II. Estimates of heritabilities and of phenotypic and genetic correlations. *Aquaculture* **80**, 25-44.

Good SP, Dodson JJ, Meekan MG, Ryan DAJ (2001) Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1187-1195.

Healey MC (1982) Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 952-957.

Heath DD, Fox CW, Heath JW (1999) Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution* **53**, 1605-1611.

Helle JH (1989) Relation between size-at-maturity and survival of progeny in chum salmon, *Oncorhynchus keta* (Walbaum). *Journal of Fish Biology* **35**, 99-107.

Herbinger CM, Doyle RW, Pitman ER, *et al.* (1995) DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. *Aquaculture* **137**, 245-256.

Holtby LB, Healey MC (1986) Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1946-1959.

Jordan WC, Youngson AF (1992) The use of genetic marking to assess the reproductive success of mature male Atlantic salmon parr (*Salmo salar*, L.) under natural spawning conditions. *Journal of Fish Biology* **41**, 613-618.

Keeley ER, McPhail JD (1998) Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). *Behaviour* **135**, 65-82.

Keenleyside MHA, Dupuis HMC (1988) Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **66**, 262-265.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* **37**, 1210-1226.

Lapointe M, Eaton B, Driscoll S, Latulippe C (2000) Modelling the probability of salmonid egg pocket scour due to floods. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1120-1130.

Metcalfe NB, Thorpe JE (1992) Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **41** (**Suppl. B**), 93-99.

Morán P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity* **77**, 655-660.

Quinn TP, Adkison MD, Ward MB (1996) Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* **102**, 304-322.

Quinn TP, Foote CJ (1994) The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* **48**, 751-761.

Quinn TP, Peterson NP (1996) The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef creek, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1555-1564.

Quinn TP, Unwin MJ, Kinnison MT (2000) Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. *Evolution* **54**, 1372-1385.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Schroder SL (1981) *The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon* Ph.D., University of Washington.

Schuett-Hames DE, Peterson NP, Conrad R, Quinn TP (2000) Patterns of gravel scour and fill after spawning by chum salmon in a Western Washington stream. *North American Journal of Fisheries Management* **20**, 610-617.

Seamons TR, Bentzen P, Quinn TP (2004) The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environmental Biology of Fishes* ***69***, 333-344.

Smith RW, Griffith JS (1994) Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* **123**, 747-756.

Smoker WW, Gharrett AJ, Stekoll MS, Joyce JE (1994) Genetic analysis of size in an anadromous population of pink salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 9-15.

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Canadian Journal of Zoology* **77**, 836-841.

Steinberg EK, Lindner KR, Gallea J, *et al.* (2002) Rates and patterns of microsatellite mutations in pink salmon. *Molecular Biology and Evolution* **19**, 1198-1202.

Taggart JB, McLaren IS, Hay DW, Webb JH, Youngson AF (2001) Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Molecular Ecology* **10**, 1047-1060.

Titus RG, Mosegaard H (1991) Selection for growth potential among migratory brown trout (*Salmo trutta*) fry competing for territories: evidence from otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 19-27.

van den Berghe EP, Gross MR (1989) Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). *Evolution* **43**, 125-140.

Ward BR, Slaney PA, Facchin AR, Land RW (1989) Size-based survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1853-1858.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Zimmerman CE, Reeves GH (2000) Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2152-2162.

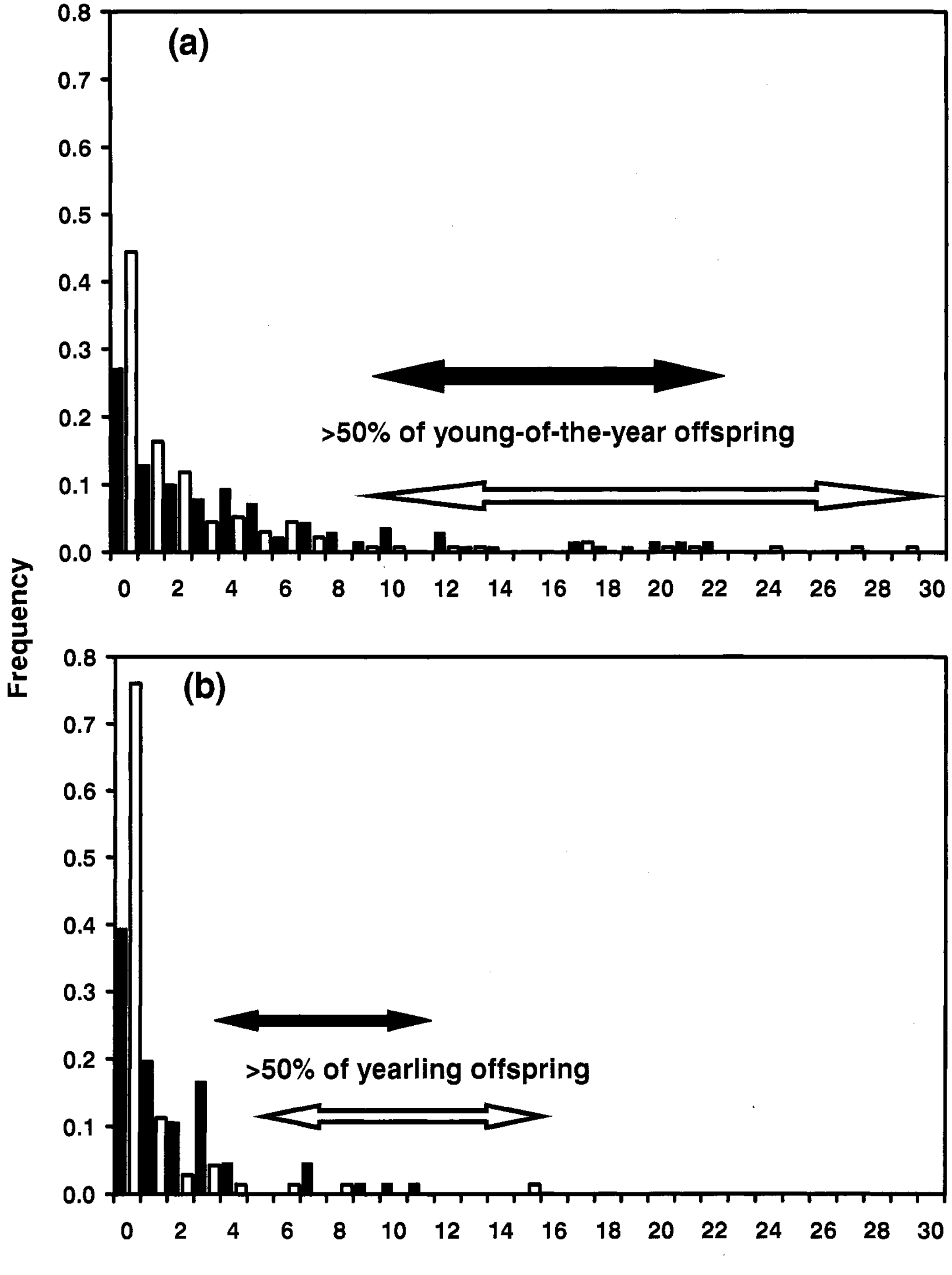

Figure 2.1. Frequency histograms showing the frequency of both maternal (■) and paternal (□) half-sibling families composed of a certain number of young-of-the-year (a) and yearling (b) offspring. Arrows encompass the range of low-frequency/large-composition families that add up to more than 50% of the total number of offspring, i.e., most of the juveniles are the offspring of a very few adults.

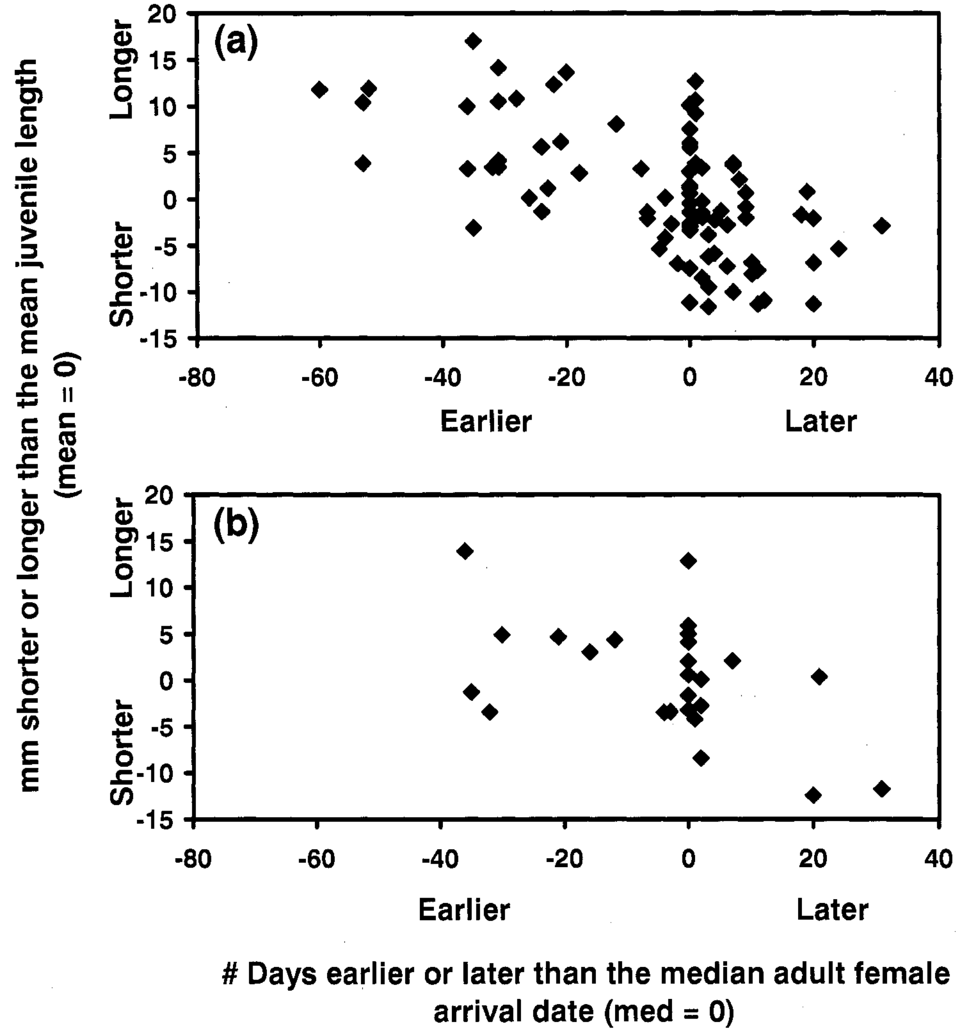

Figure 2.2. The relationship between maternal arrival date and young-of-the-year (a) and yearling (b) offspring size for all brood years. Maternal arrival date was standardized to the median arrival date in each year. Offspring sizes were standardized to the mean juvenile length per brood year. Relationships were significant at $P < 0.04$ and $P < 0.038$ for young-of-the-year (GLM; $r^2 = 0.64$) and yearling ($r^2 = 0.57$) respectively.

Table 2.1. Allelic variability at 12 microsatellite loci used for parentage assignment in four brood years of steelhead in Snow Creek. Number of alleles per locus (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability after test for goodness of fit to HWE (P_{HWE}), inbreeding coefficient (F_{IS}) and the total number of alleles across all brood years (Total N_A) are shown.

Brood Year		Locus											
		Oki23	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1997	N_A	17	12	12	14	18	19	9	11	24	24	16	12
	H_E	0.89	0.87	0.74	0.88	0.92	0.92	0.8	0.88	0.92	0.94	0.91	0.86
	H_O	0.87	0.96	0.84	0.9	1	0.9	0.73	0.87	1	0.87	0.87	0.9
	P_{HWE}	0.75	0.91	0.14	0.62	0.99	0.22	0.13	0.22	0.15	0.37	0.2	0.28
	F_{IS}	0.045	-0.094	-0.119	-0.014	-0.075	0.037	0.096	0.031	-0.073	0.093	0.064	-0.036
1998	N_A	16	12	9	15	18	24	11	14	24	38	19	16
	H_E	0.88	0.86	0.83	0.89	0.9	0.94	0.82	0.88	0.93	0.96	0.91	0.86
	H_O	0.92	0.86	0.85	0.94	0.91	0.96	0.77	0.89	0.98	0.94	0.92	0.89
	P_{HWE}	0.86	0.33	0.39	0.68	0.6	0.53	0.01	0.33	0.46	0.36	0.36	0.82
	F_{IS}	-0.037	0.006	-0.015	-0.055	0.007	-0.012	0.061	0.003	-0.043	0.025	0.003	-0.025
1999	N_A	15	15	12	20	19	11	14	17	29	41	19	18
	H_E	0.87	0.89	0.83	0.91	0.91	0.93	0.84	0.9	0.94	0.93	0.9	0.87
	H_O	0.9	0.96	0.79	0.9	0.88	0.94	0.73	0.85	0.94	0.96	0.87	0.85
	P_{HWE}	0.22	0.42	0.61	0.26	0.76	0.38	0.03	0.01	0.03	0.64	0.44	0.19
	F_{IS}	-0.029	-0.069	0.056	0.012	0.036	-0.001	0.137	0.066	0.011	0	0.052	0.037
2000	N_A	18	17	11	19	22	32	16	17	33	53	22	23
	H_E	0.9	0.88	0.8	0.89	0.9	0.95	0.79	0.9	0.94	0.96	0.91	0.83
	H_O	0.95	0.9	0.78	0.92	0.93	0.96	0.63	0.87	0.99	0.96	0.88	0.83
	P_{HWE}	0.14	0.15	0.75	0.9	0.49	0.18	0.0000[a]	0.02	0.004	0.06	0.001[a]	0.37
	F_{IS}	-0.054	-0.019	0.021	-0.033	-0.038	-0.016	0.209	0.037	-0.052	0.008	0.028	0.012
Total	N_A	20	18	15	20	25	35	17	19	39	58	25	27

[a] - significant at $\alpha = 0.05$

Table 2.2. Number of adult male and female steelhead (N), their size range (SR) and average length (Avg_L; both in millimeters), and their median arrival date (M_{AD}). For each brood year, the columns on the right side list the number of juveniles with at least one identified parent in each of three age-classes: young-of-the-year (N_{0+}), yearling (N_{1+}) and two year old (N_{2+}). The rightmost column gives the number of juveniles from each brood year for which no parents were identified (N_{NP}).

	Adults								**Juveniles**			
	Males				**Females**				**Age-class**			
Brood Year	**N**	**SR**	**Avg_L**	**M_{AD}**	**N**	**SR**	**Avg_L**	**M_{AD}**	**N_{0+}**	**N_{1+}**	**N_{2+}**	**N_{NP}**
1997	11	420 - 750	612.7	20-Mar[a]	20	595 - 830	692.3	20-Mar[a]	130	60	1	66
1998	35	600 - 735	649.6	20-Mar	18	565 - 795	638.6	21-Mar	117	22	2	23
1999	25	400 - 815	655.6	23-Mar	27	600 - 740	658.3	23-Mar	161	49		134
2000	64	430 - 785	645.4	23-Mar	75	565 - 860	640.0	25-Mar	245			13
Total	135				140				653	131	3	236

[a] - Estimated

Table 2.3. Summary of observed parental reproductive success measured as the number of offspring, by juvenile age-class, parent sex and brood year with the range, mean and standardized variance (σ^2/mean^2). No data were available for yearlings from brood year 2000.

			Brood year			
Juvenile age-class	**Parent sex**		**1997**	**1998**	**1999**	**2000**
Young-of-the-year	Male	range	1-20	1-29	1-27	1-21
		mean	10.0	4.1	6.0	3.9
		σ^2/mean^2	0.40	2.64	1.87	1.32
	Female	range	1-22	1-22	1-21	1-19
		mean	6.9	9.3	7.7	4.4
		σ^2/mean^2	0.70	0.47	0.84	0.89
Yearling	Male	range	1-15	1-4	1-3	--
		mean	5.1	2.3	1.4	--
		σ^2/mean^2	0.96	0.44	0.41	--
	Female	range	1-10	1-11	1-9	--
		mean	3.4	3.7	2.6	--
		σ^2/mean^2	0.65	1.06	0.63	--

Table 2.4. Summary of linear regression analyses for both male and female parents of both young-of-the-year (YOY) and yearling offspring. Statistically significant test results are presented; NS signifies a non-significant result ($P > 0.10$) based on sequential Bonferroni adjustments for multiple tests. No data were available for yearlings from brood year 2000.

			Male parent		**Female parent**	
Response variable	**Juvenile age-class**	**Brood Year**	**Length**	**Arrival date**	**Length**	**Arrival date**
Number	YOY	1997	NS	NS	NS	NS
		1998	$r^2 = 0.13, P = 0.06$	NS	NS	NS
		1999	NS	NS	NS	NS
		2000	NS	NS	NS	NS
	Yearling	1997	NS	NS	$r^2 = 0.13, P = 0.07$	NS
		1998	NS	NS	NS	NS
		1999	NS	NS	NS	NS
		2000	--	--	--	--
Size	YOY	1997	NS	NS	NS	$r^2 = 0.22, P = 0.05$
		1998	$r^2 = 0.54, P = 0.036$	NS	NS	$r^2 = 0.58, P < 0.05$
		1999	NS	NS	NS	$r^2 = 0.32, P = 0.05$
		2000	NS	$r^2 = 0.42, P = 0.001$	$r^2 = 0.45, P = 0.004$	$r^2 = 0.15, P = 0.004$
	Yearling	1997	NS	NS	$r^2 = 0.41, P < 0.06$	NS
		1998	NS	NS	NS	$r^2 = 0.92, P < 0.06$
		1999	NS	NS	NS	$r^2 = 0.46, P < 0.06$
		2000	--	--	--	--

Table 2.5. Average lengths (± 1 standard error) of adult male or female steelhead (*Oncorhynchus mykiss*) assigned 0, 1 - 2, 3 - 4, or 5+ juvenile offspring (young-of-the-year (YOY) or yearling). Shared superscript letters denote values which are not significantly different from one another (One-way ANOVA, Tamhane's T2 test, $P < 0.04$).

		Male parent		**Female parent**	
Age-class	**Number of offspring**	**N**	**Mean length (± 1 SE)**	**N**	**Mean length (± 1 SE)**
YOY	0	60	637.5 (± 10.9)^{a}	38	642.0 (± 8.6)^{a,b}
	1 - 2	38	641.4 (± 7.8)^{a}	32	624.8 (± 6.0)^{a}
	3 - 4	13	665.0 (± 6.8)^{a}	24	663.1 (± 11.2)^{b}
	5+	24	662.5 (± 13.0)^{a}	46	669.8 (± 9.0)^{b}
Yearling	0	55	641.5 (± 11.0)^{a}	14	673.6 (± 18.3)^{a}
	1 - 2	10	657.0 (± 21.0)^{a}	19	655.8 (± 10.4)^{a}
	3 - 4	3	676.7 (± 26.8)^{a}	13	679.6 (± 15.1)^{a}
	5+	3	661.7 (± 46.9)^{a}	6	696.7 (± 34.9)^{a}

Chapter 3: Patterns of reproductive success and selection on adult length, arrival date and juvenile offspring length at the end of offspring freshwater residence

Synopsis

Sexual selection operating before and during spawning and natural selection on offspring combine to determine patterns of reproductive success. These processes may interact to amplify or dampen variation in individual reproductive success. To test hypotheses concerning the interactions between body size and breeding date in determining reproductive success, we sampled adult steelhead (*Oncorhynchus mykiss*) in four consecutive years as they ascended a small stream to spawn, and also sampled their seaward migrating ("smolt") progeny. We linked parents to offspring using molecular methods and examined selection on parental length and arrival date (which was assumed to be closely correlated with spawning date) and natural selection on young-of-the-year (YOY) offspring, sampled in a previous study. Patterns of apparent natural and sexual selection were complex and inconsistent among brood years. Early arriving males had higher reproductive success in three of four brood years; large and early females had higher reproductive success in two of four brood years. Small YOY offspring in maternal half-sib families were favored in a one brood year. Large and early females had larger YOY offspring in that same year so the natural selection may in this case dampen the effects of sexual selection. These samples represent the end of freshwater residence for these offspring. The patterns present at this point set the stage for natural selection during ocean residency.

Introduction

Body size and the timing of key life history transitions affect survival and reproductive success in salmonid fishes, and these traits are linked in complex ways. Larger females tend to have larger eggs and higher fecundity than smaller females (Beacham, Murray, 1993). Larger females can also bury their eggs deeper in the gravel (Steen, Quinn, 1999), presumably reducing the likelihood of disturbance by the digging of other females (Essington *et al.*, 2000) and possibly protecting them from streambed scour during high flow events (Lapointe *et al.*, 2000; Schuett-Hames *et al.*, 2000). Large females may also be better able to access the best spawning sites (Fleming, Gross, 1994), though prior residence effects may outweigh size (Foote, 1990). Larger males can exclude smaller males from access to females (Dickerson *et al.*, 2002; Keenleyside, Dupuis, 1988; Quinn, Foote, 1994). However, achievement of large size may necessitate prolonged exposure to predators in risky (e.g., marine) environments, and so small individuals also occur in natural populations, especially among males (Quinn, 2005).

The effects of arrival and spawning date on salmonid reproductive success are not straightforward. Females that arrive and breed early in the season will have a wide range of nesting sites to choose from but they may risk having their redds disturbed by the digging of later-arriving females (Fukushima *et al.*, 1998), though the amount of redd disturbance varies among years as a function of density (Essington *et al.*, 2000). Early arriving and relatively small males may have more access to early breeding females, while later in the spawning season, when sex ratios may be heavily male biased, even very large males may be unable to dominate access to females (Dickerson *et al.*, 2005; Dickerson *et al.*, 2002).

Offspring size and fitness are correlated with maternal size through correlations with egg size (Beacham, Murray, 1990). Juvenile size also varies with parental spawning date (Einum, Fleming, 2000). Progeny of early-spawning adults tend to emerge from the gravel earlier than other fry of the same cohort (Beacham, Murray, 1990) and will tend to be larger at a given point in time because they will have had time to grow. In addition, for salmonid species with a freshwater residence period, early emergers may benefit by having first access to vacant feeding territories (Chandler, Bjornn, 1988). Due to their larger size and prior residence they may be better able to hold and defend feeding territories (Keeley, McPhail, 1998), though territory size may vary with food availability and density of fish (Keeley, 2001). However, early emergence may lead to poor growth if food is not available, and survival rates may be reduced if there is extended exposure to predation (Brännäs, 1995). Moreover, if food is abundant then the maternal effects of egg size and spawning date diminish over time and fry size is eventually uncoupled from these maternal traits (Heath *et al.*, 1999; Kinnison *et al.*, 1998).

In freshwater, larger juveniles typically have a survival advantage over smaller ones (Sogard, 1997) because they can evade predation due to gape limitations of predators and because their large size may benefit them during the period of limited energy during the winter. For example, larger coho salmon (*Oncorhynchus kisutch*) survived the winter at a higher rate than smaller juveniles (Quinn, Peterson, 1996), as did larger rainbow trout (*O. mykiss*), though overall survival varied with environmental conditions (Smith, Griffith, 1994). However overwinter survival rates may not always be higher for large individuals (Connolly, Petersen, 2003) and the strength and direction of selection may vary annually (Good *et al.*, 2001). During seaward migration, survival may also be size-related (Collis *et al.*, 2001), however very little research has been done on this topic. In any case, selection pressures are likely to be different from those experienced during freshwater residency or from those experienced during ocean residency (Antolos *et al.*, 2005).

The reproductive success of parents results from the processes of natural and sexual selection at spawning and natural selection throughout the lifetime of their offspring. For anadromous salmonids, the natural selection operating on offspring involves both freshwater and saltwater components that are likely to be quite different. The great majority of lifetime mortality occurs in freshwater, and the size and timing of migration to sea affect survival there (Quinn, 2005) so the freshwater life history stages are very important and probably more closely linked to parental traits than the marine stages. For species that essentially spend half of their life in freshwater and half in saltwater it is important to quantify selection in both environments, however this has rarely, if ever, been done.

In (Seamons *et al.*, 2004a) (Chapter 2) we investigated the effect of parental length and arrival date on the number of young-of-the-year (YOY) and yearling offspring in steelhead (*O. mykiss*) detected by sampling in Snow Creek. Our ability to link the production of offspring to specific parental traits was hampered by the small fraction of juveniles that could be sampled and by the necessity of sampling selected locations rather than the entire creek. Selection was only reliably detected for the time period between parental arrival on the spawning grounds to the end of the first summer of life for juvenile offspring (YOY), especially for fathers, owing to the relative scarcity of yearling aged juveniles and the apparently high reproductive success of mature parr (Chapter 1) (Seamons *et al.*, 2004b). In addition, any selection in the last winter before migration to the ocean or during the first part of the migration would have been undetected by the fall sampling.

The goals of this chapter were to continue the investigation into selection on parental and offspring phenotypic traits. More specifically, we first estimated the effect of parental length and return date on the number of emigrating steelhead smolt offspring, at the end of their freshwater residence. Second, we determined the persistence of family-specific abundance by examining the relationship between the numbers of individuals in each maternal or paternal half-sib family at the end of the first summer and the number as smolts at outmigration. Third, we tested the hypothesis that average half-sib family length at the end of the first summer determined patterns of abundance to the smolt stage (i.e., that families with larger offspring were over-represented as smolts relative to families with smaller offspring).

Methods

Sampling site, sample collection

Adult steelhead were sampled from Snow Creek, Washington, at a permanent weir operated by the Washington Department of Fish and Wildlife (WDFW; see map in (Seamons *et al.*, 2004a)). Fork length, sex, and date were recorded, scales were taken for age analysis, and fin tissue samples were taken for genetic analysis in the spring of brood years (BY) 1998 through 2001. All returning adults were marked with an opercle punch just prior to release above the weir. If an emigrating unmarked adult (kelt) was found in the downstream trap it was sampled in the same way described above. Any adults missing their adipose fin (indicating that they were probably strays from a hatchery elsewhere) were killed at the weir.

In Washington State, the great majority of steelhead spend two years in freshwater before migrating to sea as smolts (Busby *et al.*, 1996). Accordingly, in the years 2000 through 2003, steelhead smolts were sampled as they were captured in the downstream trap at the weir. Two or three times a week WDFW employees seined the pond behind the weir, forcing all fish into the downstream trap. Fish were then sorted by age (parr, smolts, kelts) and species (coho; steelhead; cutthroat, *O. clarki*). Parr and kelts were counted and released downstream of the weir. Smolts were counted and placed in a large bucket in a water-filled circular raceway, and then a random sample (with regard to length and age) was removed from the bucket and immediately anesthetized in buffered tricaine methanesulfonate. Fork length and date were recorded, scales were taken for age determination, a small caudal fin clip was taken for genetic analysis, and the fish were released downstream after recovering from the anesthetic. All fin clips from adults and smolts were preserved and stored in 95% ethanol. Age determination from scales of adults and smolts was performed by WDFW employees.

In all four years we sampled from throughout the entire emigration period. In years 2000 and 2001 smolts were sampled from every day that smolts were processed, but the number or percentage of the daily total was not fixed. Instead we attempted to sample 20% of the total run. Though we attempted to avoid any bias in sampling by date, in these years the samples may have been weighted slightly toward the latter part of the outmigration period as a proportion of the total emigrating on any one day. In 2002 and 2003, 20% of all smolts trapped on any one day were sampled, avoiding any sampling bias by date.

Genetic analysis and parentage assignment

DNA extracted from fin tissue was used to amplify 12 microsatellite loci by polymerase chain reaction (PCR; Table 1.1 in (Seamons *et al.*, 2004b). Amplified DNA was visualized on a MegaBACE 1000 automated sequencer (Amersham Pharmacia Biotech Limited, Piscataway, N.J.) according to the manufacturer's guidelines. See (Seamons *et al.*, 2004b) for a more detailed description of DNA extraction, PCR and visualization methods.

Genetic data from adults 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 (using default values for dememorization, batches and iterations per batch; (Raymond, Rousset, 1995)). 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).

Parents were assigned to individual smolts using the principles of exclusion. If all but one or two adults were genetically excluded from being a parent, i.e., they did not share an allele with the smolt individual, that adult or adults were assigned as parents. If all adults were genetically excluded from parentage, it was assumed that the true parents were not sampled and that smolt individual was dropped from further analysis. See (Seamons *et al.*, 2004a) for a more detailed description of the parentage methods.

As described previously for juvenile samples (Seamons *et al.*, 2004a), some smolt individuals were assigned only a single parent. The probability of a random match for a single parent was calculated using equation 1 in (Seamons *et al.*, 2004a). All smolts tended to be very similar in size (~150 mm), precluding any assignment of individuals to BY by length. Three smolts of unknown age (i.e., no age from scales), assigned a single parent known to have spawned in more than one BY (i.e. ambiguous age from parentage) were dropped from further analysis because the absolute and relative value of parental length and arrival date were different in each of the BY in which it spawned.

The number of adults reported for BY 1999 and 2000 differs slightly from previously reported numbers (Chapters 1 and 2) (Seamons *et al.*, 2004a). The additional adults include a repeat spawner sampled in 2000 that had also spawned without being sampled (due to flooding) in 1999 (identified by a spawning mark on its scales), a hatchery fish released upstream (rather than being killed) in 1999, and two adults previously identified as cutthroat trout but which were subsequently identified by parentage as steelhead. These last two individuals could be F1 or backcrossed hybrids (adult hybrids are difficult to correctly identify to species, (Baumstieger *et al.*, 2005)) so these adults and their offspring (if any) were dropped from further analysis.

Five of 49 adults from BY 2001 were sampled only as they exited Snow Creek. Three of the five were aged two or three (similar in age to smolts) but were substantially larger (>250 mm). These fish were included as adults in the parentage assignment tests, but not in further analyses since they probably exhibited a different life history. The remaining two of the five adults were also included in parentage assignment tests, but since their arrival dates and fork lengths were unknown they and their offspring (if any) were dropped from further analyses.

Statistical analysis

Thirteen adults were known to have returned to Snow Creek in more than one year. Because we were testing the effects of relative size and arrival timing within a brood year on reproductive success repeat spawning individuals were treated as separate individuals. The major assumption was that reproductive success in one season was independent of the next season, that is, repeat spawning individuals did not gain an advantage in their second year of spawning from having spawned in a previous year. There was no available data to either support or dispute this assumption.

Males and females were expected to have different strategies to maximize reproductive success and different selection pressures on the traits measured; accordingly, data for each parental sex were analyzed separately. In general, the data were analyzed using the methods outlined in the review by (Brodie III *et al.*, 1995). Length and arrival date were standardized to an average of zero and a standard deviation of one by subtracting the yearly mean from individual values and dividing by the standard deviation. Interaction terms and quadratic terms were calculated for both length and date and then standardized in the same way. Our measure of fitness was the relative number of offspring detected in our smolt sample per individual parent per brood year. Relative fitness was natural-log transformed to correct for heteroscedasticity in the data.

The effect of parental length and arrival date on fitness was measured by several linear regressions. The total effect of either length or arrival date was calculated by performing linear regression analysis on each trait separately, within each BY. Since length and arrival date were correlated, this analysis included indirect effects of the correlated trait. The effect of each trait without the indirect effects of the other was calculated by performing multiple regression analysis using both traits. Each trait was then tested for quadratic relationships by performing multiple regressions using the standardized trait value and the corresponding standardized quadratic value. Finally, all terms were included in a multiple regression model to estimate complex relationships between trait values and fitness.

The number of members of each family sampled at the smolt stage is dependent to some degree on the number of members of the same family sampled as YOY. As an extreme example, if a male failed to produce YOY offspring he could not produce any smolt offspring. We used least squares linear regression to test for correlation between the relative (to the within BY average) number of YOY offspring and the relative number of smolt offspring of the same family. We also included this term in multiple regressions testing the effects of family average length on the number of smolt offspring (described below).

To test the hypothesis that length of individuals within maternal and paternal half-sib families at the young-of-the-year stage (YOY; from Chapter 2 (Seamons *et al.*, 2004a) affected their survival to smoltification weighted least squares regression was performed using the standardized average within family length weighted by the inverse of the within-family variance in length against relative fitness at the smolt stage. Relative fitness was natural-log transformed to correct for heteroscedasticity. This analysis was done only for BY 1998, 1999 and 2000 as there was no YOY sample from BY 2001. Families included in this data set were limited to those with two or more members detected at the YOY stage. Family size was correlated with maternal arrival date in 1998 and 1999, with maternal length and arrival date in 2000, with paternal length in 1998 and paternal arrival date in 2000 (Chapter 2) (Seamons *et al.*, 2004a). To account for these correlations and possible non-significant correlations in other years, maternal and paternal length and arrival date were included in all models.

Results

Adult and smolt offspring characteristics

A total of 297 adult steelhead returned and were sampled in Snow Creek from 1998 to 2001 (Table 3.1). The sex ratio generally favored females (1.05 females for each male over all BYs), though in 1998 males were almost twice as numerous as females. The overall number of fish returning each year ranged from 18-78 females and 21-63 males. Lengths varied from 400 to 785 mm in males and 420 to 860 mm in females. The median arrival dates for both males and females were similar, typically in the second or third week of March, and differed by no more than 5 days among years. Average arrival date is slightly different than the median date for both males and females.

The steelhead smolt migration started in late April, peaked in May, and ended in the beginning of June. The smolts were about 150 mm in fork length, including individuals of ages one, two and three, though age two was by far the most common (78%: Table 3.2). The total number of smolts leaving from Snow Creek annually varied from 1285 to 2589 and though the percent of the total sampled varied somewhat among years, we sampled 20% of all outmigrating smolts for a total of 1664 sampled individuals (Table 3.2)

Population genetics

Three of 48 tests for deviation of genotypic proportions from Hardy-Weinberg equilibrium were significant after sequential Bonferroni corrections (Table 3.3). In BY 2000, *Omy77* and *Ots108* both showed a positive F_{IS} value associated with heterozygote deficiencies while *One2* had a heterozygote excess. Allelic assignment in *One2* is sometimes difficult because it is a dinucleotide locus with long alleles; the excess of heterozygotes may be due to homozygotes being called heterozygotes because of confusing stutter patterns. Null alleles were expected in *Omy77* (Ardren *et al.*, 1999) and suspected in *Ots108* from parentage information. 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. 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 where putative parents of these individuals appeared to be heterozygous with a null allele or putative offspring appeared to have inherited a null allele. No accommodation for null alleles was made for *Ots108* during parentage assignment.

We did not calculate genotyping error rates from the smolt samples. Given that our methodology did not change, error rates were expected to be the same as those reported in Chapter 2, 0.6% per allele (Seamons *et al.*, 2004a).

Parentage

Average observed heterozygosity was 0.88 and varied among brood years and loci from 0.61 to 0.99. Exclusion probabilities per locus varied from 0.604 (BY 2001, *Omy1004*) to 0.925 (BY 2000, *Ots107*) while the global exclusion probability was 0.99999 for each BY. Given this genetic statistical power, we were able to assign at least one parent to 84% and both parents to 43% of all smolts. Of those that had at least one parent assigned, 50% had both parents assigned, 48% had only a mother assigned and 2% had only a father assigned. The probability of drawing a single matching parent at random from any of the BY was less than 4 in 1000. Of the 167 known-age smolts that had no parents assigned, 96 (57%) were from BY 1999, a year when flooding reduced the efficiency of adult sampling.

Nearly 35% of all anadromous males and 15% of females had no detected smolt offspring (all BY pooled, Figures 1a and 1b), and most offspring were produced by a few very successful parents. Calculating the number of offspring relative to other parents in a given year may exaggerate or mask differences in absolute numbers of offspring. For example, the two males with the most offspring (Figure 3.1a) both had the largest relative number of offspring (Figure 3.1b), but the male with 39 offspring had 17 times the mean number of offspring (BY 1998) while the male with 52 offspring had only 10 times the mean number (BY 2000). Nevertheless, the overall patterns of reproductive success remained the same regardless of whether they were inferred from the relative or absolute numbers of offspring.

Selection on adult traits

Larger males produced more smolt offspring in 1998 and 2000 but not in 1999 or 2001 (Table 3.4). In 1998 length (plus indirect arrival date effects) explained nearly 10% of variation in reproductive success (RS), but the relationship appeared to be driven by the extremely high RS (17 times the mean RS) of one large, early-arriving male. There was no significant effect of length when the data were reanalyzed without that male ($p=0.79$). Early arriving males had more offspring in BY 1998 and 2000 ($p < 0.05$) and tended to have more in 1999 ($p < 0.10$), though overall, arrival date (plus indirect effects of length) explained less than 15% of the variation in RS. Arrival date was marginally related to RS in 1998 when the highly successful male was removed from the dataset. When the tendency for large males to arrive early was accounted for by multiple regression, earlier males produced more offspring in 1998, 1999 and 2000 ($p<0.05$), and longer males produced more offspring in 1998 and 2000 ($p<0.05$), though, for 1998, the effect of length disappeared when the single large, early male was excluded. With or without the outlier male the quadratic length selection gradient and the length by date interaction gradient were statistically significant, suggesting disruptive selection on length and simultaneous selection for large and early males.

Larger and earlier arriving females produced more smolt offspring in both BY 1998 and 2000 (Table 3.5). For these two BY, length was only marginally related ($p<0.10$) to RS once the covariance between length and arrival date was accounted for so the relationships primarily reflected the higher smolt production from early females. This model accounted for 66% of the variation in RS in 1998 and 20% in 2000. No other relationships in any other years were statistically significant.

Selection on YOY traits

Paternal half-sib families that had many members detected as YOY were also well represented in the smolt sample in 1998 ($p<0.05$, $r^2=0.75$) and in the pooled data ($p<0.10$, $r^2=0.28$), but not in 1999 or 2000 (Figure 3.2a, Table 3.6). No significant relationship was found of YOY length and numbers of smolt individuals in paternal half-sib families (Figure 3.3a), however two of three selection gradients were negative, suggesting that shorter families were detected in greater numbers in our smolt sample (Table 3.6).

Well represented maternal YOY half-sib families were detected in higher numbers in the smolt sample in 1998 ($r^2=0.90$), but not in 1999 or 2000 (Figure 3.2b, Table 3.6). Shorter maternal half-sib families were detected at higher numbers at smolt outmigration in BY 2000 ($r^2=0.43$; Figure 3.3).

Discussion

Remnant effects of sexual selection?

Our primary objective was to determine the effects of the timing of arrival for spawning and the size at spawning on an individual's reproductive success measured by the number of offspring reaching the smolt life history stage after about two years in fresh water. Overall, males that returned to fresh water to spawn earlier produced more smolt offspring than later arriving males. This was not wholly unexpected given that salmonid males typically arrive before females (Morbey, 2000) and that male steelhead tended to arrive before the females with whom they mated (Chapter 1) (Seamons *et al.*, 2004b). The relationship of reproductive success to male length was weaker overall and significant in only one of four brood years studied (after removal of an outlier). Though large males typically achieve dominant positions during competition for mates (Dickerson *et al.*, 2002), dominance does not appear to substantially increase reproductive success under natural conditions (Dickerson *et al.*, 2005). Indeed, numerous studies have shown that sub-dominant males (satellite males of the same age as the dominant male, younger and smaller anadromous males (jacks), or non-anadromous males) can fertilize significant fractions of the eggs produced by females (Foote *et al.*, 1997; Morán *et al.*, 1996; Schroder, 1981).

Large and early arriving females also produced more offspring, though only in two of four brood years. Large salmonid females on average produce more and larger eggs than smaller females (Beacham, Murray, 1993). Over the range in length observed in females in Snow Creek in these years (565 – 860 mm), predicted fecundity might vary by as much as 2600 eggs, the estimate for the largest female being more than twice that for the smallest ($0.0694033*\text{fork length}^{1.66088}$, $r^2 = 0.38$ – Thom Johnson, WDFW, 283236 Highway 101, Port Townsend, WA, 98368, personal communication). Large females also may confer an advantage to their offspring survival by out-competing smaller females for choice nest sites (Fleming, Gross, 1994), or by burying their eggs deep enough to prevent scour by floods or later spawning females (Steen, Quinn, 1999). However, in the same two BY, arrival date appeared to be the more important trait in determining patterns of reproductive success, as indicated by the greater statistical support and larger selection gradients. Early spawning females risk redd superimposition by later spawning females in semelparous species of salmon (Essington *et al.*, 2000). This factor maybe even more important in iteroparous species, such as steelhead, because those females do not guard the nest after spawning. On the other hand, nest disturbance may be less important in steelhead because the densities of spawning females are typically quite low (Busby *et al.*, 1996). Emergence date of fry from the gravel is a function of spawning date and water temperature (Beacham, Murray, 1990) and the conditions affecting growth and survival upon emergence (Brännäs, 1995; Einum, Fleming, 2000) are likely to vary among years, so the optimal dates for spawning and emergence are not fixed. In three of four years selection gradients were negative, suggesting that early arrival (and presumably spawning) was better. In the fourth year, however, the selection gradient, though non-significant, was positive, suggesting later was better. It is possible that the approximately normal distribution of arrival has resulted from selection that is stabilizing when averaged over many years but is directional in many individual years (i.e., in some years the early fish are most successful but in other years the late ones produce most of the offspring). In fact, there was no evidence of stabilizing selection as indicated by non-significant coefficients and gradients for quadratic terms in either the pooled data or in data from individual brood years.

Natural selection on offspring

We expected to find that families whose offspring were longer at earlier life history stages were over-represented in the smolt sample. Instead, size-selective mortality was weak or undetectable in most years, and in the single brood year with statistically significant relationships, shorter, not longer, families were favored. Larger fish may escape predation (Sogard, 1997) or survive overwinter (Quinn, Peterson, 1996) in higher numbers than smaller fish. However, the effects of size may vary and even favor small fish over large (Connolly, Petersen, 2003).

The apparent selection favoring small fish in maternal families in 2000 (and possibly other years and in paternal families) could be the result of life history decisions made by differing sexes of juveniles. Some fraction of the fast growing males may have matured rather than smolted, i.e., the sex ratio of smolts may have been skewed towards females because many males (which were likely large YOY) matured in freshwater instead of migrating to the ocean. Mature parr appear to be present in Snow Creek (Chapter 1) (Seamons *et al.*, 2004b), though their abundance is unknown. The heritability for early maturity could be quite high, and early maturity may be incompatible with smolt transformation (Thrower *et al.*, 2004). The sex ratio of Snow Creek outmigrating smolts is unknown.

Comparison to reproductive success – YOY

A much stronger relationship between paternal size and arrival timing was found in the smolt sample than in the YOY or yearling sample (Chapter 2) (Seamons *et al.*, 2004a). The difference between these results may be due to the spatial limitations of our YOY and yearling sampling, i.e., the smolt sample was more representative of the population patterns than the YOY or yearling sample. However, families that were unsampled (i.e., rare) as YOY were also unsampled or rare as smolts. Observed differences could therefore be a result of differing or random patterns of survival among families in the years between their first sampling stages. In 1998 the patterns of reproductive success between life history stages of offspring were similar, i.e., the same male appeared very successful in both YOY and smolt samples while all other males had low reproductive success. However, in 1999 size selective mortality apparently accounted for part of the patterns seen in reproductive success, and some other unmeasured factor or random effects determined patterns of reproductive success for BY 2000 fathers.

Summary

In terms of the number of offspring produced, selection appeared to favor early arriving males and large and early arriving females in the years of this study. We found no evidence of stabilizing selection for arrival date for either males or females, nor did we find any evidence supporting the idea of varying directional selection; it was selection for early arrival or no apparent selection. In some years the patterns of reproductive success were set early in the freshwater life history of offspring, in others natural selection on offspring seemed to oppose sexual selection in parents.

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References

Antolos M, Roby DD, Lyons DE, *et al.* (2005) Caspian tern predation on juvenile salmonids in the Mid-Columbia River. *Transactions of the American Fisheries Society* **134**, 466-480.

Ardren WR, Borer S, Thrower F, Joyce JE, Kapuscinski AR (1999) Inheritance of 12 microsatellite loci in *Oncorhynchus mykiss*. *Journal of Heredity* **90**, 529-536.

Baumstieger J, Hankin D, Loudenslager EJ (2005) Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identifications. *Transactions of the American Fisheries Society* **134**, 829-840.

Beacham TD, Murray CB (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Transactions of the American Fisheries Society* **119**, 927-945.

Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* **42**, 485-508.

Brännäs E (1995) First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* **9**, 411-420.

Brodie III ED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* **10**, 313-318.

Busby PJ, Wainwright TC, Bryant GJ, *et al.* (1996) Status review of west coast steelhead from Washington, Idaho, Oregon, and California, p. 261. U. S. Dep. Commer.

Chandler GL, Bjornn TC (1988) Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. *Transactions of the American Fisheries Society* **117**, 432-443.

Collis K, Roby DD, Craig DP, Ryan BA, Ledgerwood RD (2001) Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River Estuary: vulnerability of different salmonid species, stocks, and rearing types. *Transactions of the American Fisheries Society* **130**, 385-396.

Connolly PJ, Petersen JH (2003) Bigger is not always better for overwintering young-of-year steelhead. *Transactions of the American Fisheries Society* **132**, 262-274.

Dickerson BR, Brinck KW, Willson MF, Bentzen P, Quinn TP (2005) Relative importance of salmon body size and arrival time at breeding grounds to reproductive success. *Ecology* **86**, 347-352.

Dickerson BR, Quinn TP, Willson MF (2002) Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethology Ecology and Evolution* **14**, 29-44.

Einum S, Fleming IA (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* **54**, 628-639.

Essington TE, Quinn TP, Ewert VE (2000) Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 205-213.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Foote CJ, Brown GS, Wood CC (1997) Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1785-1795.

Fukushima M, Quinn TJ, Smoker WW (1998) Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 618-625.

Good SP, Dodson JJ, Meekan MG, Ryan DAJ (2001) Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1187-1195.

Heath DD, Fox CW, Heath JW (1999) Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution* **53**, 1605-1611.

Keeley ER (2001) Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* **82**, 1247-1259.

Keeley ER, McPhail JD (1998) Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). *Behaviour* **135**, 65-82.

Keenleyside MHA, Dupuis HMC (1988) Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **66**, 262-265.

Kinnison MT, Unwin MJ, Hershberger WK, Quinn TP (1998) Egg size, fecundity, and development rate of two introduced New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. *Canadian Journal of Fisheries and Aquatic Science* **55**, 1946-1953.

Lapointe M, Eaton B, Driscoll S, Latulippe C (2000) Modelling the probability of salmonid egg pocket scour due to floods. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1120-1130.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**, 639-655.

Morán P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity* 77, 655-660.

Morbey Y (2000) Protandry in Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1252-1257.

Quinn TP (2005) *The behavior and ecology of Pacific salmon and trout* University of Washington Press, Seattle, WA.

Quinn TP, Foote CJ (1994) The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* **48**, 751-761.

Quinn TP, Peterson NP (1996) The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef creek, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1555-1564.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Schroder SL (1981) *The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon* Ph.D., University of Washington.

Schuett-Hames DE, Peterson NP, Conrad R, Quinn TP (2000) Patterns of gravel scour and fill after spawning by chum salmon in a Western Washington stream. *North American Journal of Fisheries Management* **20**, 610-617.

Seamons TR, Bentzen P, Quinn TP (2004a) The effects of adult length and arrival date on individual reproductive success in wild steelhead trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Science* **61**, 185-192.

Seamons TR, Bentzen P, Quinn TP (2004b) The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environmental Biology of Fishes* ***69***, 333-344.

Smith RW, Griffith JS (1994) Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* **123**, 747-756.

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**, 1129-1157.

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Canadian Journal of Zoology* **77**, 836-841.

Thrower FP, Hard JJ, Joyce JE (2004) Genetic architecture of growth and early life-history transitions in anadromous and derived freshwater populations of steelhead. *Journal of Fish Biology* **65**, 286-307.

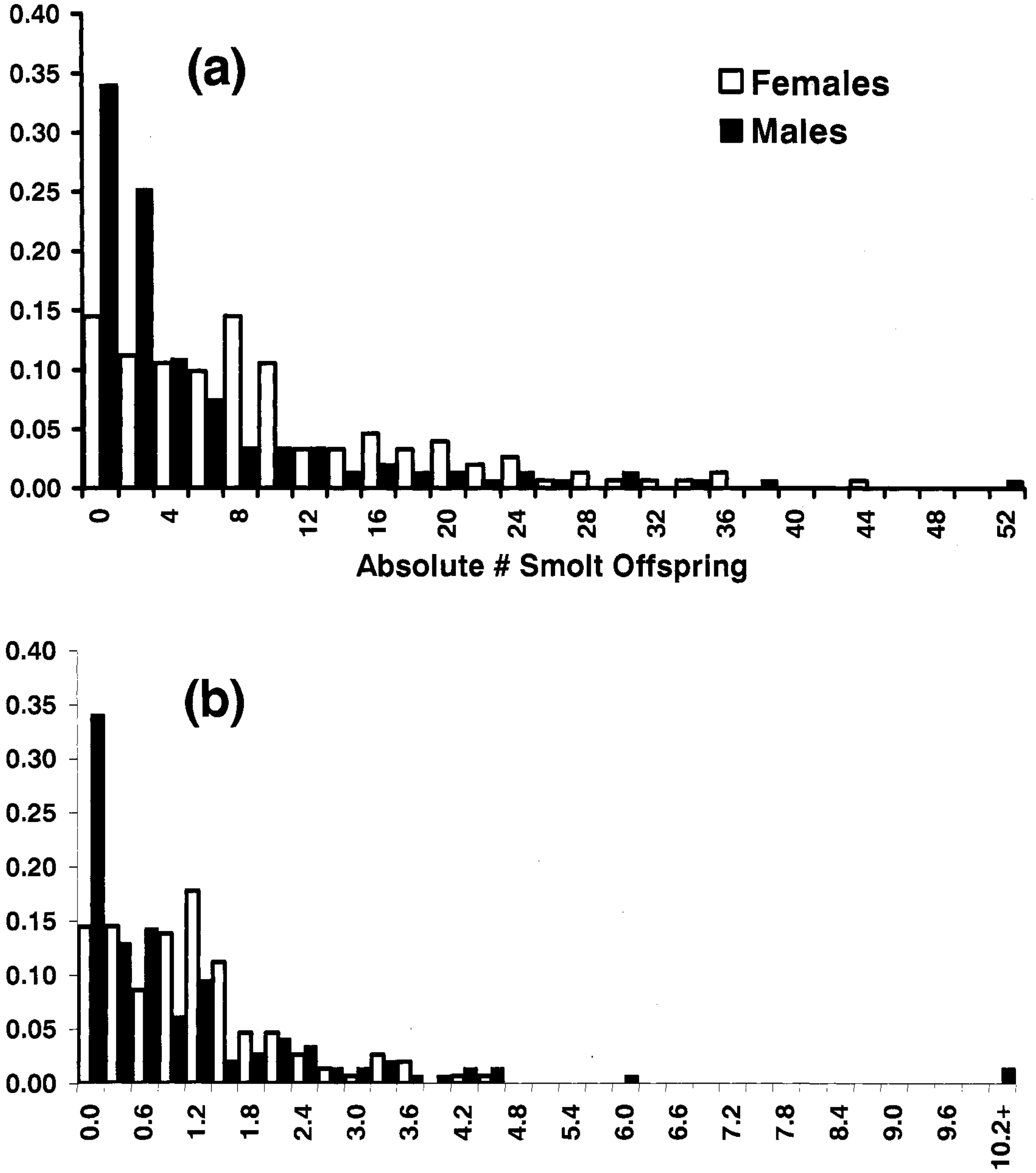

Figure 3.1. Frequency histograms showing the proportion of maternal (open bars) and paternal (solid bars) half-sib families in terms of both the absolute number of offspring per family (a) and relative to the within brood year average number of offspring per family (b).

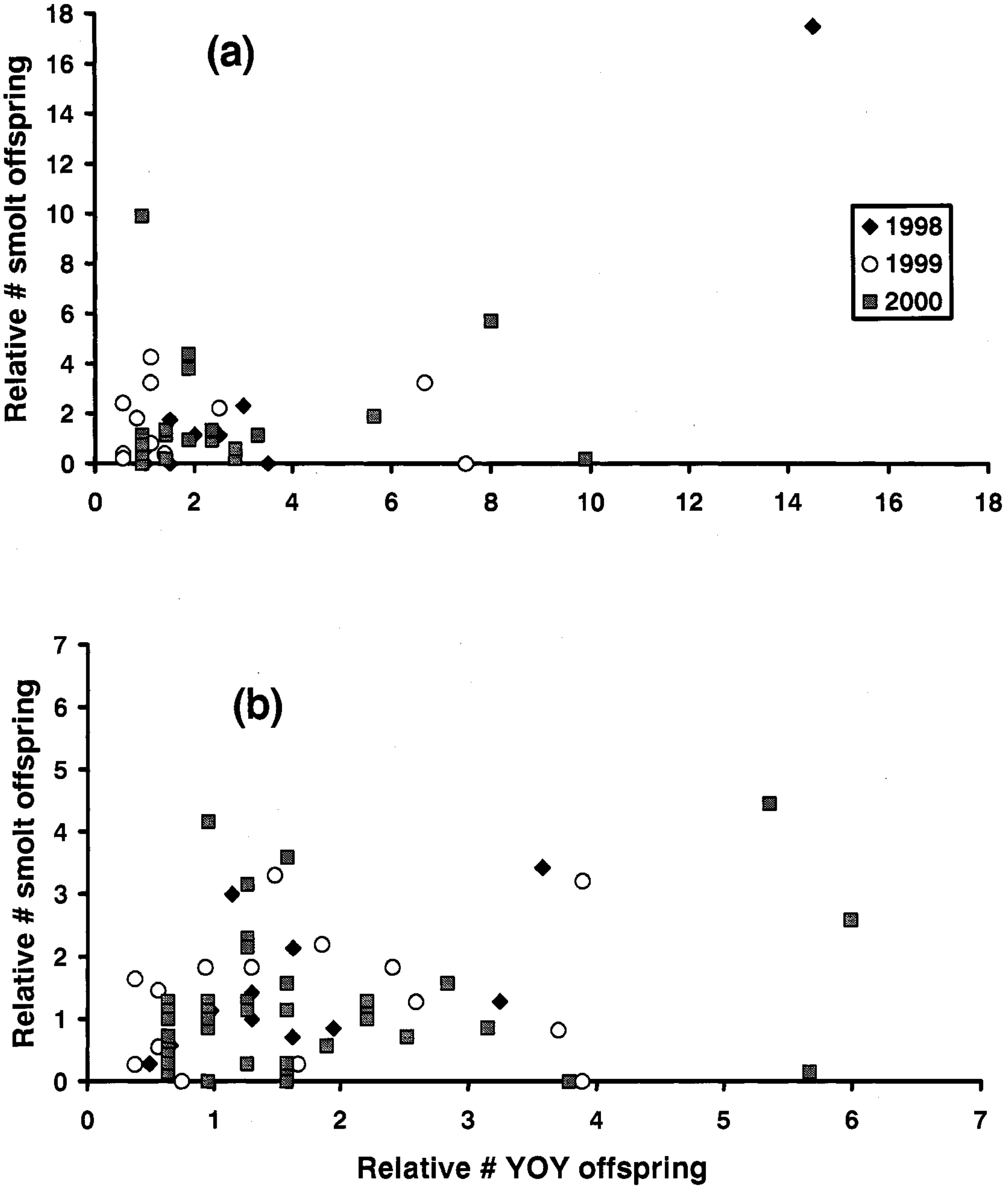

Figure 3.2. The relationship between the relative number of YOY offspring per half-sib family to the relative number of smolt offspring per half-sib family for both males (a) and females (b).

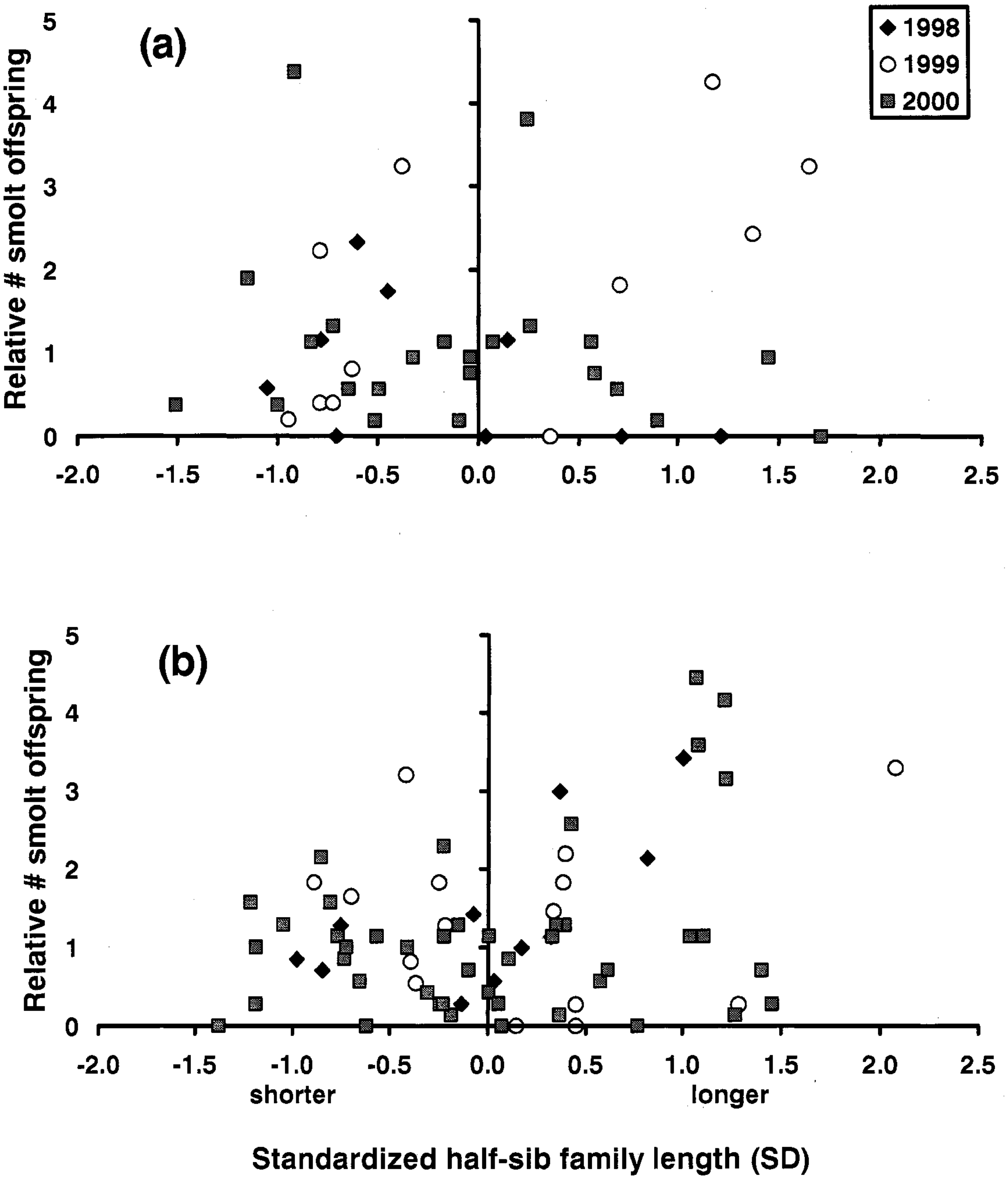

Figure 3.3. Relationship between the within paternal (a) or maternal (b) half-sib family standardized fork length (standard deviations) as young-of-the-year (YOY) and the relative number of smolt offspring per half-sib family. Data shown are unweighted.

Table 3.1. Number of adult male and female steelhead per brood year (*N*), their average fork length in millimeters (L_{AVG}), size range (SR), median date of arrival (AD_M) and average date of arrival (AD_A). Average arrival date is reported because it was used to standardize parental arrival date values.

	Males					Females				
Brood Year	*N*	L_{AVG}	SR	AD_M	AD_A	*N*	L_{AVG}	SR	AD_M	AD_A
1998	35	650	600 - 735	20-Mar	12-Mar	18	639	565 - 795	22-Mar	17-Mar
1999	26	656	400 - 815	22-Mar	14-Mar	28	658	600 - 740	20-Mar	10-Mar
2000	63	645	430 - 785	23-Mar	13-Mar	78	635	420 - 860	25-Mar	18-Mar
2001	21	667	460 - 765	19-Mar	23-Mar	28	683	610 - 775	26-Mar	26-Mar
Total	145					152				

Table 3.2. Number of smolts outmigrating in each of four years (*N*), number sampled (N_{SAMP}), the percent of the total number sampled in each outmigration year, and the age composition of outmigration smolts as a percent of the number sampled (1, 2 and 3 year old (YO)).

				Age Composition (% of N_{SAMP})		
Outmigration Year	*N*	N_{SAMP}	% of total	1 YO	2 YO	3 YO
2000	1285	231	18.0	38.3	58.1	3.6
2001	2346	336	14.3	2.5	94.3	3.2
2002	2298	505	22.0	0.8	87.4	11.7
2003	2589	592	22.9	3.3	72.1	24.6
Total	8518	1664	20.0	11.2	78.0	10.8

Table 3.3. Allelic variability at 12 microsatellite loci used for parentage analysis. The number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosities, the probability of deviations from Hardy-Weinberg equilibrium expectations after goodness of fit tests (P_{HWE}) and the inbreeding coefficient (F_{IS}) are shown with the total number of alleles across brood years (Total N_A).

		Locus											
Brood year		*Oki23*	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1998	N_A	16	12	9	15	18	24	11	14	24	38	19	16
	H_E	0.89	0.87	0.84	0.89	0.91	0.95	0.82	0.89	0.94	0.97	0.92	0.87
	H_O	0.92	0.87	0.85	0.94	0.92	0.96	0.77	0.89	0.98	0.94	0.88	0.89
	P_{HWE}	0.83	0.37	0.40	0.65	0.68	0.65	0.01	0.35	0.35	0.45	0.30	0.79
	F_{IS}	-0.037	0.006	-0.015	-0.055	-0.011	-0.012	0.061	0.003	-0.043	0.025	0.042	-0.0245
1999	N_A	15	15	13	21	19	24	14	19	30	42	20	19
	H_E	0.88	0.90	0.84	0.92	0.92	0.94	0.85	0.91	0.95	0.96	0.91	0.88
	H_O	0.91	0.94	0.78	0.91	0.89	0.94	0.74	0.85	0.94	0.96	0.85	0.85
	P_{HWE}	0.24	0.25	0.42	0.30	0.70	0.52	0.005	0.03	0.06	0.32	0.25	0.26
	F_{IS}	-0.033	-0.053	0.075	0.012	0.031	-0.001	0.128	0.065	0.009	0.000	0.066	0.031
2000	N_A	18	19	14	20	24	32	17	19	36	56	24	26
	H_E	0.90	0.89	0.81	0.89	0.90	0.95	0.80	0.91	0.95	0.97	0.91	0.85
	H_O	0.94	0.91	0.79	0.92	0.84	0.97	0.61	0.88	0.99	0.95	0.85	0.81
	P_{HWE}	0.23	0.15	0.65	0.90	0.57	0.15	0.000*	0.02	0.000*	0.03	0.000*	0.01
	F_{IS}	-0.047	-0.023	0.024	-0.028	-0.041	-0.017	0.235	0.036	-0.050	0.015	0.066	0.046
2001	N_A	17	12	13	17	21	22	13	16	28	36	28	15
	H_E	0.91	0.88	0.79	0.88	0.90	0.94	0.80	0.92	0.94	0.96	0.92	0.84
	H_O	0.87	0.91	0.80	0.82	0.94	0.94	0.65	0.81	0.91	0.96	0.89	0.86
	P_{HWE}	0.27	0.97	0.38	0.22	0.23	0.14	0.05	0.03	0.23	0.02	0.14	0.86
	F_{IS}	0.046	-0.037	-0.009	0.072	-0.045	0.004	0.185	0.110	0.036	0.0002	0.036	0.023
Total	N_A	18	20	18	22	26	34	20	21	43	66	26	28

* - indicates tests with significant ($p<0.05$) deviations from Hardy-Weinberg expectations, after sequential Bonferroni corrections.

Table 3.4. Selection differentials and gradients (1 standard error) for linear and quadratic selection for steelhead males by brood year and pooled data.

Males		Brood Year						
Linear Selection		1998	1999	2000	2001	Pooled	1998[a]	Pooled[a]
Differentials	Length	0.213 (0.100)**	0.079 (0.115)	0.155 (0.062)**	-0.205 (0.126)	0.105 (0.046)**	0.03 (0.111)	0.064 (0.045)
	Date	-0.265 (0.097)*	-0.198 (0.109)***	-0.239 (0.058)*	-0.033 (0.134)	-0.21 (0.043)*	-0.192 (0.106)***	-0.186 (0.041)*
Gradients	Length	0.195 (0.092)**	0.158 (0.113)	0.13 (0.056)**	-0.212 (0.134)	0.11 (0.043)"**	0.043 (0.108)	0.074 (0.042)***
	Date	-0.251 (0.092)*	-0.249 (0.113)**	-0.224 (0.056)*	0.025 (0.134)	-0.213 (0.042)*	-0.194 (0.108)***	-0.19 (0.041)*
Quadratic Selection								
Differentials	Length^2	0.973 (3.111)	-1.12 (0.934)	0.165 (0.46)	-1.616 (1.632)	-0.002 (0.372)	-7.617 (3.188)**	-0.029 (0.352)
	Date^2	0.064 (0.135)	-0.207 (0.168)	0.112 (0.068)	0.126 (0.141)	0.076 (0.053)	0.009 (0.151)	0.063 (0.05)
Gradients	Length^2	-7.3 (2.274)*	0.464 (1.381)	0.432 (0.43)	-3.088 (2.069)	0.175 (0.348)	-10.623 (2.83)*	0.12 (0.335)
	Date^2	0.05 (0.087)	-0.127 (0.18)	0.067 (0.071)	0.003 (0.146)	0.073 (0.052)	0.011 (0.123)	0.062 (0.05)
	Length*Date	-9.185 (1.474)*	-0.809 (0.74)	-0.879 (0.915)	3.025 (2.06)	-0.361 (0.26)	-8.643 (2.516)*	-0.208 (0.254)

[a] – results of analysis with one outlier male removed.

Probability values – * - significant at $p<0.01$, ** - significant at $p<0.05$, *** - significant at $p<0.10$

Table 3.5. Selection differentials and gradients (1 standard error) for linear and quadratic selection for steelhead females by brood year and pooled data.

Females		Brood Year				
Linear Selection		1998	1999	2000	2001	Pooled
Differentials	Length	0.322 (0.081)*	0.075 (0.088)	0.115 (0.045)**	0.049 (0.08)	0.118 (0.033)*
	Date	-0.358 (0.071)*	0.085 (0.088)	-0.17 (0.042)*	-0.044 (0.08)	-0.121 (0.033)*
Gradients	Length	0.169 (0.08)***	0.119 (0.093)	0.08 (0.042)"***	0.063 (0.083)	0.098 (0.033)*
	Date	-0.258 (0.08)*	0.127 (0.093)	-0.152 (0.042)*	-0.059 (0.083)	-0.101 (0.033)*
Quadratic Selection						
Differentials	$Length^2$	-0.94 (2.07)	-0.658 (0.693)	0.104 (0.666)	-0.903 (0.569)	-0.146 (0.196)
	$Date^2$	0.056 (0.08)	-0.071 (0.106)	0.065 (0.059)	0.011 (0.083)	0.03 (0.039)
Gradients	$Length^2$	-0.509 (2.407)	-0.9 (1.199)	0.283 (0.646)	-1.126 (0.698)	-0.194 (0.192)
	$Date^2$	0.091 (0.154)	-0.157 (0.203)	0.021 (0.065)	0.021 (0.084)	0.015 (0.084)
	Length*Date	0.668 (1.663)	-1.329 (2.352)	-0.377 (0.473)	0.271 (1.002)	-0.075 (1.002)

Probability values – * - significant at $p<0.01$, ** - significant at $p<0.05$, *** - significant at $p<0.10$

Table 3.6. Selection gradients (1 standard error) for linear selection on the average fork length of members of paternal and maternal half-sib families, measured at age 0+ (young-of-the-year; YOY). Also shown are the partial regression coefficients for the number of YOY offspring per half-sib family.

		Brood Year				
Males		1998	1999	2000	Pooled	1998[a]
	Family Average Length	-0.098 (0.218)	0.348 (0.226)	-0.146 (0.151)	0.024 (0.106)	-0.237 (0.247)
	# YOY members	0.195 (0.062)**	-0.016 (0.086)	0.037 (0.047)	0.061 (0.034)***	-0.057 (0.235)
Females						
	Family Average Length	0.037 (0.098)	-0.281 (0.223)	-0.259 (0.093)*	-0.174 (0.075)**	
	# YOY members	0.179 (0.043)*	-0.05 (0.115)	0.047 (0.038)	0.048 (0.034)	

[a] – results of analysis with one outlier male removed.

Probability values – * - significant at $p<0.01$, ** - significant at $p<0.05$

Chapter 4: Reproductive success of steelhead over nineteen generations and family-specific variation in marine survival as a function of body size and migration date

Synopsis

Sexual selection operating during spawning and natural selection on offspring combine to determine patterns of reproductive success. The direction and strength of selection may change over the lifetime of parents and their offspring or over the course of years or seasons. To test hypotheses concerning the effects of body size and breeding date on individual reproductive success and how those relationships are correlated with breeding conditions parents were genetically matched to adult offspring in 19 brood years in a small population of steelhead (*Oncorhynchus mykiss*). Overall, large and early arriving males produced more adult offspring than small or late arriving males, and large females produced more adult offspring than small females. In individual brood years, selection was consistently positive for male and female body size, while selection for arrival date for both sexes varied in direction among years. The opportunity for selection (variance in reproductive success divided by average reproductive success squared) was positively correlated with both the sex ratio and the spawning density for males and with only the spawning density for females. The strength and direction of selection on male length varied more when the sex ratio was female biased. In females, the strength of selection was unrelated to either the sex ratio or the spawning density. Marine survival of maternal and paternal half-sibling families appeared unrelated to either smolt length or date of migration, and instead appeared to be random or equal among families in the three years for which we had data. Though there was consistent selection for large body size,

this trait may be less likely to respond to selection than arrival date, at least on contemporary timescales, because of a large environmental effect on body size. Extensive yearly variation in the direction and shape of selection on both traits in concert with overlapping generations will confound response to selection on both traits, thus making predictions of evolutionary change in response to changing environments difficult.

Introduction

The direction and strength of selection can change over time. For example, beak size changed in Darwin's finches (*Geospiza* spp) on the Galapagos Islands, depending on the food source, which in turn depended on rainfall (Grant, 2003). Selection at different life history stages may oppose each other leading to trade-offs (Stearns, 1992), but presumably only if the selection pressures are constant. Understanding the processes of selection at all life history stages and across varying environments is important for predicting evolutionary response to a changing environment.

Salmonids as a group are good model systems for studying processes of evolution (Hendry, Stearns, 2004). Females and males have fundamentally different strategies for maximizing mating success: females maximize fecundity; males maximize fertilization. Body size and timing of spawning are known to have major effects on both strategies. Most straightforwardly, larger females tend to produce more eggs than smaller females (Beacham, Murray, 1993). There is considerable variation among species in the strength and shape of this relationship, among populations of the same species, and among females of the same population and species, however the trend is ubiquitous (Groot, Margolis, 1991; Quinn, 2005).

Males, on the other hand, have a more dynamic relationship between body size and their ability to fertilize eggs. Males physically compete for access to spawning females, the goal being to obtain a position directly adjacent to the female during spawning. Larger males can exclude smaller males from access to females (Dickerson *et al.*, 2002; Keenleyside, Dupuis, 1988; Quinn, Foote, 1994), however their ability to do this is dependent on the number of competitors (Dickerson *et al.*, 2002) which in turn is dependent on the timing of spawning (Quinn *et al.*, 1996). The intensity of this competition has led to the evolution of a variety of life history strategies involving the age (and body size) at maturity (Gross, 1985).

Natural selection on offspring begins at conception, but is mediated by maternal effects related to body size and spawn timing. Female salmon dig nests in streambed gravel for their developing offspring. Developing embryos are susceptible to disturbance from scour due to flooding or from the digging of other females (Essington *et al.*, 2000; Lapointe *et al.*, 2000). Large females can bury their eggs deeper in the gravel offering their offspring some extra protection from scour (Steen, Quinn, 1999). Females may physically compete for access to spawning habitat and large females may outcompete smaller females (Fleming, Gross, 1994). However, the ability of females to provide high quality habitat for their offspring is to some degree function of spawn timing (Fukushima *et al.*, 1998), but also of the predictability of environmental conditions (Seiler *et al.*, 2002).

Offspring quality is also affected by maternal body size and spawn timing. Large females produce larger eggs (Beacham, Murray, 1993), which produce larger fry (Beacham, Murray, 1990). Progeny of early-spawning females also tend to be larger due to earlier onset of feeding (Beacham, Murray, 1990) though the strength of this relationship will likely vary extensively in species with extended freshwater residence, due to territoriality (Chandler, Bjornn, 1988), predation (Brännäs, 1995) and food availability (Keeley, McPhail, 1998). Freshwater mortality tends to be size-selective (Quinn, Peterson, 1996; Smith, Griffith, 1994; Sogard, 1997) and generally, but not always (Connolly, Petersen, 2003; Good *et al.*, 2001), favors larger individuals.

The benefits of being large may extend beyond freshwater residency, though if food is abundant then the maternal effects of egg size and spawning date diminish over time and fry size is eventually uncoupled from these maternal traits (Heath *et al.*, 1999; Kinnison *et al.*, 1998). The limited research on the topic suggests that survival during seaward migration may be size-related (Collis *et al.*, 2001), and evidence exists supporting the idea that bigger is better during marine residence (Henderson, Cass, 1991; Holtby *et al.*, 1990; Moss *et al.*, 2005; Ward, Slaney, 1988; Ward *et al.*, 1989; Yamamoto *et al.*, 1999), however the importance of size may be overshadowed by other environmental variables (Quinn *et al.*, 2005; Ward, 2000).

The intensity of sexual selection depends on the ecological distribution of reproductive resources, that is, the density, patchiness, and the demography of breeding (Shuster, Wade, 2003). Semelparous Pacific salmon (*Oncorhynchus* spp.) are generally found in high density on the breeding grounds, clustered in both time (1 or 2 months) and space (Quinn, 2005). For any population the overall, seasonal sex ratio may be 1:1. However, males tend to arrive before females (Morbey, 2000), and after spawning the females guard nests while males move on to court other females. Therefore, over the spawning season, the operational sex ratio becomes male biased as females die or become reproductively unavailable (Quinn *et al.*, 1996). Iteroparous Pacific trout (such as steelhead, *O. mykiss*) are generally found in lower density on breeding grounds than the semelparous species (Quinn, 2005). They may be somewhat clustered in space, but often have a protracted spawning season of 3 or 4 months. Spawning occurs relatively rapidly preventing much spatial clustering.

Salmonid parity presents the opportunity for energetic trade-offs between bouts of reproduction (Crespi, Teo, 2002). Indeed, semelparous and iteroparous salmonids behave differently in ways that might affect their reproductive success. After spawning, semelparous female salmon guard their nests until they die, presumably in order to prevent nest re-use by other females. Iteroparous females do not guard their nest and may actually leave freshwater soon after spawning. Semelparous male salmon mate with as many females as they can before they die on the spawning grounds. Iteroparous males also try to spawn as much as possible, but can and may leave freshwater at any time.

Males of both semelparous and iteroparous species may mature early (Quinn, 2005). Jacks, males that migrate to sea but mature younger than the typical age at maturity exist in both groups, however, mature parr, males that mature prior (or maybe instead of) migrating to sea, also are found in both groups (Erkinaro *et al.*, 1994; Morán *et al.*, 1996; Seamons *et al.*, 2004b; Shapovalov, Taft, 1954; Thomaz *et al.*, 1997; Unwin *et al.*, 1999); however, they appear to be more common in iteroparous species (Quinn, 2005).

The goals of this chapter were 1), to determine the extent to which parental body size and date of arrival on the spawning grounds determined the number of returning adult offspring over two decades in a wild population of steelhead trout, 2), to determine trends among years in the direction and strength of selection on parental size and arrival date in relation to the sex ratio and abundance of females, 3), to determine the relationship between the opportunity for sexual selection and the observed sex ratio. The fourth goal, utilizing four years of data, was to determine the extent to which the average body size and migration date of half-sib families of smolts determined patterns of marine survival to return as adults.

Methods

Sampling site, sample collection

Adult steelhead were sampled from Snow Creek, Washington by employees of Washington Department of Fish and Wildlife (WDFW). Most fish were captured in a trap in a permanent weir found 0.95 km from the mouth of the creek (see map in (Seamons *et al.*, 2004b). Returning adults have been captured since the weir was installed in 1977, and trapping has continued since then. A small number of adults spawn below the weir every year. In one brood year (BY) these fish were captured by electrofishing in addition to the adults trapped at the weir. For all adults captured, fork length, sex, date and origin (hatchery/wild) were recorded and scales were taken for age analysis. Scales were dried in a folded piece of paper inserted into a small paper envelope, and later mounted on gummed scale cards for age analysis by WDFW employees. These scales and surplus scales were made available for DNA extraction and analysis. From 1997 to 2004, fin tissue samples were taken, in addition to the previous information, and stored in 95% ethanol for DNA analysis. All captured arriving adults were also given an opercle punch just before being released upstream of the weir. All unmarked adults captured in the downstream trap (kelts) were sampled as described above. As a matter of current WDFW policy, all stray hatchery steelhead are killed at the weir. However, in the years covered by our dataset a small number of hatchery adults bypassed the weir during high flows or were purposely released (upstream or downstream) after capture (8.5% of total number of adults). Cutthroat trout (*O. clarki*) were also sampled and included in parentage analysis (see below) since hybridization with steelhead is known to occur (Young *et al.*, 2001) and visual identification of adult hybrids is difficult (Baumstieger *et al.*, 2005). Information on sampling of smolts can be found in Chapter 3.

Genetics

Scales were used for DNA extraction and analysis for all adults without fin tissue sample. Scales were available for some fish before 1982 but the collections were incomplete so only scales from adults from 1982 through 1996 were used. Most of the scales we used came from envelopes as more scales were usually taken than were necessary to age the fish. When these loose, ungummed scales were unavailable, one or two scales per individual were carefully peeled from the gummed scale card for use in DNA analysis. DNA was extracted from these scales and from fin clips (collected from

1997 through 2004) using Qiagen DNeasy Tissue 96-well extraction kits (Qiagen Inc., Valencia, CA, USA) following the manufacturers guidelines.

Polymerase chain reactions to amplify 12 microsatellite loci were carried out as described in Chapter 1 (Seamons *et al.*, 2004b). For scale samples, the total number of cycles in the thermal cycler profile was raised from 25 to 30. Alleles were visualized and size fractionated using the methods described in Chapter 1 (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 using default values for dememorization, batches and iterations per batch (Raymond, Rousset, 1995). 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

Some spawning occurs in Snow Creek below the weir every year. Since both males and females may spawn below and above the weir (Thom Johnson, WDFW, 283236 Highway 101, Port Townsend, WA, 98368 – personal communication) all adults that were captured and sampled in Snow Creek were used as potential parents. This includes putative stray hatchery fish, which might have spawned below the weir even if they were subsequently killed. Adult cutthroat trout (37 total) and large (>250mm fork length) steelhead (approximately 60 total) that were outmigrating with the smolts but were distinctly larger than smolts 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 one allele at each locus with the putative offspring were finally called the true parents. When two adults matched, 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 brood years before the BY of the adult offspring were considered possible parents when performing parentage analysis. No accommodation was made for genotyping (or other) errors. See Chapter 1 (Seamons *et al.*, 2004a) for more information on the parentage methods.

Some adults were assigned only a single parent. The chance of matching a single parent is much higher than matching two parents simultaneously (Marshall *et al.*, 1998). We calculated the largest probability of a random match of a single parent using equation 1 in Chapter 2 (Seamons *et al.*, 2004a). This value puts an upper bound on the probability of matching a single parent by chance, i.e., all other probabilities will be smaller. In addition, if the single parent had spawned in multiple years and the age of the offspring was unknown (see Chapter 2) (Seamons *et al.*, 2004a) the offspring would not be able to be included in analysis. However, no adult steelhead offspring were matched to a single parent that had spawned in multiple years.

Statistical analysis

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. In some cases the arrival date could be estimated to within a day (e.g., if the upstream trap was not fishing for three days, the middle date was the estimate) so the estimated date was used as the arrival date. However, in cases where the date was more ambiguous, the arrival date was left blank. Our dataset 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 all anadromous males in a brood year). These data were included in the pooled analysis, but could not be analyzed by individual brood year.

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

Selection on parental traits was investigated using least squares linear regression analysis as outlined in (Lande, Arnold, 1983), summarized by (Brodie III *et al.*, 1995). The measure of fitness was the number of returning adult offspring per parent (parental reproductive success). Formal selection analyses require that traits are standardized to a mean of zero and a standard deviation of unity. The observed values were therefore adjusted by dividing by the average number of returning adult offspring detected per parental sex per brood year. The traits in question, fork length at arrival for spawning and date of arrival, were calculated as millimeters longer or shorter than the mean per sex per brood year or days before or after the mean per sex per brood year (i.e., relative trait values) to facilitate comparisons among years that differed in average length and/or average return date. Relative length and date were then standardized to a standard deviation of one by dividing each trait value by the standard deviation. Quadratic and interaction terms were calculated using the relative length or date as the raw trait value. Squared terms and the interaction term were both calculated then standardized to a mean of zero and a standard deviation of one. We analyzed the pooled data including brood year as a fixed effect using generalized linear model (GLM) analysis. This term was non-significant ($p > 0.10$) in all tests in which it was included so it was dropped from analysis and data were analyzed 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, co-varying 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 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 the partial regression coefficient for the squared term and the interaction term from a multiple regression including each trait, its squared value, and the interaction term. It should be noted that our estimates of direct selection included any indirect selection from unmeasured phenotypic traits that were correlated with body size and arrival date.

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

We also examined trends in the strength of selection across brood years. 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.

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 sexual selection for females might expected to be higher in brood years when many females returned (higher spawning density) and lower in years when few females returned (lower 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. Spawning density was estimated by first, estimating the average number of days females spent above the weir in several brood years. All adults returning in some brood years were either physically tagged by WDFW employees (1977-1989) or sampled for tissue as they arrived and again as they left (2001-2004). Tagged fish were observed leaving on a particular date; twice sampled adults were matched by genotype. 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 females and estimated an outmigration date by adding this value to the arrival date for females in BY 1982 – 2000. Next, we calculated the number of females upstream of the weir on any one day of the spawning period by subtracting the number of females that left from the number that arrived on any one day. Finally, we used the value from the date with maximum number of females upstream of the weir as our estimate of spawning density.

We determined the relationship between the opportunity for sexual selection for males (I_{males}) and females ($I_{females}$, (Shuster, Wade, 2003)) and the observed sex ratio using least squares regression. The opportunity for sexual selection was calculated as the variance in male (or female) reproductive success divided by the squared average male (or female) reproductive success within a BY. I_{males} was natural log transformed to correct for heteroscedasticity.

We used a set of weighted least squares regression to determine the relationship between half-sib family outmigration date or length and the number of returning adult members of the same family (total selection). Individual smolt outmigration date and length were standardized in the same way as described above for adult traits. After standardization, the average within paternal or maternal half-sib family outmigration date or length was calculated and this value was used in the regression analysis. The precision of within half-sib families varied with the numbers of individuals in each half-sib family, thus the data were weighted by the inverse of the variance in the average outmigration date or length.

Separate multiple regression analyses were conducted to determine direct selection on smolt length and outmigration date. Because the number of returning adult members of a particular half-sib family is not independent of the numbers of smolts leaving (e.g., families that produced no smolts cannot produce any adult offspring) we included the relative number of smolt offspring per half-sib family in multiple regression analysis. Significant correlations existed between parental standard length or standard arrival date and smolt length or outmigration date (data not shown), so parental traits were included in the multiple regressions. Parental traits were standardized as described above, however they were standardized to means and standard deviations of only those adults that had smolt offspring detected.

Results

Parental characteristics

A total of 1094 anadromous, wild and hatchery steelhead returned and were sampled at Snow Creek in BY 1982 through 2004 (Table 4.1). The number of adults returning in any one year varied considerably, ranging from 6 (BY 1990) to 143 (BY 2000; Figure 4.1) with an average return of 49 adults per BY. Overall, more females than males returned to Snow Creek (1.15 females per male), though the observed sex ratio varied considerably among brood years (Figure 4.1). 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.

A great deal of variation in length at return was apparent in Snow Creek steelhead (Table 4.1). Fork length of individual males ranged from 330 to 885 mm and length of females ranged from 302 to 860 mm (Table 4.1). On average, anadromous males were shorter than females, 634 mm vs. 648 mm, though the largest adult in our dataset was male and the shortest was female. Average male fork length within years ranged from 573.8 mm (BY 1993) to 676.5 mm (BY 1996). Average female fork length ranged from 568.8 mm (BY 2003) to 692.5 mm (BY 1990).

A large amount of variation was also apparent in steelhead arrival timing (Table 4.1). Individuals arrived as early as November and as late as the end of April. There was no consistent arrival pattern (median or mean date) related to the sex of adults within and among BY, though it was generally near the third week of March (Table 4.1).

The average period spent upstream of the weir per female varied between 16.7 and 78.3 days with an overall average of 49.3 days (Table 4.2). On average males spent between 26.8 and 83.5 days upstream of the weir with an overall average of 48 days (Table 4.2).

Population genetics

Seven of 276 individual locus tests showed significant departures from HWE after sequential Bonferroni corrections (Table 4.3). *Omy77* deviated from HWE in brood years 1983, 2000 and 2003 probably from a substantial number of null alleles (Ardren *et al.*, 1999). The remaining loci with significant deviations from HWE were *Ots107* and *Ots108* in three brood years before 1995. *Ots107* had a very large size range with alleles as long as 440 base pairs. Since we were amplifying DNA from scale samples it is likely that the deviations were due to upper allele dropout. A qualitative scan of parentage assignments suggested that *Ots108* had low frequency null alleles (several matching potential parents that were homozygous as was the offspring), though no formal analysis of this was completed. We did not calculate allelic genotyping error with this dataset, but we do not expect it to be substantially different than 0.6% per allele, reported in Chapter 2 (Seamons *et al.*, 2004a).

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. 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 where putative parents of these individuals appeared to be heterozygous with a null allele or putative offspring appeared to have inherited a null allele. Locus *One108* also appeared to have null alleles present at low frequency based on consistent marginally significant positive F_{IS} values (Table 4.3) and observation during parentage assignment. Null alleles in *One108* were taken into account in parentage assignments as described for *Omy77*.

Average observed heterozygosity across all BY and all loci was 0.88, ranging from a low of 0.52 in BY 1995, *Omy77* to a high of 1.00 in several BY and loci (Table 4.3). The global exclusion probability across all BY and all loci was 1.00 for both parents. Across loci the exclusion probability for all BY combined ranged from a low of 0.641 in *Omy1004* to a high of 0.936 in *Ots107*. Within BY across loci the exclusion probabilities ranged from a low of 0.502 in *Omy77* in BY 1990 to a high of 0.926 in *Ots107* in BY 2000.

We did not expect to be able to assign parents to cutthroat, hatchery origin steelhead or to steelhead spawned before 1982. After considering species, origin, and parental brood year (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 BY 1990 (0.013), the year when only 6 fish returned to spawn. The next lower probability of a random match was 0.006 (BY 1988), which was more representative of the probabilities seen in all other BY (average = 0.002). Successful parental assignment to known aged adult offspring varied by brood year from 48% to 95% (average = 73%) across all 19 parental brood years (1982 – 2000). As was expected, assignment success was lower for adults from brood years when the trap was compromised by high flows.

On average over all brood years, sixty percent of females and 75% of males produced no adult offspring (Figure 4.2a). Two females produced the highest absolute number of offspring (8, Figure 4.2a), while a male produced the highest relative number of offspring (38 times the average, Figure 4.2b).

Selection analysis

Overall, larger and earlier-arriving males had higher reproductive success (more returning adult offspring) than smaller and later-arriving males in terms of total and direct linear selection ($p<0.05$, pooled data, Table 4.4). However, these relationships explained very little of the variation ($r^2=0.01$, all tests). Within individual BY, larger males had more offspring than smaller males in 1991 (differential, $p<0.10$) and 1998 ($p<0.05$, differential, $p<0.10$, gradient) and smaller males had more offspring than larger ones in 1992 ($p<0.05$, gradient; Table 4.4). No statistically significant linear relationships between arrival date and reproductive success were found in individual BY except in BY 1998 when early arriving males had more offspring than later arrivals ($p<0.05$, gradient; Table 4.4). Males of intermediate length had fewer offspring in 1988 ($p<0.01$, differential) and more offspring in 1996 ($p<0.05$, differential; Table 4.4) than large or small males, however after accounting for correlations with date there was no significant non-linear selection apparent in either length or arrival date ($p>0.10$, gradients; Table 4.4). Correlational selection favoring combinations of length and arrival date was found only in BY 1998 when large and early fish had more offspring ($p<0.10$, gradient; Table 4.4).

Larger females had more offspring than smaller females overall ($p<0.05$, total and direct linear selection – pooled data, Table 4.5), but, as for males, length explained very little of the variation in offspring number ($r^2=0.01$, both tests). Arrival date was unrelated to the number of offspring produced ($p>0.10$). Among BY, large females had more offspring in 1989 and 1985 ($p<0.05$, total and direct linear selection; Table 4.5); no other linear relationship with length was statistically significant. Earlier arriving females had more offspring in 1990, 1999 ($p<0.05$, total and direct linear selection; Table 4.5) and in 1983 ($p<0.10$, total linear selection; Table 4.5); no other linear relationship with arrival date was statistically significant ($p>0.10$). Females of intermediate length had less offspring in 1996 and 1985 ($p<0.05$, both tests) and more offspring in 1983 ($p<0.05$) than larger and smaller females (total univariate non-linear selection, Table 4.5). Females of intermediate length had fewer offspring in 1988 ($p<0.10$) and 1985 ($p<0.05$) and more offspring in 1995 ($p<0.05$) than larger and smaller females when covariance with other traits was factored out (direct univariate non-linear selection, Table 4.5). Females arriving early and late had more offspring than females arriving near the mean arrival date in 2000 ($p<0.05$, total and direct univariate non-linear selection; Table 4.5), while the opposite was true in 1990 ($p<0.05$, total univariate non-linear selection) and 1995 ($p<0.10$, direct univariate non-linear selection; Table 4.5). Large and late females had more offspring in 1988 ($p<0.05$) and large and early females had more offspring in 1982 ($p<0.05$) and 1995 ($p<0.10$, direct bivariate non-linear selection; Table 4.5).

Selection for male length was consistently positive across BY ($p<0.05$; total and direct linear selection, Figure 4.3). No other test was significantly different from expectations of half positive, half negative. Selection for female length was consistently positive across BY ($p<0.05$; total and direct linear selection, Figure 4.4). No other test was significantly different from random expectations of half positive and half negative selection. Selection (absolute value of selection coefficient) appeared stronger on male length and than on female length (Mann-Whitney test, $p<0.05$ – differentials, $p<0.10$ – gradients), and selection on male arrival date was stronger than that on female arrival date (Mann-Whitney test, $p<0.10$ – gradients).

Large, positive selection differentials (total selection) for male length (indicating selection favoring large males) were found more often when the observed sex ratio was skewed towards males ($p<0.05$, one-tailed test). This relationship disappeared when indirect selection from arrival date was accounted for (Figure 4.5a, $p=0.15$, one-tailed test). Male length selection differentials and gradients varied more when the sex ratio was skewed towards females (one-tailed F-test, $p<0.10$; Figure 4.5a). There was no significant relationship between male arrival date differentials or gradients with the observed sex ratio ($p>0.24$, two-tailed test), and though the male arrival date selection gradients appeared to vary more when the sex ratio was skewed toward females (Figure 4.5b) there was no statistically significant difference in variance (one-tailed F-test, p=0.53). The observed sex ratio was not related to female length or arrival date differentials or gradients (p>0.15 all tests, one-tailed tests), nor did there appear to be any relationship of sex ratio and the variance of the differentials or gradients.

Maximum density did not appear to be related to the value of selection differentials for female length (p=0.12, two-tailed test) or arrival date (p=0.84, two-tailed test), nor did density appear to be related to the value of selection gradients for length (p=0.19, Figure 4.6a) or arrival date (p=0.86; Figure 4.6b). The apparent increase in variability in selection gradients for female length (Figure 4.6a) and arrival date (Figure 4.6b) with a decrease in the estimated density is likely due to chance because of small sample size (low overall numbers of females = low estimated density). No relationship was found between the number of males returning and the strength of selection on male length or arrival date (p<0.30, all tests).

The opportunity for sexual selection in males (I_{males}, (Shuster, Wade, 2003)) was higher in years with a male-skewed total observed sex ratio (r^2= 0.31, p=0.001 – ln transformed data, Figure 4.7a – untransformed data), while the opportunity for sexual selection for females ($I_{females}$, (Shuster, Wade, 2003)) was unrelated to the observed total sex ratio (p=0.95, Figure 4.7b). I_{males} was higher in years in which more males returned (linear regression, p<0.001, r^2=0.74, Figure 4.8a) and $I_{females}$ was higher in years in which more females returned (linear regression, r^2=0.30, p<0.01, Figure 4.8b).

The frequency of mother-only parental assignments increased with an increasingly female biased sex ratio (logistic regression, p<0.001, r^2=0.65; Figure 4.9). Regressions of the sex ratio on the frequency of father-only parental assignments was non-significant ($p>0.90$) as were regressions of the frequency of mother-only parental assignments with the number of males ($p>0.10$), the number of females ($p>0.47$), or the total number of returning adults ($p>0.22$).

Considerable variation was found between brood years in the relationships between half-sib family smolt characteristics and the number of members detected as adults (Table 4.6). In 1998, though large and late migrating paternal half-sib families and late migrating maternal half-sib families had more detected adult members when indirect selection was included (differentials, $p<0.01$), neither smolt length nor migration date explained any of the variation in returning adults when parental traits and the number of individuals per smolt family were included in the model ($p>0.10$, Table 4.6). In 1999, indirect and direct selection on migration date favored early migrating families, however direct selection appeared to be equal or random across paternal and maternal half-sib families as the number of members detected as smolts explained much of the variation ($r^2=0.56$ – paternal, $r^2=0.38$ – maternal) in number of members detected as adults ($p<0.01$, Table 4.6). In terms of indirect selection, in 2000, late (differentials, $p<0.10$) migrating smolts in paternal half-sib families were detected in higher numbers as adults ($r^2=0.33$). Conversely, early outmigrating maternal half-sib families were detected in higher numbers as adults (differentials, $r^2=0.12$, $p<0.01$). However, overall, the number of individuals found in paternal and maternal smolt half-sib families determined the number detected as adults (direct selection, $p<0.01$, paternal; $p<0.10$, maternal), but because of the variation in effect among brood years, number of smolts explained very little of the variation in adult members detected ($r^2=0.01$ both tests).

Discussion

Our primary objective was to determine the effects of parental length at spawning and arrival date on the spawning grounds (in and of itself important, but here assumed to be related to spawning date) on the number of returning adult offspring. By genetically inferring parentage we directly enumerated the number of mature, returning adult offspring of adult steelhead from 19 brood years of a small population of steelhead.

Most adult offspring were produced by a few adults, the same pattern observed in Chapter 2 (Seamons *et al.*, 2004a) with young-of-the-year and yearling offspring and in Chapter 3 with smolt offspring. The only obvious, and not unexpected, difference was that the percentage of males and females with no detected offspring increased. The additional mortality in the ocean may have caused more half-sib families to disappear, especially the rare families. It may also be because the offspring of small parents matured as parr, which we did not sample, and were lost from the anadromous population (Thrower *et al.*, 2004). This has the potential to bias our results if smaller anadromous adults (male and female) are more likely to produce mature parr. Offspring of small anadromous male (but not female) Atlantic salmon (*Salmo salar*) are more likely to mature as parr (Duston *et al.*, 2005), and coho salmon (*O. kisutch*) jacks are more likely to produce jacks (Appleby *et al.*, 2003). Anadromous *O. mykiss* adults can produce mature parr (Thrower *et al.*, 2004) and mature parr produce anadromous adults (this paper), but the likelihood of small anadromous *O. mykiss* adults producing mature parr is unknown.

Remnant effects of selection during breeding?

Are these few adults that produced more offspring larger or smaller adults? Larger males consistently (regression coefficients were positive in all but three BY) produced more offspring than smaller males overall (statistically significant in the pooled data and in some individual BY). This was not entirely unexpected as male salmonids engage in physical contests to acquire and dominate access to spawning females (Keenleyside, Dupuis, 1988; Quinn, Foote, 1994), and large males typically obtain the best position, closest to the female, during spawning (Dickerson *et al.*, 2002). Those males closest are thought to fertilize the most eggs and hence have higher reproductive success, though it is not always the case (Dickerson *et al.*, 2005).

Larger females also consistently produced more adult offspring than smaller females. Larger females have higher fecundity (Beacham, Murray, 1993) and may also provide better developing and rearing habitat (maternal effects) by digging deeper redds that may be less susceptible to disturbance (Steen, Quinn, 1999). Larger females also may produce higher quality (e.g., larger) offspring (Beacham, Murray, 1990) that may survive in higher numbers when size-selective mortality favors large size (Sogard, 1997).

Do these few adults that produced more offspring arrive on the spawning grounds earlier or later than those that produced fewer or no adult offspring? Overall, earlier arriving males produced more offspring than later arriving males. Pacific salmon males generally arrive before females (Morbey, 2000), and male steelhead arrived before (rather than simultaneous with) the females they mated with (Chapter 1) (Seamons *et al.*, 2004b). Early arriving males may benefit from a lack of competitors (Quinn *et al.*, 1996) or dominance established by prior residence (Foote, 1990). However, the relationship is affected by the operational sex ratio because the ability of early arriving males to dominate diminishes with an increase in the number of males on the spawning grounds relative to the number of females (Dickerson *et al.*, 2005). Further, even later arriving male steelhead were reproductively successful, they just needed to arrive before a female (Chapter 1) (Seamons *et al.*, 2004b), and the apparent lack of consistency in the direction of selection suggests that sometimes arriving later is advantageous.

We found no apparent general advantage to any arrival timing for females; evidence existed for an advantage for early, early and late, and intermediate arrival timing in various brood years. In winter steelhead, arrival timing may be more closely related to spawn timing for females than in males due to the fact that, unlike Pacific salmon, most adult steelhead live through the entire spawning period. The benefits, then, of arrival timing for females were probably more closely tied to the spawning environment. The nests of early spawning females may be susceptible to disturbance from later arriving females (Essington *et al.*, 2000). The nests of all females, regardless of timing, were, to some degree, susceptible to disturbance from floods (Lapointe *et al.*, 2000), the frequency, duration and timing of which were unpredictable brood year to brood year (Seiler *et al.*, 2002).

Trends in strength and direction of selection

Our second objective was to determine trends in direction and strength of observed selection in parental length and arrival date, especially in relation to the observed sex ratio and female spawning density. Although the half positive, half negative regression coefficients across BY might be observed 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. Regression coefficients for selection on length in females and males were almost always positive, and negative only in years of low population size. In their review of selection on size, Kingsolver and Pfennig (2004) found that the majority of studies found positive selection for size, a possible explanation for the tendency for organisms in the fossil record to evolve large body size. Blanckenhorn (2000) suggested several mechanisms by which body size may stay small, all involving costs of being large. Though the overall trend was for larger body size, we found evidence that the direction and shape of selection varied among brood years, suggesting that there are sometimes costs associated with being large. For anadromous salmonids there is likely a cost to getting bigger in terms of additional mortality occurring while at sea for an extra year.

Selection on male and female steelhead traits was stronger than that reported for organisms in general in the review by (Kingsolver *et al.*, 2001). When compared with their dataset, our median differential for male length selection differentials was 60th percentile, the median gradient for male length was 64th percentile. Our median male arrival date differential fit in the 63rd percentile, while the median male arrival date gradient was in the 69th percentile. Female selection differentials were closer to or below the median reported by Kingsolver et al. (43rd for length and 51st for arrival date). Selection gradients, however, fell into the 62nd and 64th percentiles for length and arrival date respectively. Contrary to Kingsolver et al., median selection differentials and gradients for arrival timing (phenology) were almost always greater than those for length (morphology). Measurement error is not likely to be the cause of the observed difference since arrival date is actually a rough estimate of spawning date (though arrival date is also a trait unto itself) while length was measured within millimeters. It seems more likely that the variable and unpredictable effects of arrival timing may have a more significant impact on fitness; one large flood may mean an entire nest is destroyed rather than slightly reducing the survival of offspring.

Male opportunity for sexual selection increased with an increasing male bias in the observed sex ratio. Male opportunity for selection was expected to be related to the operational sex ratio (Quinn *et al.*, 1996). Presumably, when the sex ratio is female biased it is easier for males to acquire mates, or perhaps more correctly, it is more difficult for one or a few males to dominate access to spawning females. This was also reflected in the higher reproductive success of mature parr. The opportunity for selection may also be higher when spawning density is high (Fleming, Gross, 1994). In Snow Creek steelhead, male opportunity for sexual selection also increased when more males returned and explained more variation in opportunity than the sex ratio. It could be that the number of males returning is also a measure of the level of competition. If the ability to dominate access to a female does not change with the number of competitors (perhaps because of low spawning density), more males will go without spawning (i.e., zero reproductive success) increasing the overall variance in reproductive success and thus the opportunity for selection. Though we found a significant effect, the magnitude of this effect may be smaller than measured to due to autocorrelation between the variables.

What determined the sex ratio? The overall sex ratio (for anadromous adults) generally favored females, likely because many males matured as parr (Chapter 1) (Seamons *et al.*, 2004b) and may have been lost to the anadromous population (Thrower *et al.*, 2004). However, the sex ratio varied widely among brood years. The cause of the variation in the sex ratio is unknown. It is difficult to imagine that there was sex specific mortality in the ocean. The sex ratio could be tied to environmental conditions during freshwater life history of a cohort. The decision to either smolt or mature is made based on growth specific variables, part of which are environmentally influenced (Thrower *et al.*, 2004).

Female opportunity for selection was expected to increase with increasing density of females on the spawning grounds (Fleming, Gross, 1994). Indeed, we saw an increase in the opportunity for selection in females in years when more females returned; however the amount of variation explained by the number of females was substantially less than that for males. Female Pacific salmon compete for access to good spawning sites (van den Berghe, Gross, 1989) and the intensity of this competition might be expected to increase as female density in space and time increases. We might also see this relationship because of density dependent effects. Snow Creek appeared to be able to support a certain number of individuals to smolt age regardless of the number of females (Johnson, Cooper, 1995). When few females spawn the number of offspring per female is closer to being equal among females. In spite of this relationship, we found no relationship between our estimate of spawning density and the strength of selection. With over ten kilometers of stream accessible, and to some degree available as spawning habitat, even our greatest estimate of female spawning density, 6.5 females per kilometer (perhaps an order of magnitude less than for coho (*O. kisutch*) in Snow Creek), seems unlikely to have caused serious competition among females.

Natural selection on traits of smolt offspring

Larger smolts may survive in higher frequency during migration (Collis *et al.*, 2001) or during marine residence (Henderson, Cass, 1991; Holtby *et al.*, 1990; Ward, Slaney, 1988; Ward *et al.*, 1989). As in adults, selection on migration timing within years is likely to be variable (Bilton *et al.*, 1982), though the overall pattern of Spring migration has likely evolved due to strong selection for a Spring migration related to food availability or other ecological ocean conditions. Though there was some significant indirect selection on smolt size and timing, direct selection was weak or absent in our data. Instead, overall patterns of reproductive success appeared to be set in the first half (i.e., freshwater) of their life history, i.e., equal or random family survival at sea. Selection coefficients for the number of smolt family members were almost always positive implying that more numerous smolt families produce more adults. Conversely, selection coefficients for smolt length and arrival date were both positive and negative suggesting that the direction or strength of selection varies among years as was seen in Keogh River steelhead (Ward, 2000).

Patterns of selection during spawning and early life history

Most mortality occurs in freshwater for Pacific salmonids (Quinn, 2005), and as seen here, selection during spawning and early life history determined overall patterns of reproductive success. Males and females mated multiply – up to 10 different mates – and failed to successfully mate (Chapter 1) (Seamons *et al.*, 2004b). There was no evidence of size assortative mating, but females spawned with males that arrived before they themselves arrived (Chapter 1) (Seamons *et al.*, 2004b). Non-anadromous males fertilized approximately 30-40% of eggs (Chapter 1) and their offspring survived and migrated to the ocean in approximately the same proportions as smolts (48%, Chapter 3) and returned as spawning adults (~40%, this chapter).

Large and early males had more young-of-the-year offspring (YOY) (Chapter 2 – coefficients not shown), more yearling offspring (Chapter 2 – coefficients not shown), more smolt offspring (Chapter 3) and more adult offspring. Larger females had more YOY and yearling offspring in 1997, 1998 and more YOY offspring in 2000 and fewer YOY and yearling offspring in 1999 (Chapter 2 – coefficients not shown). Larger females had more smolt offspring in 1998 and 2000 (Chapter 3). Larger females had more adult offspring in 1997, 1998 and 2000 and fewer in 2000, the same as observed for YOY offspring (indirect selection, Table 4.5). When indirect selection was accounted for the pattern shifted such that larger females had more offspring in 1999 and fewer in 1998 (direct selection, Table 4.5), though overall, the trend was for larger females to have more adult offspring.

The direction of selection on arrival timing of females changed according to the age of offspring. Later arriving females had more YOY offspring in 1997, 1999 and fewer in 1998; the coefficient for BY 2000 was zero (Chapter 2 – coefficients not shown). Patterns observed with smolt and adult offspring are the same in 1998 and 1999; later arriving females had more offspring in 1999 and fewer offspring in 1998 (Chapter 3). In BY 2000 earlier arriving females had more smolt and adult offspring. The difference in measured selection may be due to a small juvenile sample size per family in BY 2000. We sampled a relatively fixed number of juvenile offspring each year, ~300 (Chapter 2). Brood year 2000 saw the largest number of females returning in the years for which we have data, 78. Assuming equal reproductive success among females the number of offspring sampled per family in BY 2000 was a little less than four, compared to 10 or more for BY 1997-1999. In this case patterns seen in smolt offspring are likely the best estimate of selection in the early life history.

Often the goal of research such as this is to understand how populations may respond to changing environmental conditions (e.g., global warming) or to management policies (e.g., size selective fisheries). Making predictions about evolution of body size or breeding date in steelhead is difficult if not impossible. The strength and direction of selection varied between the sexes within years. When the selection differs between the sexes the selection applied to the population is the average of the selection of the males and females (Falconer, Mackay, 1996). The strength and direction of selection also varied among years. This alone is not a problem if the general trend is in one direction, such as we saw with length in this study. However steelhead have overlapping generations which, even in the absence of variation in selection pressure, reduces the response to selection (Falconer, Mackay, 1996). In our case, differently aged offspring of a single parent might be subject to very different selection pressures, e.g., arrival date, compounding the effects of overlapping generations.

Summary

Larger and earlier arriving male steelhead and larger female steelhead had more returning adult offspring than smaller or later arriving fish. Selection was consistently positive for larger males and females, but varied in shape and direction for arrival timing. Variance in reproductive success was related to the sex ratio for males, but not for females, while variance in reproductive success for both males and females was positively influenced by the overall spawning numbers. The strength of selection on male length varied more when the sex ratio was biased for females. Selection on smolt traits varied in direction and intensity, but mainly the numbers of smolt individuals in a family determined the number of returning adults in a family. In spite of this selection, Snow Creek steelhead were unlikely to show any significant evolution in adult length or arrival date due to a large environmental influence on the expression of length and on the selection on arrival date. Extensive yearly variation in the direction and shape of selection on both traits in concert with overlapping generations will also confound response to selection on both traits making predictions of evolutionary change in response to changing environments difficult.

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References

Appleby AE, Tipping JM, Seidel PR (2003) The effect of using two-year-old male coho salmon in hatchery broodstock on adult returns. *North American Journal of Fisheries Management* **65**, 60-62.

Ardren WR, Borer S, Thrower F, Joyce JE, Kapuscinski AR (1999) Inheritance of 12 microsatellite loci in *Oncorhynchus mykiss*. *Journal of Heredity* **90**, 529-536.

Baumstieger J, Hankin D, Loudenslager EJ (2005) Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identifications. *Transactions of the American Fisheries Society* **134**, 829-840.

Beacham TD, Murray CB (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Transactions of the American Fisheries Society* **119**, 927-945.

Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* **42**, 485-508.

Bilton HT, Alderdice DF, Schnute JT (1982) Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Canadian Journal of Fisheries and Aquatic Science* **39**, 426-447.

Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology* **75**, 385-407.

Brännäs E (1995) First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* **9**, 411-420.

Brodie III ED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* **10**, 313-318.

Chandler GL, Bjornn TC (1988) Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. *Transactions of the American Fisheries Society* **117**, 432-443.

Collis K, Roby DD, Craig DP, Ryan BA, Ledgerwood RD (2001) Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River Estuary: vulnerability of different salmonid species, stocks, and rearing types. *Transactions of the American Fisheries Society* **130**, 385-396.

Connolly PJ, Petersen JH (2003) Bigger is not always better for overwintering young-of-year steelhead. *Transactions of the American Fisheries Society* **132**, 262-274.

Crespi BJ, Teo R (2002) Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. *Evolution* **56**, 1008-1020.

Dickerson BR, Brinck KW, Willson MF, Bentzen P, Quinn TP (2005) Relative importance of salmon body size and arrival time at breeding grounds to reproductive success. *Ecology* **86**, 347-352.

Dickerson BR, Quinn TP, Willson MF (2002) Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethology Ecology and Evolution* **14**, 29-44.

Duston J, Astatkie T, MacIsaac PF (2005) Genetic influence of parr versus anadromous sires on the life histories of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2067-2075.

Erkinaro J, Shchurov IL, Saari R, Niemelä E (1994) Occurrence of Atlantic salmon parr in redds at spawning time. *Journal of Fish Biology* **45**, 899-900.

Essington TE, Quinn TP, Ewert VE (2000) Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 205-213.

Falconer DS, Mackay TFC (1996) *Introduction to quantitative genetics*, 4th edn. Prentice Hall, Essex, England.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Fukushima M, Quinn TJ, Smoker WW (1998) Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 618-625.

Good SP, Dodson JJ, Meekan MG, Ryan DAJ (2001) Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1187-1195.

Grant BR (2003) Evolution in Darwin's finches: a review of a study on Isla Daphne Majòr in the Galapagos Archipelago. *Zoology* **106**, 255-259.

Groot C, Margolis L (1991) *Pacific salmon life histories* University of British Columbia Press, Vancouver, British Columbia.

Gross MR (1985) Disruptive selection for alternative life histories in salmon. *Nature* **313**, 47-48.

Heath DD, Fox CW, Heath JW (1999) Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution* **53**, 1605-1611.

Henderson MA, Cass AJ (1991) Effect of smolt size on smolt-to-adult survival for Chilko Lake sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 988-994.

Hendry AP, Stearns SC (2004) Evolution illuminated: salmon and their relatives. Oxford University Press, New York, New York.

Holtby LB, Andersen BC, Kadowaki RK (1990) Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon

(*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 2181-2194.

Johnson TH, Cooper R (1995) Anadromous game fish research and planning annual report, p. 33. State of Washington Department of Fish and Wildlife, Olympia.

Keeley ER, McPhail JD (1998) Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). *Behaviour* **135**, 65-82.

Keenleyside MHA, Dupuis HMC (1988) Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **66**, 262-265.

Kingsolver JG, Hoekstra HE, Hoekstra JM, *et al.* (2001) The strength of phenotypic selection in natural populations. *The American Naturalist* **157**, 245-261.

Kingsolver JG, Pfennig DW (2004) Individual-level selection as a cause of Cope's Rule of phyletic size increase. *Evolution* **58**, 1608-1612.

Kinnison MT, Unwin MJ, Hershberger WK, Quinn TP (1998) Egg size, fecundity, and development rate of two introduced New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. *Canadian Journal of Fisheries and Aquatic Science* **55**, 1946-1953.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* **37**, 1210-1226.

Lapointe M, Eaton B, Driscoll S, Latulippe C (2000) Modelling the probability of salmonid egg pocket scour due to floods. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1120-1130.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**, 639-655.

Morán P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity* **77**, 655-660.

Morbey Y (2000) Protandry in Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1252-1257.

Moss JH, Beauchamp DA, Cross AD, *et al.* (2005) Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. *Transactions of the American Fisheries Society* **134**, 1313-1322.

Quinn TP (2005) *The behavior and ecology of Pacific salmon and trout* University of Washington Press, Seattle, WA.

Quinn TP, Adkison MD, Ward MB (1996) Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* **102**, 304-322.

Quinn TP, Dickerson BR, Vøllestad LA (2005) Marine survival and distribution patterns of two Puget Sound hatchery populations of coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. *Fisheries Research* **76**, 209-220.

Quinn TP, Foote CJ (1994) The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* **48**, 751-761.

Quinn TP, Peterson NP (1996) The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef creek, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1555-1564.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Seamons TR, Bentzen P, Quinn TP (2004a) The effects of adult length and arrival date on individual reproductive success in wild steelhead trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Science* **61**, 185-192.

Seamons TR, Bentzen P, Quinn TP (2004b) The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environmental Biology of Fishes* ***69***, 333-344.

Seiler D, Volkhardt G, Fleischer L, Kishimoto L (2002) 2000 Cedar River sockeye salmon fry production evaluation. Washington Department of Fish and Wildlife, Olympia, WA.

Shapovalov L, Taft AC (1954) Life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. *California Department of Fish and Game, Fisheries Bulletin* **98**, 375.

Shuster SM, Wade MJ (2003) *Mating systems and strategies* Princeton University Press, Princeton, New Jersey.

Smith RW, Griffith JS (1994) Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* **123**, 747-756.

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**, 1129-1157.

Stearns SC (1992) *The evolution of life histories* Oxford University Press, New York.

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Canadian Journal of Zoology* **77**, 836-841.

Thomaz D, Beall E, Burke T (1997) Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceedings of the Royal Society London Series B* **264**, 219-226.

Thrower FP, Hard JJ, Joyce JE (2004) Genetic architecture of growth and early life-history transitions in anadromous and derived freshwater populations of steelhead. *Journal of Fish Biology* **65**, 286-307.

Unwin MJ, Kinnison MT, Quinn TP (1999) Exceptions to semelparity: Postmaturation survival, morphology, and energetics of male chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1172-1181.

van den Berghe EP, Gross MR (1989) Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). *Evolution* **43**, 125-140.

Ward BR (2000) Declivity in steelhead (*Oncorhynchus mykiss*) recruitment at the Keogh River over the past decade. *Canadian Journal of Fisheries and Aquatic Science* **57**, 298-306.

Ward BR, Slaney PA (1988) Life history and smolt-to-adult survival of Keogh river steelhead trout (*Salmo gairdneri*) and the relation to smolt size. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1110-1122.

Ward BR, Slaney PA, Facchin AR, Land RW (1989) Size-based survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1853-1858.

Yamamoto S, Morita K, Goto A (1999) Marine growth and survival of white-spotted charr, *Salvelinus leucomaenis*, in relation to smolt size. *Ichtyological Research* **46**, 85-92.

Young WP, Ostberg CO, Keim P, Thorgaard GH (2001) Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). *Molecular Ecology* **10**, 921-930.

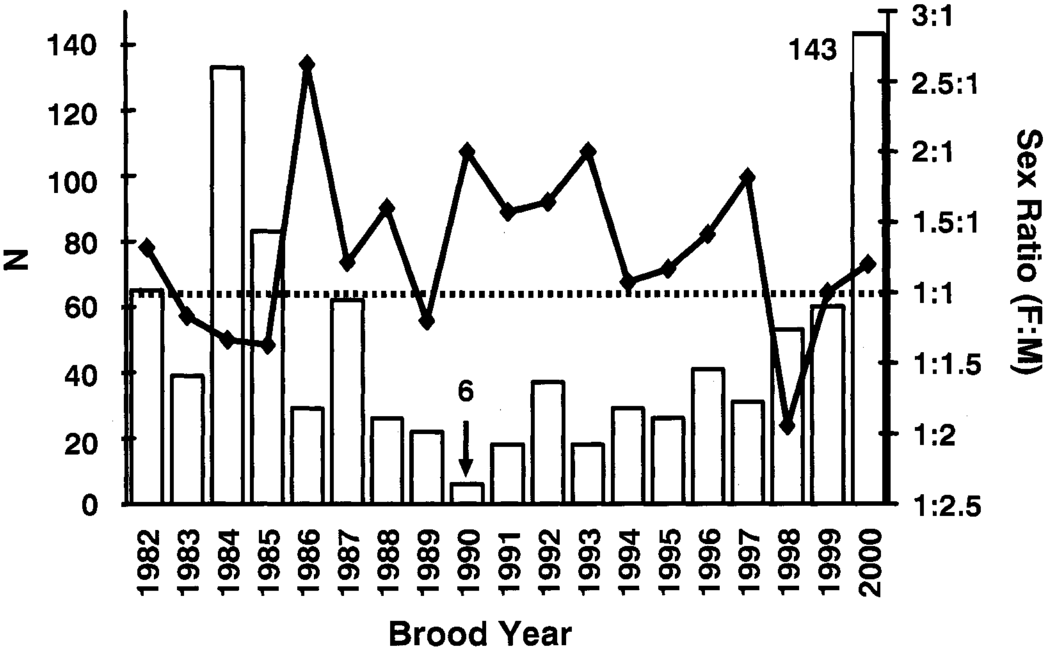

Figure 4.1. Number of adult Snow Creek steelhead returning to spawn and the observed sex ratio over the entire breeding season for brood years 1982 through 2000. Open bars represent the number of adults returning, the solid line represents the sex ratio (#females:#males). The dotted line highlights a sex ratio of 1:1.

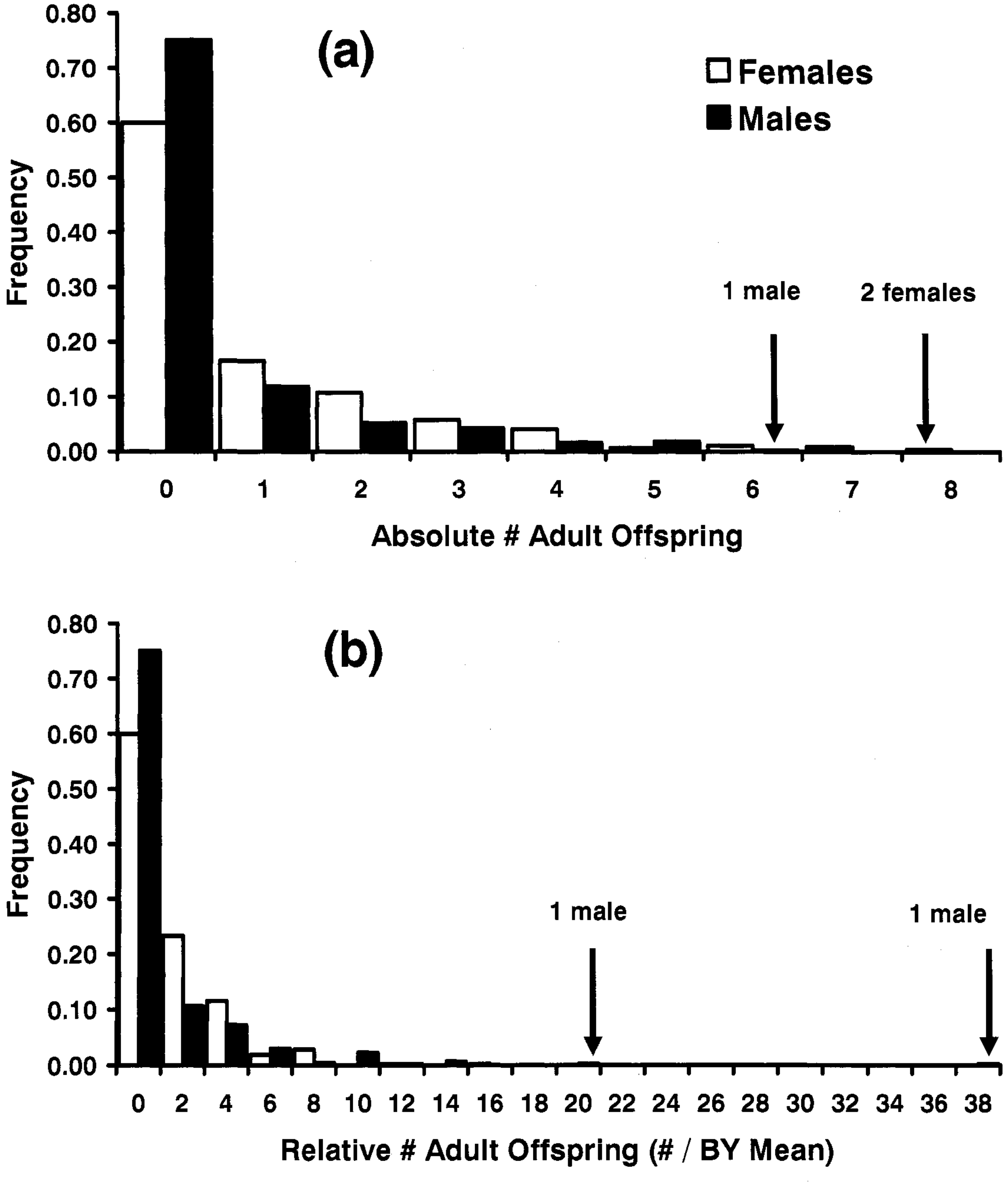

Figure 4.2. Frequency histograms showing the frequency of maternal (open bars) and paternal (solid bars) half-sib families in terms of both the absolute number of adult offspring per family (a) and relative to the within brood year average number of adult offspring per family (b).

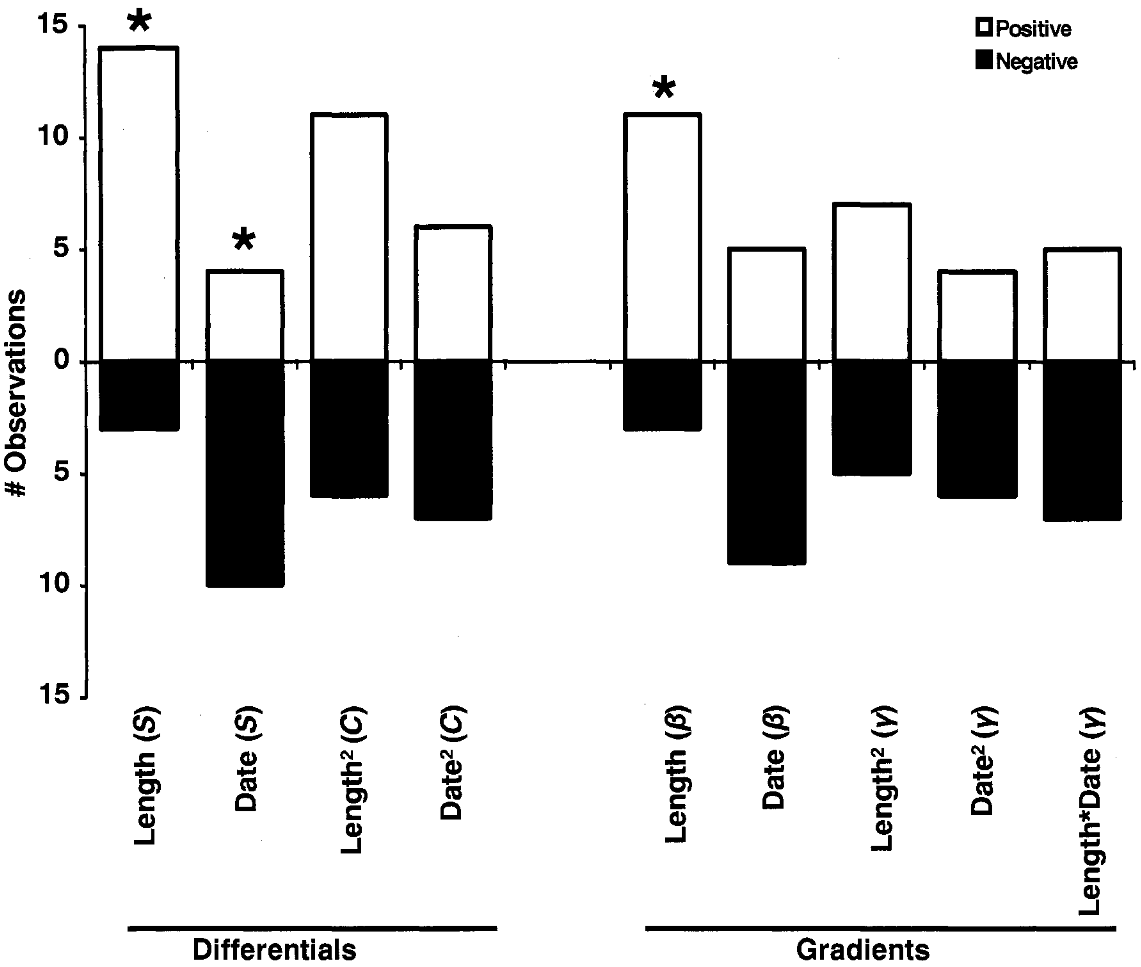

Figure 4.3. Trends in the sign (positive or negative) of the linear (*S* and β) and quadratic (*C* and γ) selection coefficients on fork length and arrival date of adult male Snow Creek steelhead in 19 brood years. * indicates significance at $p<0.05$ from a Chi-squared test with expectations of 1:1, positive to negative.

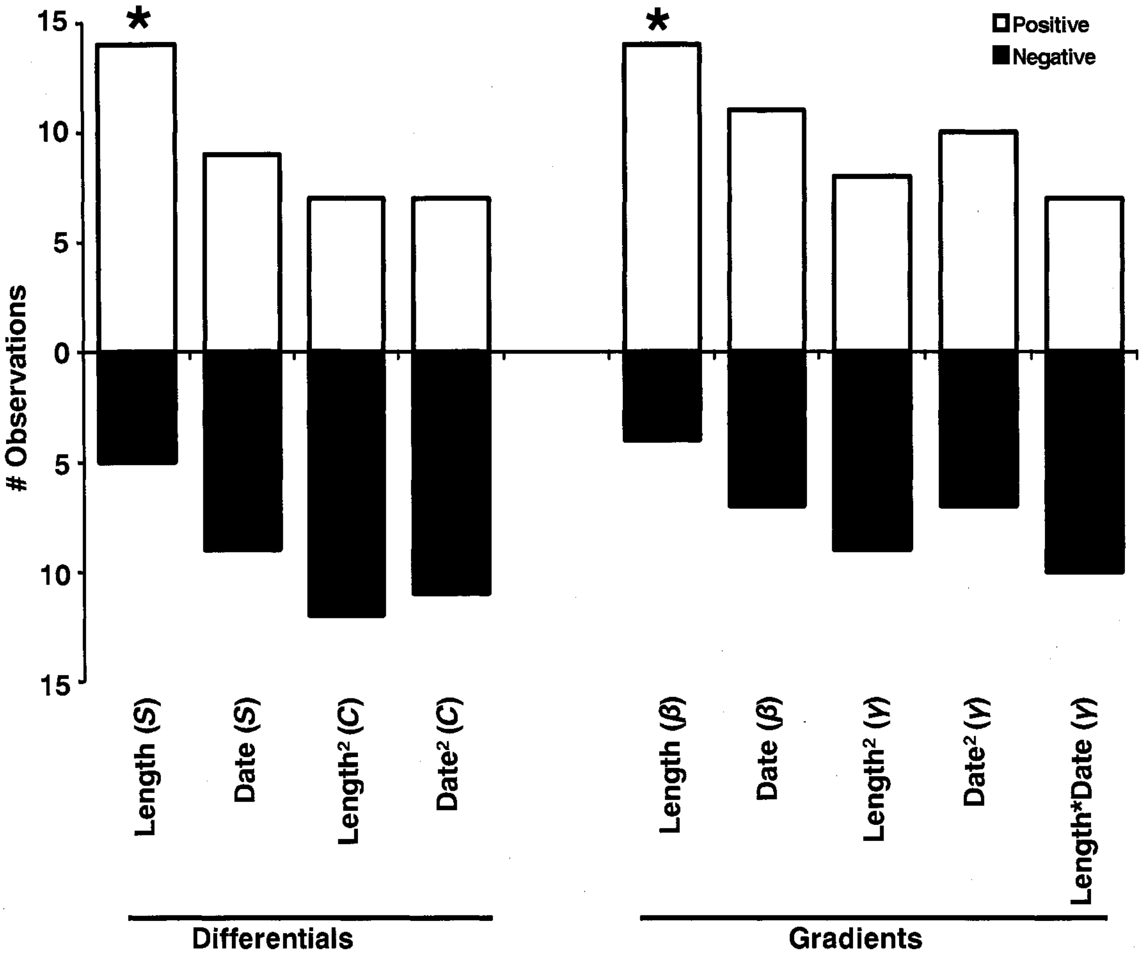

Figure 4.4. Trends in the sign (positive or negative) of the linear (*S* and *β*) and quadratic (*C* and *γ*) selection coefficients on fork length and arrival date of adult female Snow Creek steelhead in 19 brood years. * indicates significance at $p<0.05$ from a Chi-squared test with expectations of 1:1, positive to negative.

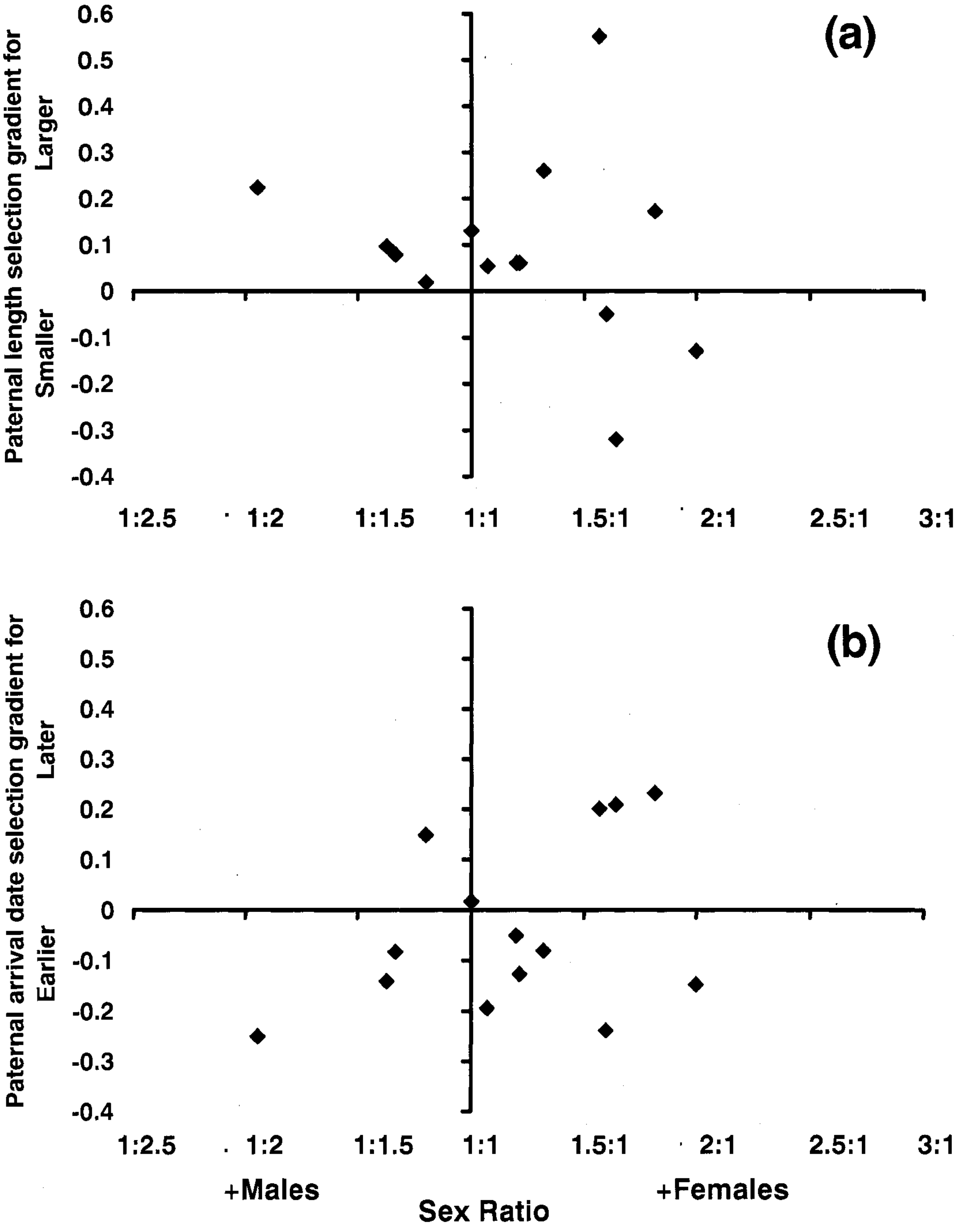

Figure 4.5. The relationship between the observed total adult sex ratio and directional selection gradients for paternal fork length (a) and arrival date (b).

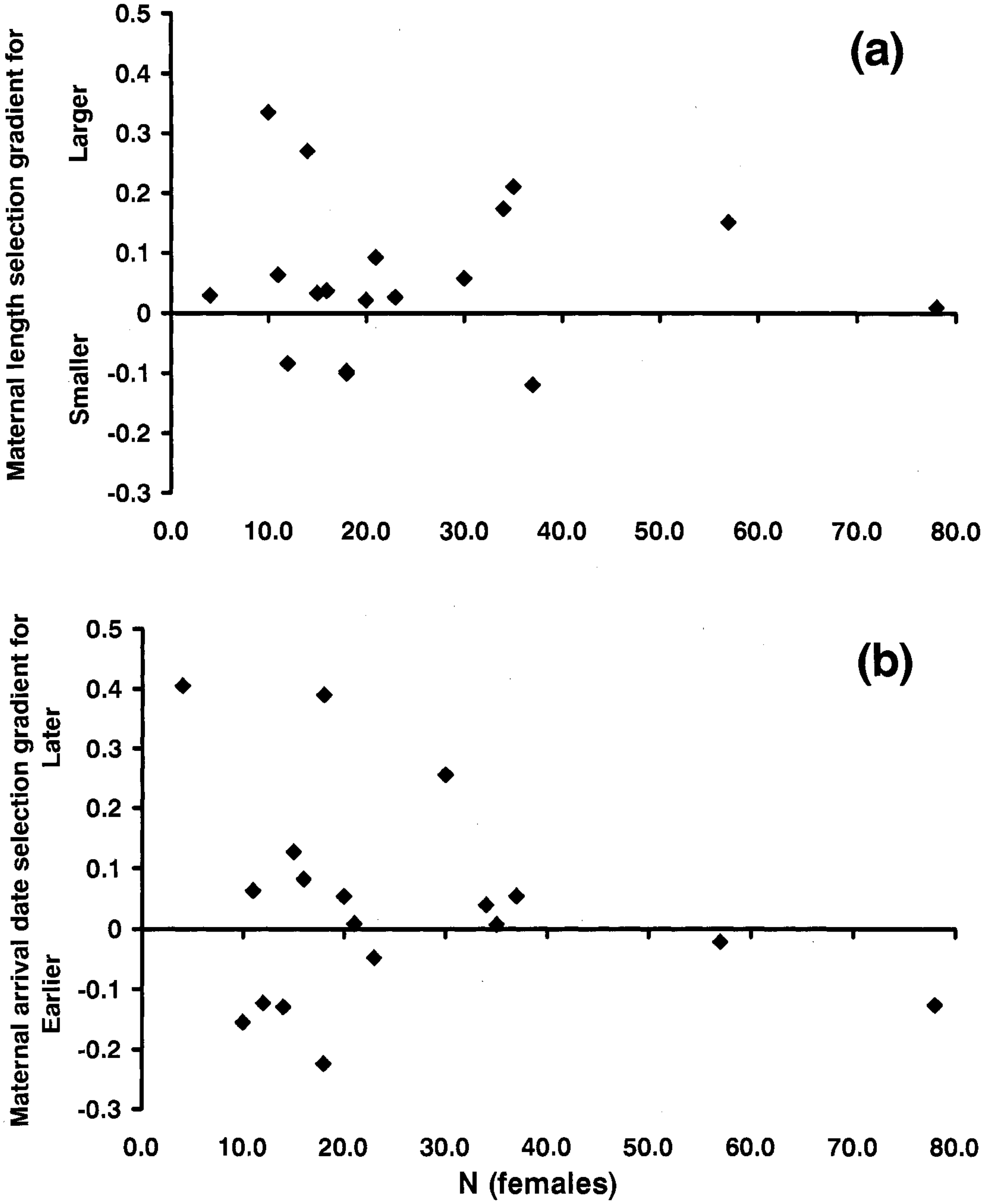

Figure 4.6. The relationship between the total number of females returning per brood year and directional selection gradients for maternal fork length (a) and arrival date (b).

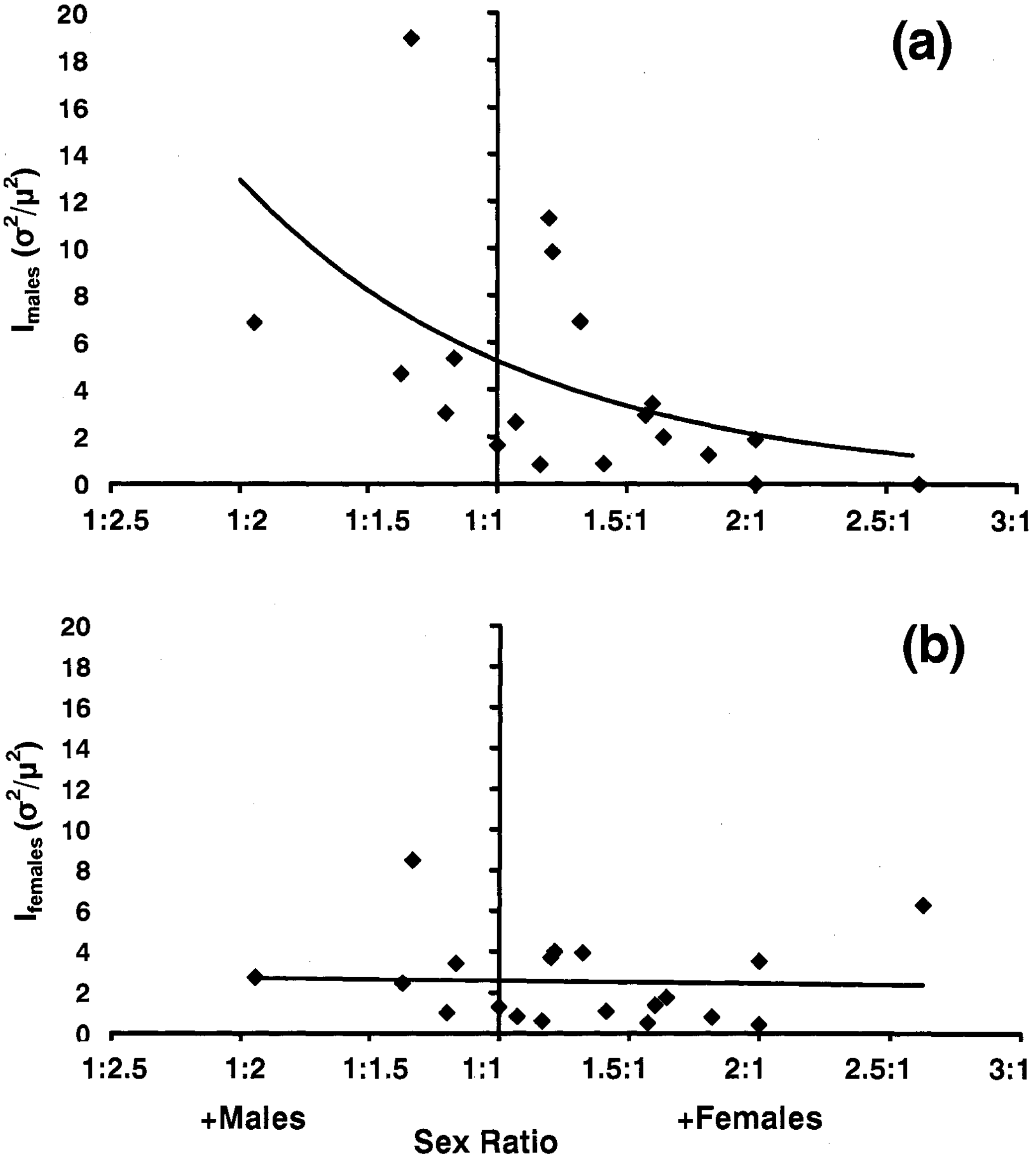

Figure 4.7. The relationship between the observed total adult sex ratio and the observed opportunity for sexual selection for adult male (a) and female (b) steelhead from Snow Creek, Washington. Line is predicted from least squares regression analysis. Data shown are untransformed.

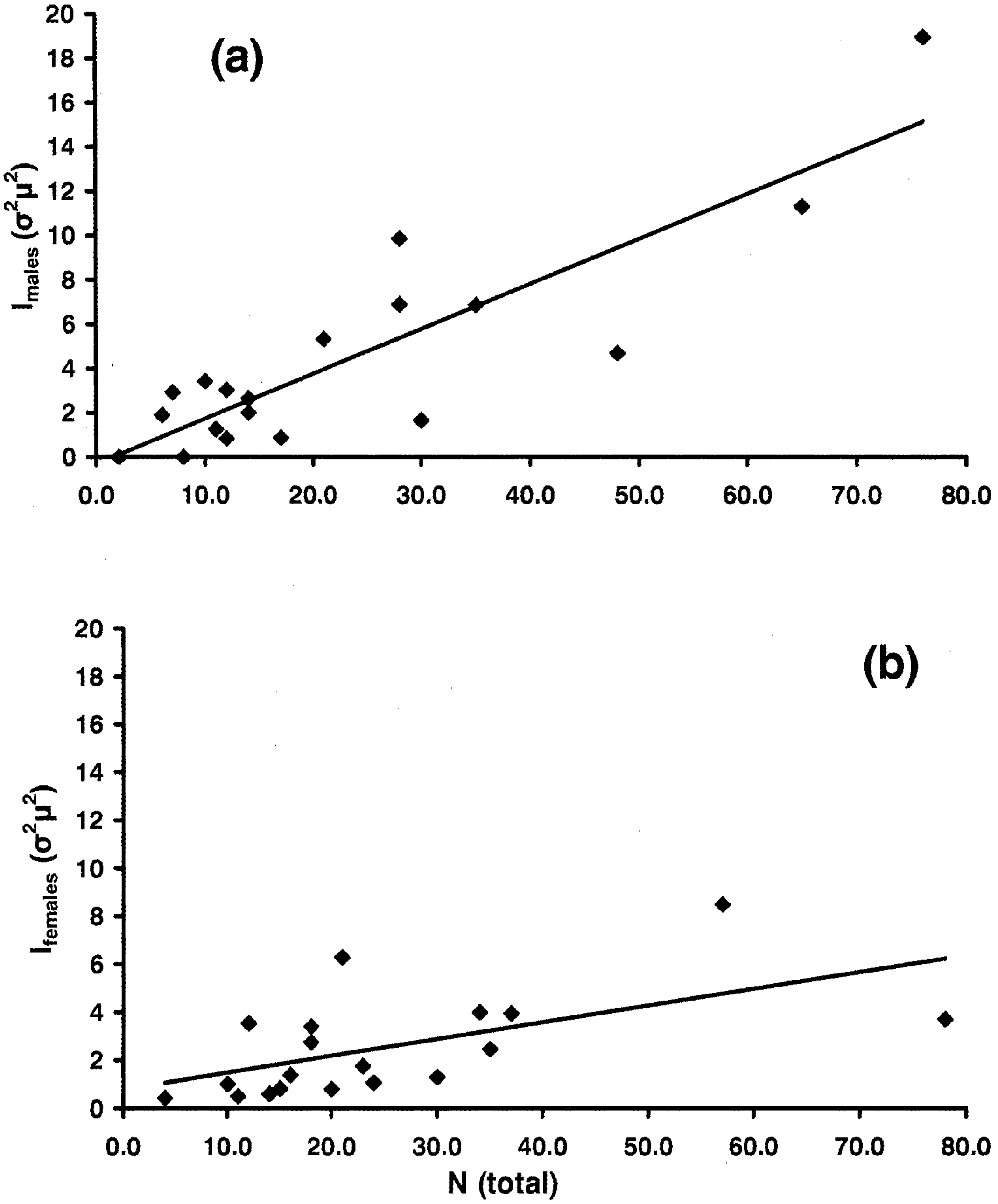

Figure 4.8. The relationship of the number of returning adult males (a) or females (b) on the measured opportunity for selection (I, σ^2/μ^2).

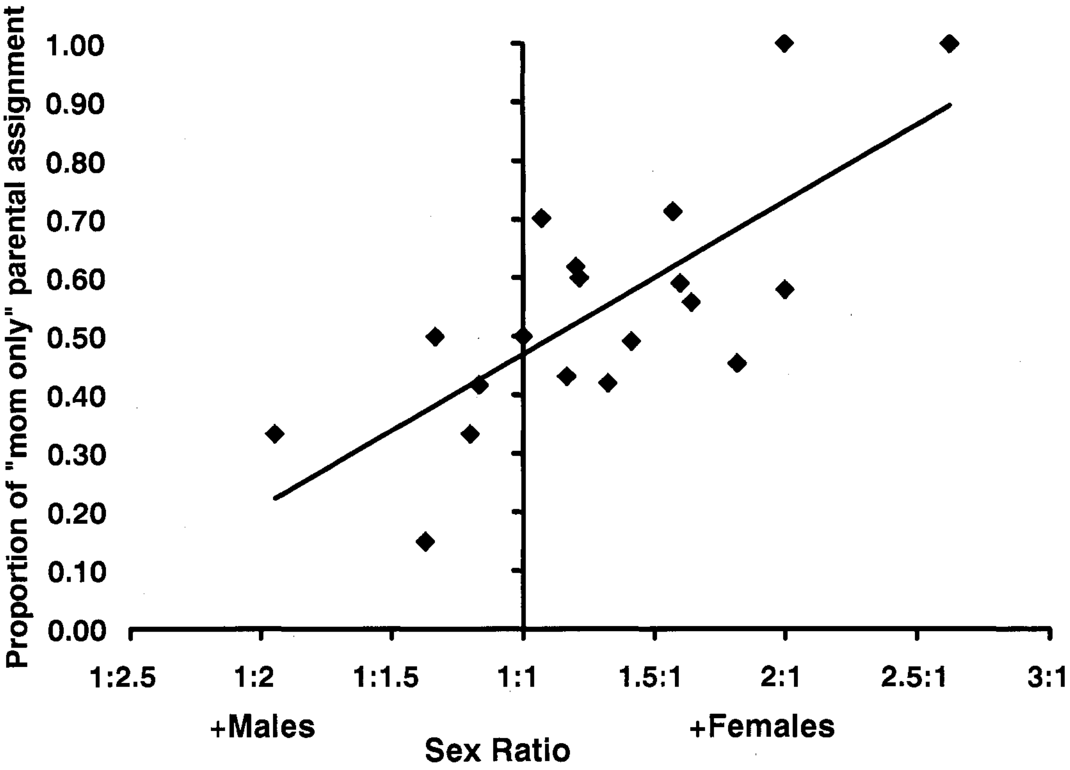

Figure 4.9. The relationship between the sex ratio and the proportion of parental assignments where only a mother was assigned. Line represents predicted values from least squares regression analysis.

Table 4.1. Total number and number of hatchery origin (#HO) adult male and female steelhead from Snow Creek, Washington with average fork length (L_{AVG}), range of fork lengths (SR), median arrival date (AD_M), mean arrival date (AD_A) and the range of arrival dates (DR) for 23 brood years. Also shown are the sex ratio (F/M) and the number of anadromous adult cutthroat trout (N_{CT}).

	Males						Females							
Brood Year	N (#HO)	L_{AVG}	SR	AD_M	AD_A	DR	N (#HO)	L_{AVG}	SR	AD_M	AD_A	DR	F/M	N_{CT}
1982	28 (6)	606	355 - 708	21-Feb	27-Feb	13-Jan - 22-Apr	37 (5)	642	345 - 785	4-Mar	10-Mar	16-Feb - 5-Apr	1.32	2
1983	21 (3)	614	360 - 825	11-Mar	11-Mar	11-Mar - 11-Mar	18 (2)	643	550 - 719	17-Feb	17-Feb	19-Jan - 13-Mar	0.86	1
1984	76 (23)	618	393 - 795	20-Mar	17-Mar	14-Nov - 20-Apr	57 (10)	640	474 - 810	20-Mar	8-Mar	11-Nov - 20-Apr	0.75	2
1985	48 (4)	639	477 - 790	8-Apr	3-Apr	15-Feb - 26-Apr	35 (1)	674	595 - 810	8-Apr	2-Apr	5-Mar - 25-Apr	0.73	1
1986	8 (3)	581	420 - 675	27-Mar	2-Apr	27-Mar - 14-Apr	21 (1)	650	570 - 760	7-Mar	9-Mar	28-Feb - 27-Mar	2.63	0
1987	28 (6)	634	430 - 885	18-Mar	18-Mar	26-Jan - 13-Apr	34 (2)	639	565 - 750	13-Mar	18-Mar	3-Mar - 13-Apr	1.21	1
1988	10 (3)	640	525 - 748	21-Mar	19-Mar	1-Mar - 6-Apr	16 (0)	684	603 - 819	23-Mar	23-Mar	1-Mar - 11-Apr	1.60	0
1989	12 (0)	634	330 - 740	25-Feb	27-Feb	22-Feb - 8-Mar	10 (0)	634	400 - 770	8-Mar	3-Mar	23-Feb - 10-Mar	0.83	1
1990	2 (1)	623	615 - 630	15-Feb	27-Feb	27-Feb - 27-Feb	4 (1)	693	595 - 820	16-Mar	17-Mar	15-Mar - 20-Mar	2.00	0
1991	7 (0)	603	555 - 655	25-Mar	25-Mar	20-Mar - 30-Mar	11 (0)	619	560 - 740	20-Mar	19-Mar	22-Feb - 4-Apr	1.57	0
1992	14 (1)	653	530 - 720	8-Mar	11-Mar	28-Feb - 29-Mar	23 (1)	636	558 - 745	18-Mar	14-Mar	22-Feb - 30-Mar	1.64	0
1993	6 (1)	574	430 - 665	17-Mar	21-Mar	6-Mar - 16-Apr	12 (0)	655	585 - 770	16-Mar	13-Mar	5-Mar - 24-Mar	2.00	3
1994	14 (6)	662	570 - 760	15-Mar	9-Mar	24-Jan - 28-Mar	15 (2)	646	565 - 800	23-Mar	21-Mar	20-Feb - 31-Mar	1.07	6
1995	12 (0)	650	610 - 720	24-Mar	24-Mar	8-Mar - 9-Apr	14 (0)	639	595 - 690	10-Mar	13-Mar	8-Mar - 7-Apr	1.17	0
1996	17 (1)	676	540 - 790	***	***	***	24 (1)	645	560 - 780	***	***	***	1.41	0
1997	11 (1)	623	420 - 750	21-Mar	21-Mar	21-Mar - 21-Mar	20 (0)	692	595 - 830	21-Mar	21-Mar	7-Mar - 24-Mar	1.82	0
1998	35 (0)	650	600 - 735	20-Mar	12-Mar	3-Feb - 2-Apr	18 (0)	638	565 - 795	22-Mar	17-Mar	13-Feb - 14-Apr	0.51	0
1999	30 (5)	647	400 - 815	22-Mar	16-Mar	15-Dec - 26-Apr	30 (2)	658	600 - 740	20-Mar	10-Mar	13-Dec - 23-Apr	1.00	4
2000	65 (1)	645	430 - 785	23-Mar	13-Mar	25-Jan - 14-Apr	78 (0)	635	420 - 860	25-Mar	17-Mar	25-Jan - 14-Apr	1.20	3
2001	21 (0)	667	460 - 765	19-Mar	21-Mar	9-Mar - 7-Apr	28 (0)	683	610 - 775	26-Mar	26-Mar	9-Mar - 11-Apr	1.33	4
2002	5 (0)	669	615 - 740	11-Mar	3-Mar	19-Feb - 12-Mar	8 (0)	670	565 - 805	12-Mar	10-Mar	20-Feb - 18-Mar	1.60	1
2003	29 (0)	642	445 - 720	13-Mar	11-Mar	14-Feb - 8-Apr	57 (0)	569	302 - 815	13-Mar	10-Mar	14-Feb - 8-Apr	1.97	6
2004	9 (0)	637	585 - 730	26-Mar	23-Mar	18-Feb - 16-Apr	16 (0)	627	397 - 780	26-Mar	22-Mar	18-Feb - 16-Apr	1.78	2
Total	508 (65)						586 (28)						1.15	37

*** - 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).

Table 4.2. The average and standard deviation of the number of days spent upstream of the weir by male and female steelhead in Snow Creek in several brood years. Data are shown for all brood years in which individual arrival and outmigration date were known.

			Number of days upstream	
Adult Sex	Brood year	N	μ	σ
Female	1977	22	40.7	24.57
	1978	40	66.4	33.49
	1979	17	78.3	75.76
	1980	27	52.9	16.30
	1981	25	51.9	33.69
	1982	21	62.5	28.91
	1989	10	56.9	11.06
	2001	20	16.7	8.20
	2002	5	39.8	24.19
	2003	8	49.5	29.27
	2004	9	26.6	18.62
	Average		49.3	
Male	1977	9	36.0	19.51
	1978	32	44.0	27.52
	1979	11	41.5	20.82
	1980	29	60.8	24.43
	1981	6	48.2	18.16
	1982	11	83.5	40.09
	1989	9	62.6	5.68
	2001	13	26.8	18.72
	2002	3	46.7	2.08
	2003	8	43.5	16.12
	2004	4	34.3	16.24
	Average		48.0	

Table 4.3. Allelic variability at 12 microsatellite loci in 23 brood years of adult steelhead returning to Snow Creek, Washington. Number of alleles per locus (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability after test for goodness of fit to HWE (P_{HWE}), inbreeding coefficient (F_{IS}) and the total number of alleles across all brood years (Total N_A) are shown.

Brood		Locus											
Year		*Oki23*	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1982	N_A	16	16	14	20	20	30	15	21	32	41	21	19
	H_E	0.91	0.90	0.80	0.92	0.93	0.96	0.87	0.92	0.96	0.96	0.92	0.86
	H_O	0.86	0.87	0.78	0.93	0.90	0.95	0.70	0.88	1.00	0.95	0.90	0.80
	P_{HWE}	0.559	0.223	0.588	0.185	0.097	0.296	0.040	0.876	0.148	0.104	0.671	0.160
	F_{IS}	0.049	0.039	0.022	-0.019	0.036	0.007	0.193	0.044	-0.040	0.016	0.025	0.068
1983	N_A	16	15	10	13	16	25	12	18	24	34	17	15
	H_E	0.91	0.89	0.83	0.89	0.93	0.95	0.85	0.92	0.95	0.97	0.92	0.83
	H_O	0.86	0.83	0.80	0.86	0.91	0.94	0.62	0.83	1.00	0.94	0.86	0.83
	P_{HWE}	0.459	0.107	0.470	0.648	0.196	0.531	0.000	0.150	0.149	0.214	0.037	0.241
	F_{IS}	0.059	0.072	0.034	0.033	0.013	0.005	0.275	0.100	-0.059	0.027	0.069	0.008
1984	N_A	17	18	19	23	27	32	18	22	43	62	22	22
	H_E	0.91	0.88	0.79	0.90	0.93	0.95	0.84	0.92	0.95	0.97	0.92	0.86
	H_O	0.88	0.87	0.85	0.91	0.89	0.94	0.67	0.87	0.98	0.97	0.91	0.87
	P_{HWE}	0.680	0.156	0.780	0.320	0.030	0.097	0.002	0.078	0.015	0.296	0.510	0.483
	F_{IS}	0.040	0.022	-0.071	-0.014	0.044	0.011	0.203	0.054	-0.027	0.007	0.005	-0.016
1985	N_A	17	15	12	19	22	28	15	20	35	46	24	18
	H_E	0.92	0.89	0.79	0.88	0.92	0.95	0.82	0.91	0.95	0.97	0.92	0.84
	H_O	0.94	0.87	0.69	0.83	0.92	0.98	0.74	0.87	0.94	0.90	0.90	0.86
	P_{HWE}	0.051	0.011	0.192	0.014	0.088	0.470	0.009	0.222	0.051	0.000*	0.072	0.117
	F_{IS}	-0.025	0.021	0.126	0.057	0.004	-0.027	0.102	0.046	0.007	0.066	0.013	-0.018
1986	N_A	13	12	10	12	17	21	12	12	18	29	17	13
	H_E	0.90	0.90	0.83	0.88	0.94	0.96	0.87	0.89	0.92	0.97	0.94	0.86
	H_O	0.96	0.78	0.78	0.74	0.96	0.95	0.83	0.87	0.91	0.96	0.87	0.87
	P_{HWE}	0.581	0.019	0.070	0.453	0.305	0.636	0.426	0.418	0.244	0.702	0.449	0.417
	F_{IS}	-0.067	0.131	0.058	0.159	-0.018	0.002	0.054	0.019	0.006	0.017	0.075	-0.016
1987	N_A	16	15	13	20	21	31	15	18	27	35	20	16
	H_E	0.90	0.89	0.76	0.91	0.92	0.96	0.86	0.92	0.95	0.95	0.91	0.86
	H_O	0.86	0.78	0.69	0.90	0.93	0.95	0.69	0.81	0.95	0.86	0.81	0.76
	P_{HWE}	0.834	0.407	0.091	0.039	0.801	0.518	0.035	0.014	0.033	0.049	0.000*	0.123
	F_{IS}	0.045	0.031	0.088	0.018	-0.012	0.008	0.194	0.118	0.005	0.095	0.104	0.112

Table 4.3 continued

Brood Year		Locus											
		Oki23	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1988	N_A	14	14	12	17	18	20	11	13	23	26	13	14
	H_E	0.89	0.89	0.75	0.92	0.92	0.95	0.74	0.91	0.96	0.96	0.89	0.77
	H_O	0.88	0.80	0.81	0.96	0.88	0.96	0.63	0.65	0.92	1.00	0.92	0.77
	P_{HWE}	0.978	0.216	0.596	0.844	0.032	0.069	0.126	0.006	0.056	0.200	0.184	0.796
	F_{IS}	0.009	0.100	-0.077	-0.041	0.043	-0.015	0.153	0.286	0.042	-0.038	-0.029	0.001
1989	N_A	13	12	10	15	17	21	13	16	19	32	15	16
	H_E	0.92	0.91	0.84	0.92	0.92	0.95	0.88	0.93	0.95	0.98	0.93	0.89
	H_O	0.95	0.83	0.87	0.87	0.91	1.00	0.83	0.86	1.00	1.00	0.78	0.96
	P_{HWE}	0.710	0.068	0.499	0.561	0.101	0.196	0.265	0.108	0.578	1.000	0.138	0.996
	F_{IS}	-0.043	0.089	-0.035	0.053	0.011	-0.055	0.064	0.074	-0.052	-0.022	0.162	-0.080
1990	N_A	5	9	6	7	10	10	5	8	10	11	8	5
	H_E	0.82	0.95	0.86	0.89	0.97	0.97	0.80	0.92	0.97	0.98	0.92	0.80
	H_O	1.00	1.00	0.83	1.00	1.00	1.00	0.67	0.83	1.00	0.83	1.00	0.67
	P_{HWE}	1.000	1.000	0.011	1.000	1.000	1.000	0.764	0.510	1.000	0.124	1.000	0.312
	F_{IS}	-0.250	-0.053	0.038	-0.132	-0.034	-0.034	0.184	0.107	-0.034	0.167	-0.091	0.184
1991	N_A	11	11	7	14	12	21	10	10	22	24	16	12
	H_E	0.89	0.88	0.77	0.92	0.92	0.96	0.84	0.90	0.97	0.95	0.94	0.88
	H_O	0.89	0.89	0.72	0.89	0.94	0.89	0.82	0.78	1.00	1.00	0.94	0.89
	P_{HWE}	0.362	0.383	0.824	0.415	0.901	0.047	0.525	0.707	0.334	1.000	0.685	0.006
	F_{IS}	0.005	-0.011	0.069	0.030	-0.025	0.075	0.026	0.139	-0.036	-0.057	-0.009	-0.015
1992	N_A	15	10	10	14	19	25	10	15	25	30	14	11
	H_E	0.89	0.88	0.80	0.88	0.92	0.95	0.80	0.91	0.96	0.96	0.89	0.84
	H_O	0.95	0.95	0.86	0.95	0.92	0.95	0.57	0.89	0.95	0.97	0.95	0.86
	P_{HWE}	0.560	0.370	0.719	0.927	0.245	0.100	0.006	0.270	0.123	0.705	0.942	0.865
	F_{IS}	-0.066	-0.073	-0.088	-0.071	-0.004	0.009	0.292	0.022	0.011	-0.010	-0.064	-0.034
1993	N_A	10	11	10	14	12	20	11	16	18	24	16	11
	H_E	0.89	0.88	0.79	0.89	0.90	0.95	0.84	0.94	0.94	0.96	0.94	0.85
	H_O	1.00	0.90	0.81	0.90	0.81	0.90	0.70	0.90	0.90	1.00	0.90	0.95
	P_{HWE}	0.317	0.103	0.633	0.173	0.005	0.144	0.049	0.522	0.564	0.099	0.018	0.062
	F_{IS}	-0.124	-0.030	-0.026	-0.013	0.101	0.050	0.166	0.040	0.037	-0.041	0.036	-0.130

Table 4.3 continued

Brood Year		Locus											
		Oki23	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
1994	N_A	14	14	17	17	22	21	9	17	26	34	19	19
	H_E	0.90	0.89	0.89	0.93	0.95	0.95	0.82	0.92	0.95	0.97	0.91	0.89
	H_O	0.88	0.94	0.94	0.88	0.97	0.91	0.61	0.88	0.88	0.94	0.78	0.85
	P_{HWE}	0.653	0.527	0.824	0.278	0.676	0.066	0.024	0.114	0.062	0.000*	0.010	0.419
	F_{IS}	0.024	-0.059	-0.053	0.052	-0.023	0.043	0.265	0.040	0.073	0.035	0.148	0.041
1995	N_A	10	11	9	10	15	23	9	15	21	26	15	13
	H_E	0.88	0.86	0.82	0.87	0.91	0.95	0.80	0.92	0.94	0.96	0.92	0.86
	H_O	0.88	0.88	0.80	0.78	0.92	1.00	0.52	0.84	1.00	0.96	0.84	0.84
	P_{HWE}	0.556	0.547	0.854	0.499	0.819	0.750	0.017	0.303	0.844	0.187	0.439	0.089
	F_{IS}	-0.006	-0.026	0.029	0.099	-0.013	-0.051	0.355	0.092	-0.060	0.004	0.085	0.027
1996	N_A	16	11	9	17	17	20	11	13	25	31	16	13
	H_E	0.90	0.88	0.75	0.88	0.92	0.93	0.82	0.92	0.93	0.96	0.92	0.82
	H_O	0.90	0.87	0.79	0.95	0.92	0.97	0.62	0.85	0.95	1.00	0.95	0.82
	P_{HWE}	0.202	0.280	0.720	0.881	0.043	0.053	0.002	0.130	0.218	0.142	0.628	0.852
	F_{IS}	-0.001	0.008	-0.066	-0.077	-0.007	-0.047	0.250	0.078	-0.021	-0.039	-0.037	-0.005
1997	N_A	17	12	12	14	18	19	9	11	24	24	16	12
	H_E	0.91	0.89	0.75	0.89	0.93	0.94	0.81	0.90	0.93	0.96	0.93	0.87
	H_O	0.87	0.97	0.84	0.90	1.00	0.90	0.73	0.87	1.00	0.87	0.87	0.90
	P_{HWE}	0.729	0.938	0.163	0.632	0.989	0.074	0.135	0.146	0.332	0.272	0.144	0.338
	F_{IS}	0.045	-0.094	-0.119	-0.014	-0.075	0.037	0.096	0.031	-0.073	0.093	0.064	-0.036
1998	N_A	16	12	9	15	18	24	11	14	23	38	19	15
	H_E	0.89	0.87	0.84	0.89	0.91	0.95	0.82	0.89	0.94	0.97	0.92	0.86
	H_O	0.92	0.87	0.85	0.94	0.92	0.96	0.77	0.89	0.98	0.94	0.90	0.87
	P_{HWE}	0.835	0.329	0.398	0.625	0.620	0.680	0.004	0.380	0.455	0.611	0.308	0.760
	F_{IS}	-0.037	0.006	-0.015	-0.055	-0.011	-0.012	0.061	0.003	-0.044	0.025	0.023	-0.008
1999	N_A	15	15	14	21	20	24	16	20	30	46	21	19
	H_E	0.88	0.89	0.84	0.92	0.92	0.95	0.84	0.91	0.95	0.97	0.90	0.89
	H_O	0.89	0.92	0.77	0.92	0.87	0.93	0.72	0.85	0.93	0.97	0.85	0.87
	P_{HWE}	0.102	0.100	0.346	0.223	0.336	0.461	0.005	0.008	0.025	0.434	0.229	0.302
	F_{IS}	-0.005	-0.026	0.082	0.007	0.059	0.013	0.146	0.064	0.021	0.000	0.058	0.023

Table 4.3 continued

Brood Year		Locus											
		Oki23	*Omy1001UW*	*Omy1004UW*	*Omy1011UW*	*Omy1191UW*	*Omy1212UW*	*Omy77*	*One108*	*One2*	*Ots107*	*Ots108*	*Ssa85*
2000	N_A	18	20	14	20	24	32	17	19	35	57	25	26
	H_E	0.90	0.89	0.80	0.89	0.90	0.95	0.80	0.91	0.95	0.97	0.92	0.84
	H_O	0.95	0.91	0.78	0.92	0.94	0.97	0.62	0.88	0.99	0.95	0.88	0.81
	P_{HWE}	0.200	0.121	0.603	0.869	0.480	0.151	0.000*	0.073	0.001	0.022	0.000*	0.043
	F_{IS}	-0.046	-0.024	0.027	-0.027	-0.040	-0.016	0.229	0.035	-0.050	0.015	0.042	0.042
2001	N_A	17	12	14	17	21	24	13	16	29	39	22	18
	H_E	0.91	0.88	0.80	0.88	0.91	0.94	0.81	0.92	0.95	0.96	0.92	0.85
	H_O	0.86	0.89	0.81	0.82	0.93	0.95	0.65	0.82	0.93	0.98	0.89	0.84
	P_{HWE}	0.302	0.963	0.509	0.283	0.233	0.097	0.023	0.011	0.218	0.011	0.289	0.410
	F_{IS}	0.056	-0.020	-0.012	0.066	-0.023	-0.005	0.205	0.109	0.017	-0.018	0.031	0.007
2002	N_A	13	12	11	15	12	15	7	11	15	18	15	11
	H_E	0.93	0.92	0.89	0.94	0.93	0.95	0.81	0.92	0.94	0.96	0.93	0.90
	H_O	0.92	0.92	0.85	0.92	0.83	1.00	0.69	0.92	0.85	1.00	0.85	0.77
	P_{HWE}	0.809	0.940	0.580	0.735	0.353	0.673	0.366	0.917	0.277	0.545	0.155	0.166
	F_{IS}	0.016	-0.007	0.050	0.024	0.106	-0.054	0.150	0.000	0.105	-0.047	0.096	0.155
2003	N_A	16	18	16	20	24	29	16	23	30	45	22	22
	H_E	0.88	0.89	0.84	0.92	0.93	0.95	0.83	0.92	0.95	0.96	0.93	0.87
	H_O	0.84	0.93	0.80	0.82	0.95	0.94	0.74	0.84	0.90	0.92	0.87	0.80
	P_{HWE}	0.086	0.747	0.029	0.040	0.410	0.568	0.000*	0.065	0.003	0.016	0.002	0.807
	F_{IS}	0.042	-0.044	0.049	0.102	-0.025	0.017	0.111	0.082	0.053	0.047	0.063	0.077
2004	N_A	14	12	10	18	18	22	11	16	25	31	17	15
	H_E	0.91	0.88	0.83	0.90	0.91	0.96	0.82	0.91	0.95	0.96	0.93	0.86
	H_O	0.89	0.91	0.86	0.83	1.00	0.93	0.71	0.77	0.94	1.00	0.88	0.71
	P_{HWE}	0.336	0.732	0.910	0.046	0.984	0.419	0.248	0.018	0.960	0.689	0.617	0.238
	F_{IS}	0.023	-0.044	-0.027	0.078	-0.105	0.029	0.130	0.151	0.008	-0.039	0.048	0.170
Total	N_A	20	24	31	28	38	45	23	31	53	94	30	36

* - significant at α=0.05 after sequential Bonferroni corrections

Table 4.4. 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.

	Differentials				Gradients				
Brood Year	Length	Date	Length^2	Date^2	Length	Date	Length^2	Date^2	Length*Date
1982	0.193 (0.132)	-0.073 (0.147)	0.316 (0.256)	-0.292 (0.160)	0.261 (0.200)	-0.08 (0.145)	0.256 (0.472)	-0.366 (0.167)	-0.222 (0.252)
1983[a]	0.181 (0.159)	NA	-0.378 (0.184)	NA	NA	NA	NA	NA	NA
1984	0.062 (0.078)	-0.065 (0.078)	0.126 (0.092)	-0.266 (0.097)	0.08 (0.080)	-0.082 (0.080)	0.262 (0.105)	-0.27 (0.107)	-0.136 (0.099)
1985	0.126 (0.101)	-0.159 (0.100)	0.054 (0.106)	0.02 (0.126)	0.098 (0.102)	-0.14 (0.102)	0.134 (0.115)	0.144 (0.182)	0.178 (0.167)
1986[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1987	0.039 (0.143)	-0.115 (0.141)	-0.248 (0.144)	-0.042 (0.160)	0.062 (0.146)	-0.126 (0.146)	-0.218 (0.176)	-0.102 (0.175)	-0.106 (0.172)
1988	-0.176 (0.242)	-0.263 (0.232)	1.122 (0.150)*	0.238 (0.244)	-0.048 (0.293)	-0.238 (0.293)	0.932 (0.415)	-0.346 (0.266)	0.364 (0.501)
1989	0.011 (0.223)	0.148 (0.218)	-0.186 (0.238)	-1.22 (0.294)***	0.02 (0.231)	0.15 (0.231)	-0.344 (0.406)	-1.246 (0.395)	-0.132 (0.419)
1990[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1991	0.528 (0.228)***	0.138 (0.322)	0.476 (0.225)	-2.762 (0.812)	0.552 (0.236)	0.202 (0.236)	1.162 (1.101)	NA	-0.364 (0.592)
1992[c]	-0.08 (0.170)	-0.1 (0.132)	0.2 (0.209)	0.026 (0.174)	-0.319 (0.084)**	0.21 (0.104)	NA	NA	NA
1993[c]	-0.189 (0.301)	-0.2 (0.299)	0.222 (0.447)	-0.634 (0.564)	-0.128 (0.372)	-0.147 (0.372)	NA	NA	NA
1994	0.032 (0.174)	-0.187 (0.165)	0.148 (0.182)	0.052 (0.183)	0.055 (0.173)	-0.193 (0.173)	0.096 (0.199)	0.516 (0.293)	-0.732 (0.380)
1995[a]	0.226 (0.135)	NA	0.186 (0.174)	NA	NA	NA	NA	NA	NA
1996[a]	0.002 (0.124)	NA	-0.48 (0.112)**	NA	NA	NA	NA	NA	NA
1997[d]	0.094 (0.172)	0.174 (0.166)	-0.38 (0.195)	NA	0.173 (0.176)	0.233 (0.176)	-0.262 (0.202)	NA	0.374 (0.179)
1998	0.243 (0.118)**	-0.266 (0.117)**	0.156 (0.140)	0.398 (0.159)	0.225 (0.112)***	-0.249 (0.112)**	-0.182 (0.149)	0.406 (0.148)	-0.47 (0.129)***
1999	0.132 (0.110)	0.025 (0.113)	-0.19 (0.141)	-0.496 (0.157)	0.131 (0.113)	0.018 (0.113)	-0.238 (0.173)	-0.53 (0.166)	0.092 (0.211)
2000	0.067 (0.091)	-0.056 (0.091)	0.044 (0.114)	0.074 (0.107)	0.062 (0.092)	-0.05 (0.092)	0.104 (0.132)	0.092 (0.119)	0.224 (0.149)
Pooled	0.096 (0.032)*	-0.088 (0.035)**	0.042 (0.034)	-0.076 (0.038)	0.091 (0.035)*	-0.091 (0.035)*	0.104 (0.038)	-0.088 (0.038)	-0.012 (0.038)

NA – data not available for reasons listed below

[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] – Little variation in arrival date so the Date^2 and Date values exceeded colinearity threshold in statistical analysis

Probability values – * - $p<0.01$, ** - $p<0.05$, *** - $p<0.10$

Table 4.5. 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.

	Differentials				Gradients				
Brood Year	Length	Date	Length^2	Date^2	Length	Date	Length^2	Date^2	Length*Date
1982	-0.028 (0.118)	0.063 (0.127)	-0.11 (0.136)	0.169 (0.143)	-0.119 (0.176)	0.055 (0.129)	0.244 (0.590)	0.143 (0.145)	-0.262 (0.124)**
1983	0.009 (0.183)	0.397 (0.208)***	-0.383 (0.161)**	0.16 (0.219)	-0.1 (0.255)	0.391 (0.223)	-0.101 (0.787)	0.147 (0.350)	-0.111 (0.473)
1984	0.15 (0.095)	-0.008 (0.098)	0.075 (0.099)	-0.063 (0.115)	0.152 (0.097)	-0.021 (0.097)	0.082 (0.102)	0.043 (0.157)	-0.129 (0.143)
1985	0.211 (0.103)**	-0.009 (0.109)	0.298 (0.132)**	-0.04 (0.136)	0.211 (0.105)**	0.008 (0.105)	0.338 (0.144)**	0.075 (0.129)	0.072 (0.124)
1986	0.071 (0.199)	-0.066 (0.177)	-0.312 (0.283)	-0.446 (0.293)	0.093 (0.245)	0.009 (0.378)	-0.476 (0.382)	-0.806 (0.906)	0.28 (0.885)
1987	0.168 (0.178)	0.015 (0.126)	0.201 (0.129)	-0.081 (0.163)	0.174 (0.125)	0.04 (0.125)	0.246 (0.154)	-0.107 (161)	0.028 (0.163)
1988	-0.005 (0.141)	0.064 (0.140)	-0.189 (0.169)	-0.029 (0.154)	0.038 (0.169)	0.083 (0.169)	0.751 (0.390)***	0.295 (0.171)	1.068 (0.400)**
1989	0.346 (0.106)**	-0.176 (0.149)	0.133 (0.119)	0.034 (0.220)	0.336 (0.097)**	-0.154 (0.097)	0.064 (0.145)	-0.071 (0.168)	-0.072 (0.141)
1990[a]	0.259 (0.237)	0.423 (0.018)*	-0.186 (0.318)	-0.213 (0.004)**	0.03 (0.007)	0.406 (0.007)**	NA	NA	NA
1991	0.056 (0.128)	0.056 (0.128)	-0.047 (0.187)	-0.128 (0.132)	0.064 (0.135)	0.064 (0.135)	0.092 (0.297)	-0.265 (0.272)	-0.204 (0.389)
1992	0.11 (0.122)	-0.042 (153)	-0.034 (0.131)	0.049 (0.192)	0.027 (0.228)	-0.047 (0.166)	-0.15 (0.600)	0.025 (0.230)	-0.115 (0.368)
1993	-0.013 (0.194)	-0.074 (0.193)	-0.169 (0.339)	-0.276 (0.198)	-0.083 (0.247)	-0.122 (0.247)	-0.088 (0.341)	-0.189 (0.222)	0.325 (0.240)
1994	0.04 (0.123)	0.132 (0.127)	0.234 (0.152)	-0.146 (0.261)	0.034 (0.134)	0.128 (0.134)	0.089 (0.211)	0.253 (0.453)	0.294 (0.281)
1995	0.094 (0.125)	-0.222 (0.185)	0.037 (0.131)	-0.784 (1.066)	0.271 (0.182)	-0.129 (0.180)	-0.445 (0.097)**	-5.95 (1.482)***	-4.822 (1.611)***
1996[a]	-0.023 (0.120)	NA	0.263 (0.122)**	NA	NA	NA	NA	NA	NA
1997	0.032 (0.110)	0.058 (0.110)	-0.031 (0.116)	0.542 (0.339)	0.022 (0.114)	0.054 (0.114)	-0.03 (0.113)	0.598 (0.363)	0.181 (0.117)
1998	0.036 (0.168)	-0.165 (0.163)	-0.17 (0.316)	-0.063 (0.186)	-0.096 (0.209)	-0.223 (0.209)	-0.287 (0.468)	-0.163 (0.405)	-0.195 (0.536)
1999	-0.013 (0.099)	0.241 (0.087)*	-0.084 (0.102)	0.013 (0.113)	0.058 (0.092)	0.257 (0.092)*	-0.049 (0.120)	0.027 (0.132)	-0.007 (0.128)
2000	0.039 (0.082)	-0.128 (0.081)	-0.034 (0.128)	0.221 (0.106)**	0.01 (0.083)	-0.126 (0.083)	-0.065 (0.129)	0.214 (0.120)**	-0.045 (0.093)
Pooled	0.075 (0.030)**	-0.002 (0.032)	0.01 (0.032)	0.008 (0.034)	0.083 (0.033)**	0.014 (0.032)	0.003 (0.039)	0.013 (0.034)	-0.036 (0.033)

NA – data not available for reasons listed below

[a] – Little or no arrival date data available

Probability values – * - $p<0.01$, ** - $p<0.05$, *** - $p<0.10$

Table 4.6. Directional selection (1 standard error) on length at outmigration (MSL) and date of outmigration (MSD) in smolt half-sibling families. Also shown is the partial regression coefficient for the number of members of smolt half-sibling families (NS).

		Differentials		Gradients		
Parent sex	Brood Year	MSL	MSD	MSL	MSD	NS
Male	1998	3.23 (0.77)*	0.88 (0.34)*	0.79 (2.40)	0.41 (0.54)	-1.32 (1.01)
	1999	0.41 (1.31)	-0.84 (0.40)***	-0.10 (0.45)	-0.15 (0.21)	1.03 (0.27)*
	2000	-0.74 (0.52)	0.58 (0.33)***	-0.44 (0.32)	0.46 (0.28)	0.28 (0.38)
	Pooled	-0.01 (0.43)	-0.02 (0.19)	-0.21 (0.27)	0.21 (0.19)	0.67 (0.25)*
Female	1998	0.35 (0.37)	0.88 (0.28)*	0.23 (0.38)	0.81 (0.72)	0.55 (0.41)
	1999	-0.24 (0.30)	0.32 (0.22)	-0.23 (0.14)	0.29 (0.25)	0.21 (0.13)
	2000	0.12 (0.11)	-0.51 (0.17)*	-0.01 (0.27)	-0.08 (0.19)	0.05 (0.15)
	Pooled	0.24 (0.10)**	0.02 (0.10)	-0.04 (0.13)	-0.05 (0.14)	0.19 (0.10)***

Probability values – * - p<0.01, ** - p<0.05, *** - p<0.10.

Chapter 5: Summary

Mating systems reflect the amount of sexual selection in the past, but also, to some degree, determine the amount of contemporary sexual selection. Most adult steelhead, male or female, had multiple mates, though patterns of mate numbers varied among brood years (Chapter 1). In addition, within a given brood year some individuals seemed to mate (or at least produced detectable offspring) with only one or two individuals, whereas others were involved in extended half-sib breeding complexes. Subsequent investigation indicated that changes in the number of mates among brood years were likely determined by variation in the density and sex ratio of spawning anadromous steelhead (Chapter 4) since the number of offspring per parent reflects, at least in part, the number of mates per parent. Sexual selection was more intense (i.e., more variation in reproductive success) for males than for females especially when the sex ratio was male biased or when the spawning density of males was high (Chapter 4). This presumably reflected differences in strategies between males and females for maximizing reproductive success. Variance in female reproductive success also increased with spawning density of females (Chapter 4). This is likely a result of density dependent effects on juvenile survival in Snow Creek.

The next logical question was, “Does mate number depend on body size or arrival timing?” Unfortunately, the inferred number of mates depended on the number of offspring detected, precluding an unbiased estimate of the number of mates. Thus, the proper question was, “Did the number of offspring depend on parental body size or arrival timing?” In short, the answer was “yes”, however the strength and direction of the relationships varied by brood year and by the life history stage of sampled offspring. Larger females tended to have larger and more numerous young-of-the-year (YOY) offspring (Chapter 2). However, the relationship of maternal size with YOY number was weak, suggesting that larger offspring had less of an advantage than was expected (Chapter 2). Larger females also appeared to have more smolt and adult offspring overall (Chapters 3 and 4), but within individual brood years the direction, shape and strength of the relationship varied (Chapter 4). Maternal arrival timing, in spite of describing a significant part of the variation in YOY offspring size (Chapter 2), had no consistent relationship with the number of YOY, yearling, smolt or adult offspring (Chapters 2, 3 and 4). This implies that the size of juveniles depended on when they emerged from the gravel (hence on when they were spawned) but that this effect did not confer a large survival advantage.

Paternal length was unrelated to the number of YOY or yearling offspring (Chapter 2) or smolt offspring (Chapter 3), except when one particularly large and reproductively successful male from brood year 1998 was included in the analysis. Large males had more adult offspring, however the relationship was weak (Chapter 4). In most brood years, longer males had more offspring (Chapter 4), however the direction, shape and strength of the relationship varied among brood years (Chapter 4). At least part of that variation may have been a result of variation in the sex ratio of spawning adults among brood years (Chapter 4).

The first indication that arrival timing was important for males came during the description of the mating system. When the arrival dates of steelhead known to have mated with one another were compared, I found that males tended to (but not always) arrive before the female with whom they mated (Chapter 1). This translated into more adult offspring in general for earlier arriving males (Chapter 4), however, as seen with body size, the relationships varied in direction, shape and strength across years (Chapters 3 and 4).

In the data set, not all offspring could be assigned to two parents, and only a fraction of the juveniles in the population could be sampled as fry, parr and smolts. Perfect information on all parents and offspring might have changed or clarified the nature of the relationships with parental body size and arrival date with the number of offspring produced. Mature male parr would be unsampled as potential parents, and they appeared to be an important part of the mating system in Snow Creek (Chapter 1), fathering ~30% of YOY and yearling offspring (Chapter 1). Though the true size of these fish was unknown, they were probably ~200 mm – less than half the length of the smallest anadromous male. If these males were included in the statistical tests I probably would have seen a positive quadratic coefficient in all tests, indicating that small or large males had higher reproductive success than average sized males. Mature parr were offspring as well as parents. If small anadromous males produced more mature male parr offspring than large anadromous males the overall weak relationship of reproductive success with size may disappear altogether. Interestingly, the reproductive success of mature male parr (as inferred from the offspring with no known male parent) varied with the sex ratio of anadromous adults, increasing when the sex ratio was female biased (Chapter 4).

Overall, I have shown that selection on parental length and arrival date was weak, even though considerable variation in reproductive success existed within and among brood years and within and between the sexes. Response to selection might be enhanced if mating is assortative, however there was no evidence of size assortative mating (Chapter 1); and though arrival date was important for males, mating was not strictly assortative temporally – early arriving males spawned throughout the spawning season, not only with early arriving females.

I have shown that, though body size and timing of breeding are important in determining reproductive success in experimental salmonid populations, the effects are mostly compensatory through the entire life history of offspring in a wild steelhead population. Does the variance in reproductive success translate into evolution of these traits? Evolution in a trait such as body size or arrival date also depends on the degree of genetic influence on the trait (i.e., heritability, h^2). The pedigree information established in this dataset would permit estimation of h^2 for length and arrival date of adults. These estimates could support the formulation and testing of the evolution of length and arrival data in Snow Creek steelhead.

Bibliography

Antolos M, Roby DD, Lyons DE, *et al.* (2005) Caspian tern predation on juvenile salmonids in the Mid-Columbia River. *Transactions of the American Fisheries Society* **134**, 466-480.

Appleby AE, Tipping JM, Seidel PR (2003) The effect of using two-year-old male coho salmon in hatchery broodstock on adult returns. *North American Journal of Fisheries Management* **65**, 60-62.

Ardren WR, Borer S, Thrower F, Joyce JE, Kapuscinski AR (1999) Inheritance of 12 microsatellite loci in *Oncorhynchus mykiss*. *Journal of Heredity* **90**, 529-536.

Ardren WR, Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (Ne) reveals genetic compensation in steelhead trout. *Molecular Ecology* **12**, 35-49.

Baumstieger J, Hankin D, Loudenslager EJ (2005) Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identifications. *Transactions of the American Fisheries Society* **134**, 829-840.

Beacham TD, Murray CB (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Transactions of the American Fisheries Society* **119**, 927-945.

Beacham TD, Murray CB (1993) Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). *Journal of Fish Biology* **42**, 485-508.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows ™ pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier (France).

Bentzen P, Olsen JB, McLean JE, Seamons TR, Quinn TP (2001) Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. *Journal of Heredity* **92**, 127-136.

Berejikian BA, Tezak EP, LaRae AL (2000) Female mate choice and spawning behaviour of chinook salmon under experimental conditions. *Journal of Fish Biology* **57**, 647-661.

Bilton HT, Alderdice DF, Schnute JT (1982) Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Canadian Journal of Fisheries and Aquatic Science* **39**, 426-447.

Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP (1990) Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology* **27**, 315-324.

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection, p. 826. Academic Press, London.

Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology* **75**, 385-407.

Brännäs E (1995) First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* **9**, 411-420.

Brodie III ED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* **10**, 313-318.

Busby PJ, Wainwright TC, Bryant GJ, *et al.* (1996) Status review of west coast steelhead from Washington, Idaho, Oregon, and California, p. 261. U. S. Dep. Commer.

Chandler GL, Bjornn TC (1988) Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. *Transactions of the American Fisheries Society* **117**, 432-443.

Chebanov NA, Varnavskaya NV, Varnavskiy VS (1983) Effectiveness of spawning of male sockeye salmon *Oncorhynchus nerka* (Salmonidae), of differing hierarchichal rank by means of genetic-biochemical markers. *Journal of Ichthyology* **23**, 51-55.

Collis K, Roby DD, Craig DP, Ryan BA, Ledgerwood RD (2001) Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River Estuary: vulnerability of different salmonid species, stocks, and rearing types. *Transactions of the American Fisheries Society* **130**, 385-396.

Connolly PJ, Petersen JH (2003) Bigger is not always better for overwintering young-of-year steelhead. *Transactions of the American Fisheries Society* **132**, 262-274.

Crespi BJ, Teo R (2002) Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. *Evolution* **56**, 1008-1020.

de Gaudemar B, Bonzom JM, Beall E (2000) Effects of courtship and relative mate size on sexual motivation in Atlantic salmon. *Journal of Fish Biology* **57**, 502-515.

DeWoody JA, Avise JC (2001) Genetic perspectives on the natural history of fish mating systems. *Journal of Heredity* **92**, 167-172.

Dickerson BR, Brinck KW, Willson MF, Bentzen P, Quinn TP (2005) Relative importance of salmon body size and arrival time at breeding grounds to reproductive success. *Ecology* **86**, 347-352.

Dickerson BR, Quinn TP, Willson MF (2002) Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethology Ecology and Evolution* **14**, 29-44.

Duston J, Astatkie T, MacIsaac PF (2005) Genetic influence of parr versus anadromous sires on the life histories of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2067-2075.

Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society London Series B* **266**, 2095-2100.

Einum S, Fleming IA (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* **54**, 628-639.

Erkinaro J, Shchurov IL, Saari R, Niemelä E (1994) Occurrence of Atlantic salmon parr in redds at spawning time. *Journal of Fish Biology* **45**, 899-900.

Essington TE, Quinn TP, Ewert VE (2000) Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 205-213.

Falconer DS, Mackay TFC (1996) *Introduction to quantitative genetics*, 4th edn. Prentice Hall, Essex, England.

Fields RD, Johnson KR, Thorgaard GH (1989) DNA fingerprints in rainbow trout detected by hybridization with DNA of bacteriophage M13. *Transactions of the American Fisheries Society* **118**, 78-81.

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 59-76.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Foote CJ (1988) Male mate choice dependent on male size in salmon. *Behaviour* **106**, 63-80.

Foote CJ (1989) Female mate preference in Pacific salmon. *Animal Behaviour* **38**, 721-722.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Foote CJ, Brown GS, Wood CC (1997) Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1785-1795.

Fukushima M, Quinn TJ, Smoker WW (1998) Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 618-625.

Garant D, Dodson JJ, Bernatchez L (2001) A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* **92**, 137-145.

Gjerde B (1986) Growth and reproduction in fish and shellfish. *Aquaculture* **57**, 37-55.

Gjerde B, Gjedrem T (1984) Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* **36**, 97-110.

Gjerde B, Schaeffer LR (1989) Body traits in rainbow trout II. Estimates of heritabilities and of phenotypic and genetic correlations. *Aquaculture* **80**, 25-44.

Good SP, Dodson JJ, Meekan MG, Ryan DAJ (2001) Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1187-1195.

Grant BR (2003) Evolution in Darwin's finches: a review of a study on Isla Daphne Major in the Galapagos Archipelago. *Zoology* **106**, 255-259.

Groot C, Margolis L (1991) *Pacific salmon life histories* University of British Columbia Press, Vancouver, British Columbia.

Gross MR (1985) Disruptive selection for alternative life histories in salmon. *Nature* **313**, 47-48.

Hanson AJ, Smith HD (1967) Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age-groups. *Journal of the Fisheries Research Board of Canada* **24**, 1955-1977.

Healey MC (1982) Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 952-957.

Healey MC, Prince A (1998) Alternative tactics in the breeding behaviour of male coho salmon. *Behaviour* **135**, 1099-1124.

Heath DD, Fox CW, Heath JW (1999) Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution* **53**, 1605-1611.

Helle JH (1989) Relation between size-at-maturity and survival of progeny in chum salmon, *Oncorhynchus keta* (Walbaum). *Journal of Fish Biology* **35**, 99-107.

Henderson MA, Cass AJ (1991) Effect of smolt size on smolt-to-adult survival for Chilko Lake sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 988-994.

Hendry AP, Stearns SC (2004) Evolution illuminated: salmon and their relatives. Oxford University Press, New York, New York.

Herbinger CM, Doyle RW, Pitman ER, *et al.* (1995) DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. *Aquaculture* **137**, 245-256.

Holtby LB, Andersen BC, Kadowaki RK (1990) Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 2181-2194.

Holtby LB, Healey MC (1986) Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1946-1959.

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology* **79**, 383-399.

Hutchings JA, Myers RA (1988) Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia* **75**, 169-174.

Johnson TH, Cooper R (1995) Anadromous game fish research and planning annual report, p. 33. State of Washington Department of Fish and Wildlife, Olympia.

Jones AG, Avise JC (2001) Mating systems and sexual selection in male-pregnant pipefishes and seahorses: insights from microsatellite-based studies of maternity. *Journal of Heredity* **92**, 150-158.

Jordan WC, Youngson AF (1992) The use of genetic marking to assess the reproductive success of mature male Atlantic salmon parr (*Salmo salar*, L.) under natural spawning conditions. *Journal of Fish Biology* **41**, 613-618.

Keeley ER (2001) Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* **82**, 1247-1259.

Keeley ER, McPhail JD (1998) Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). *Behaviour* **135**, 65-82.

Keenleyside MHA, Dupuis HMC (1988) Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **66**, 262-265.

Kingsolver JG, Hoekstra HE, Hoekstra JM, *et al.* (2001) The strength of phenotypic selection in natural populations. *The American Naturalist* **157**, 245-261.

Kingsolver JG, Pfennig DW (2004) Individual-level selection as a cause of Cope's Rule of phyletic size increase. *Evolution* **58**, 1608-1612.

Kinnison MT, Unwin MJ, Hershberger WK, Quinn TP (1998) Egg size, fecundity, and development rate of two introduced New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. *Canadian Journal of Fisheries and Aquatic Science* **55**, 1946-1953.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* **37**, 1210-1226.

Lapointe M, Eaton B, Driscoll S, Latulippe C (2000) Modelling the probability of salmonid egg pocket scour due to floods. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1120-1130.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**, 639-655.

Martinez JL, Moran P, Perez J, *et al.* (2000) Multiple paternity increases effective size of southern Atlantic salmon populations. *Molecular Ecology* **9**, 293-298.

Metcalfe NB, Thorpe JE (1992) Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **41 (Suppl. B)**, 93-99.

Morán P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity* **77**, 655-660.

Morbey Y (2000) Protandry in Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1252-1257.

Moss JH, Beauchamp DA, Cross AD, *et al.* (2005) Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. *Transactions of the American Fisheries Society* **134**, 1313-1322.

Needham PR, Taft AC (1934) Observations on the spawning of steelhead trout. *Transactions of the American Fisheries Society* **64**, 333-338.

Quinn TP (2005) *The behavior and ecology of Pacific salmon and trout* University of Washington Press, Seattle, WA.

Quinn TP, Adkison MD, Ward MB (1996) Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* **102**, 304-322.

Quinn TP, Dickerson BR, Vøllestad LA (2005) Marine survival and distribution patterns of two Puget Sound hatchery populations of coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. *Fisheries Research* **76**, 209-220.

Quinn TP, Foote CJ (1994) The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* **48**, 751-761.

Quinn TP, Peterson NP (1996) The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef creek, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1555-1564.

Quinn TP, Unwin MJ, Kinnison MT (2000) Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. *Evolution* **54**, 1372-1385.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Schroder SL (1981) *The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon* Ph.D., University of Washington.

Schuett-Hames DE, Peterson NP, Conrad R, Quinn TP (2000) Patterns of gravel scour and fill after spawning by chum salmon in a Western Washington stream. *North American Journal of Fisheries Management* **20**, 610-617.

Seamons TR, Bentzen P, Quinn TP (2004a) The effects of adult length and arrival date on individual reproductive success in wild steelhead trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Science* **61**, 185-192.

Seamons TR, Bentzen P, Quinn TP (2004b) The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environmental Biology of Fishes* ***69***, 333-344.

Seiler D, Volkhardt G, Fleischer L, Kishimoto L (2002) 2000 Cedar River sockeye salmon fry production evaluation. Washington Department of Fish and Wildlife, Olympia, WA.

Shapovalov L, Taft AC (1954) Life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. *California Department of Fish and Game, Fisheries Bulletin* **98**, 375.

Shuster SM, Wade MJ (2003) *Mating systems and strategies* Princeton University Press, Princeton, New Jersey.

Sillero-Zubiri C, Gottelli D, Macdonald DW (1996) Male philopatry, extra-pack copulations and inbreeding avoidance in Ethiopian wolves (*Canis simensis*). *Behavioral Ecology and Sociobiology* **38**, 331-340.

Smith RW, Griffith JS (1994) Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* **123**, 747-756.

Smoker WW, Gharrett AJ, Stekoll MS, Joyce JE (1994) Genetic analysis of size in an anadromous population of pink salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 9-15.

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**, 1129-1157.

Stearns SC (1992) *The evolution of life histories* Oxford University Press, New York.

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Canadian Journal of Zoology* **77**, 836-841.

Steinberg EK, Lindner KR, Gallea J, *et al.* (2002) Rates and patterns of microsatellite mutations in pink salmon. *Molecular Biology and Evolution* **19**, 1198-1202.

Taggart JB, McLaren IS, Hay DW, Webb JH, Youngson AF (2001) Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Molecular Ecology* **10**, 1047-1060.

Tautz AF, Groot C (1975) Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **32**, 633-642.

Thomaz D, Beall E, Burke T (1997) Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceedings of the Royal Society London Series B* **264**, 219-226.

Thrower FP, Hard JJ, Joyce JE (2004) Genetic architecture of growth and early life-history transitions in anadromous and derived freshwater populations of steelhead. *Journal of Fish Biology* **65**, 286-307.

Titus RG, Mosegaard H (1991) Selection for growth potential among migratory brown trout (*Salmo trutta*) fry competing for territories: evidence from otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 19-27.

Tsiger VV, Skirin VL, Krupyanko NI, Kashlin KA, Semenchenko AY (1994) Life history form of male masu salmon (*Oncorhynchus masou*) in South Primor'e, Russia. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 197-208.

Unwin MJ, Kinnison MT, Quinn TP (1999) Exceptions to semelparity: Postmaturation survival, morphology, and energetics of male chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1172-1181.

van den Berghe EP, Gross MR (1989) Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). *Evolution* **43**, 125-140.

Ward BR (2000) Declivity in steelhead (*Oncorhynchus mykiss*) recruitment at the Keogh River over the past decade. *Canadian Journal of Fisheries and Aquatic Science* **57**, 298-306.

Ward BR, Slaney PA (1988) Life history and smolt-to-adult survival of Keogh river steelhead trout (*Salmo gairdneri*) and the relation to smolt size. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1110-1122.

Ward BR, Slaney PA, Facchin AR, Land RW (1989) Size-based survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1853-1858.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Westneat D (1987) Extra-pair fertilizations in a predominantly monogamous birds: genetic evidence. *Animal Behaviour* **35**, 877-886.

Yamamoto S, Morita K, Goto A (1999) Marine growth and survival of white-spotted charr, *Salvelinus leucomaenis*, in relation to smolt size. *Ichtyological Research* **46**, 85-92.

Young WP, Ostberg CO, Keim P, Thorgaard GH (2001) Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). *Molecular Ecology* **10**, 921-930.

Zar JH (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Upper Saddle River, New Jersey.

Zimmerman CE, Reeves GH (2000) Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2152-2162.

VITA

Todd R. Seamons was born in Salt Lake City, Utah, where most of his extended family still lives. His early interests were in accounting, anthropology and bottle-tipping, but a desire to catch more fish, more often led him to pursue an undergraduate degree in Fisheries and Wildlife from Utah State University. There he honed his fly-fishing skills on the Logan River and developed a taste for fish genetics. After graduating in 1996 he pursued Audrey Stephens, now Dr. Audrey Seamons, following her to University of Washington in 1999 to study steelhead genetics. There, while ostensibly studying steelhead, he perfected his fly-casting technique, landing over eight steelhead. Todd bypassed a Masters degree in 2003 and in 2005 he earned a Doctor of Philosophy from the School of Aquatic and Fishery Sciences at the University of Washington. He currently resides in Seattle, Washington with his wife, Audrey and his son, Joshua.