Reproductive success in wild pink salmon, Oncorhynchus gorbuscha.

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A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington

2003

Program Authorized to Offer Degree: School of Aquatic and Fishery Sciences

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Abstract

Reproductive success in wild pink salmon, *Oncorhynchus gorbuscha*

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Reproductive success in pink salmon was investigated by examining the complex interactions among life history, morphological, and behavioral traits and their influence on offspring production. Reproductive success was measured directly (using DNA microsatellites in a parentage analysis) in adult pink salmon (*O. gorbuscha*) in a small Alaskan creek during the 1997-2000 spawning seasons. Females showed strong positive relationships between body size and both fecundity and egg size, with a trade off between these traits. Duration of nest defense was associated with entry date (early arrivals lived longer) and bear predation, which also affected the proportion of females completing egg deposition. Larger males were more dominant but the benefits of large size were overshadowed by arrival timing. Earlier small males were more dominant than

larger later males. The reproductive life span of males was reduced by bear predation but not influenced by body size or arrival timing. Both body size and arrival date affected potential reproductive success but predation also appeared important. When these same traits were compared to the number of adult offspring produced we found that males that arrived earlier, lived longer in the stream, and were more often observed in dominant positions courting females had significantly more offspring, although the importance of dominance was overshadowed by the influence of arrival timing. Female reproductive success was not closely linked to any measured trait (e.g., egg size, instream longevity, or fecundity) but stabilizing selection on arrival timing and length was inferred with intermediate values of these most productive. Size-assortative mating, although seen in many salmonids, was not apparent in this population. Heritabilities from parent-offspring regressions for arrival timing in male offspring (dam 0.72 and sire 0.58) and body size in female offspring (sire 0.48) were estimated and were within the range of those found for controlled breeding experiments. Entry timing showed the greatest influence on reproductive success. It is possible that the optimal entry timing shifts due to environmental factors (predation pressure, temperature, food availability, etc.) and thus is under shifting selective pressures and is not as easily driven to an optimum as traits such as egg size may be.

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ACKNOWLEDGEMENTS

Over the last 6 years I have had the benefit of the support and advise of a large number of people, the following is a far from exhaustive list. I would like to start by thanking the far-sighted scientists that were key to the conception as well as completion of this project, my advisor, Thomas Quinn, my molecular advisor, Paul Bentzen, and my financial supporter as well as scientific collaborator, Mary Willson. Without their support this project would have never come to be. Thomas Quinn was a wonderful mentor, his support and confidence in me allowed me to stay focused and plow ahead even when I felt overwhelmed.

I would also like to thank the other members of my committee, John Emlen and Robert Francis who were helpful in reviewing ideas and manuscripts, overall improving this body of work. I would like to thank Richard Ladner who served as my Graduate Student Representative.

Although the support of the faculty is key to any graduate program, I feel it is the fellow graduate students that often got me through the day. For their help with statistical analysis, lab assistance and instruction, field assistance, manuscript review, and emotional support I would like to thank Scott Gende, Kevin Brinck, Andrew Hendry, Micheal Kinnison, Todd Seamons, Pam Jensen, Jason Dunham, Kerim Aydin, Gretchen Nowak, Margaret Schwertner, Jennifer McLean and everyone at MMBL (University of Washington, Marine Molecular Biotechnology Lab).

For their logistic support that went well above and beyond the call of duty I thank everyone at the Hoonah Ranger District in particular JR Fiske and Paul Matter.

A big round of thanks is due to all of the people who gathered data for me both in the field and the lab, including Morgan Heim, Michael Humling, Jeff Nichols, Todd Rinaldi, Dietrick Schmidt, Lea Scheldahl, Ray Vinkey, Cathy Reidy, Dina Spangenberg, Justin Crawford, and Anna Elz. A special thanks to Bill Jones and Faith Stankevich.

During this work I was supported primarily by the Forest Sciences Lab, U.S.D.A. Forest Service, Mary Willson and later Tom Hanley. I also received several supplemental awards from the University of Washington, School of Aquatic and Fishery Sciences including; the Egdvedt scholarship and the Gilbert Journalism scholarship.

DEDICATION

To Gary L. Vinyard
1949-1998

Introduction

Understanding the factors affecting variation in reproductive success among individuals is key to understanding the selective pressures driving adaptation and ultimately furthering our understanding of the processes of evolution (Roff 1992; Stearns 1992; Andersson 1994). As a result, reproductive success has been the focus of a great deal of research in many different organisms (eg. Clutton-Brock 1988). Reproductive success has often been correlated with body size through direct effects on fecundity and egg size in females (Roff 1992; Stearns 1992) and competitive ability in males (e.g., Mathis 1991; Madsen et al. 1993; reviewed by Kodric-Brown 1990; Kirkpatrick and Ryan 1991; Cunningham and Birkhead 1998). As a result, the importance of adult size in reproductive success has been widely recognized and examined but the timing of arrival on the breeding grounds and reproduction within a season may also be correlated with breeding opportunity and success of broods.

The breeding system of Pacific salmon makes them particularly well suited for testing hypotheses regarding reproductive success. These anadromous, semelparous fishes achieve most of their adult body size while feeding at sea, do not feed or grow during the breeding season, home to their natal stream to breed at very discrete times of the year, and die upon completion of spawning, allowing researchers to estimate an individual's lifetime reproductive output in one season (see reviews in Groot and Margolis 1991). Due to the difficulty of directly measuring reproductive success in salmon it has typically been estimated by indirect methods using surrogate traits such as observations of courtship, spawning events, and physical attributes of the fish such as size or shape (Gross 1985; Keenleyside and Dupuis 1988; Fleming and Gross 1994; Quinn and Foote 1994; Mangel 1996). Recent advances in molecular biology such as DNA microsatellites provide more powerful tools for kinship analysis and the direct measurement the reproductive success of salmon (Wright and Bentzen 1994; McConnell et al. 1995; O'Reilly and Wright 1995). Our approach was to test hypotheses concerning the actual reproductive success of the parents in the most appropriate measure, the number of mature progeny.

As with most studies of reproductive success most of the past research on reproductive success in salmonids has emphasized the importance of adult body size. Females can benefit from large size by producing larger and more numerous eggs (Beacham and Murray 1993; Dickerson et al. 2002), successfully compete for access to high quality breeding sites (Foote 1990), and dig deeper (hence safer) redds (Steen and Quinn 1999). Males may benefit from large size by an increased competitive ability allowing greater access to spawning females (Gross 1985; Keenleyside and Dupuis 1988; Quinn and Foote 1994; Kitano 1996; Dickerson et al. 2002). Though less thoroughly investigated in salmonids, timing of arrival on the spawning grounds may have as great or greater influence on reproductive success than body size, potentially affecting nest security in females and dominance in males.

Another potential affect of body size on the spawning grounds is size-assortative mating. Larger males often have a competitive advantage over smaller males allowing greater choice among females (Kodric-Brown 1990). Larger females produce more numerous and larger offspring, and may acquire higher quality breeding or nesting sites.

These female traits would be expected to result in higher offspring survival, hence large males should choose large females when possible (e.g., Grant et al. 1995) and be able to dominate access to them (Morris et al. 1992). Females are more receptive to large males (e.g. Schroder 1981), perhaps because size is often heritable and females that mate with larger males may have larger offspring. In some cases, this competition and choice combine to produce size-assortative mating (the tendency for individuals of similar relative sizes to mate together; e.g., lampreys: Malmqvist 1983). Size-assortative mating has been found in many species of salmonids (eg. Hanson & Smith 1967; Chebanov 1979; Foote 1988). Although, is not always observed (Schroder 1981; Quinn and Foote 1994), under natural conditions, choice and competition may be influenced by the ripeness of available females, vigor of the males, threat of predation, and other factors that might reduce the importance of relative size.

The theory behind evolutionary biology relies heavily on the assumption that variation within most morphological, life history and behavioral traits has some genetic basis, allowing species to evolve in changing environments. The genotype contributes to the overall phenotypic variation, acting in combination with diverse environmental factors. The level of heritability among traits reflects the balance between genetic and environmental controls, and so indicates the extent to which selection affects the population. Therefore, estimates of heritability provide us with valuable information as to the current selective pressures acting on a population, the potential responses of a species to ecological changes, and also clues to past selective pressures (Prout and Barker 1989).

The following is the objectives of this study carried out on a wild population of pink salmon. Among the Pacific salmon, pink salmon are particularly well suited for a study of reproductive success. Pink salmon are semelparous and have a strict two-year life cycle allowing lifetime reproductive success to be measured in one season and all offspring to be sampled in two years (Groot and Margolis 1991). Also, upon emergence pink salmon travel directly to sea, removing factors related to instream rearing further simplifying our study (Groot and Margolis 1991

The objective of chapter 1 was to investigate the interactions between body size, arrival date and other factors that influence potential reproductive success in a natural salmon population: fecundity, egg size, and duration of nest defense in females, and access to females and reproductive lifespan in males. The hypotheses were as follows: (1) Larger females produce larger and more numerous eggs than smaller females but females with larger eggs for their size produce fewer eggs. (2) Larger females live longer in the stream unless preyed upon by bears than smaller females, and females arriving earlier live longer and are larger than later-arriving females. (3) Larger males and those with larger secondary sexual characteristics are more often dominant than smaller males, and earlier arriving males are more often dominant than later arriving males. (4) Larger males live longer than smaller males unless they are more vulnerable to predation, and earlier arriving males live longer than those arriving later. The study was conducted in pink salmon (*O. gorbuscha*), whose reproductive behavior is similar to the other salmon species spawning at high densities (Quinn 1999).

The objective of chapter 2 was to examine the relationship between arrival timing, body size, and other associated traits (fecundity, egg size, dominance, and longevity) and the number of adult progeny produced in pink salmon. We hypothesized that more offspring would be produced by: (1) females that are larger, have higher fecundity and larger eggs than females that are smaller, less fecund and have smaller eggs, (2) longer lived females compared to shorter lived females (although no female's life expectancy spans the entire run thus early arriving females may see a smaller benefit from longer life than later arriving fish), (3) longer lived males compared to males that lived a shorter time on the breeding grounds, and (4) larger males and those with higher dominance scores compared to smaller and less dominant males, (5) males that arrive early in the breeding season compared to later arrivals. Finally, based on the nature of the competitive breeding system, we predicted greater variation in reproductive success among males than among females.

The objective of chapter 3 was to test for size-assortatvie mating among individuals observed engaging in courtship as well as among successful pairs (pairs that produced adult offspring). To support our the validity of our observations we determined the extent to which apparent dominance corresponded to reproductive success, assessed by parentage analysis of the offspring produced by the matings in the first two years of the study.

The objective of chapter 4 was to examine relationship in body size and shape, longevity, egg size, and arrival timing between parents and offspring from wild spawning and rearing pink salmon and to calculate heritabilities based on these relationships.

Chapter 1: Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*

Introduction

Understanding the factors affecting variation in reproductive success among individuals is a key pursuit in behavioral ecology and life-history theory (Clutton-Brock 1988; Roff 1992; Stearns 1992; Andersson 1994). Potential reproductive success is most fundamentally affected by fecundity (or litter or clutch size) in females and access to reproductively active females in males, in most species. These factors determine the number of offspring produced by males and females but other factors also affect offspring survival, including progeny size (related to egg size or maternal investment), nesting habitat quality, and parental care. Reproductive success is often correlated with body size (through direct effects on fecundity and egg size: Roff 1992; Stearns 1992) and secondary sexual traits through mate choice or intrasexual competition (e.g., Mathis 1991; Madsen et al. 1993; reviewed by Kodric-Brown 1990; Kirkpatrick and Ryan 1991; Cunningham and Birkhead 1998). The importance of adult size in reproductive success is widely recognized but the timing of arrival on the breeding grounds and reproduction within a season may also be correlated with breeding opportunity and success of broods, and patterns of selection can vary over the breeding season in many organisms including insects (McLain et al. 1993), birds (e.g., Hatchwell 1991; Norris 1993, Wiggins et al. 1994, Verhulst et al. 1995) and fishes (Schultz 1993; Warlen 1994; Secor and Houde 1995; Cargnelli and Gross 1996).

The breeding system of anadromous salmonid fishes is ideal for testing hypotheses regarding the relative influences of size and arrival date on reproductive opportunities, and the interactions between these attributes, because both size and timing have plausible connections to reproductive success. These fishes achieve most of their adult body size feeding at sea, do not feed or grow during the breeding season, and breed at very discrete sites and times of the year (see reviews in Groot and Margolis 1991). The high densities of breeders and rapidly changing operational sex ratios result in extreme competition and sexual selection (Quinn et al. 1996). In the semelparous species, notably Pacific salmon (*Oncorhynchus* spp.), all individuals die within a few weeks of commencement of spawning, allowing estimation of an individual's lifetime reproductive output in one season. In these fishes, pre- and post-hatchling embryos develop in streambed gravel for many months. Juveniles emerge and spend a variable period of time rearing in fresh water, and then they migrate to sea, grow, mature, and return (Groot and Margolis 1991).

The distinct sex roles of male and female salmonids result in differences in behavior, energy allocation, and selection on life-history traits on the spawning grounds. Females compete with each other for breeding territories, prepare a nest and deposit their eggs in a series of pockets (collectively termed a redd) soon after entering the stream. In semelparous species they spend the rest of their lives guarding the nest (Fleming 1998; McPhee and Quinn 1998). In contrast, males compete for access to females and provide no parental care (nest building or defense), and they can spawn repeatedly over their entire lives in the stream (Schroder 1981, Fleming and Gross 1994).

Most studies of reproductive success in salmonids have emphasized the importance of adult body size. Large females produce larger and more numerous eggs (though the relationships can be quite variable; Beacham and Murray 1993), they can reduce mortality of embryos by digging deeper (hence safer) redds (Steen and Quinn 1999), and perhaps guard their redds longer from disturbance by other females (Van den Berghe and Gross 1986). Body size also affects reproductive opportunities in males. Males fight for proximity to females and success within this tactic is positively correlated with body size (Gross 1985; Keenleyside and Dupuis 1988; Quinn and Foote 1994; Kitano 1996) and the extent of sexual dimorphism for a given size (Quinn and Foote 1994). Proximity to the female during gamete release affects the number of eggs fertilized by a given male, and dominant males fertilize more eggs, on average, than lower status males, though there may be considerable variation (Schroder 1981; Hutchings and Myers 1988; Thompson et al. 1998). In contrast to the large literature emphasizing the importance of size in salmonid mating systems, arrival date has received less attention. However, earlier-arriving fish may be larger and live longer than later arrivals (McPhee and Quinn 1998; Hendry et al. 1999), confounding analysis of size and reproductive success. Some studies (e.g., Keenlyside and Dupuis 1988) did not consider arrival date at all and others (e.g., Quinn and Foote 1994) eliminated arrival date as a factor by studying only fish that arrived simultaneously. A further complication in natural systems is predation. Previous studies have shown that bears tend to prey on larger individuals and can kill a significant fraction of the population (Gard 1971; Quinn and Kinnison 1999; Ruggerone et al. 2000; Gende et al. 2001). The probability of a salmon being killed also depends on salmon density (Quinn, unpublished data), and hence on arrival date.

The objective of this study was to investigate the interactions between body size, arrival date and other factors that influence potential reproductive success in a natural salmon population: fecundity, egg size, and duration of nest defense in females, and access to females and reproductive lifespan in males. The hypotheses were as follows: (1) Larger females produce larger and more numerous eggs than smaller females but females with larger eggs for their size produce fewer eggs. (2) Larger females live longer in the stream unless preyed upon by bears than smaller females, and females arriving earlier live longer and are larger than later-arriving females. (3) Larger males and those with larger secondary sexual characteristics are more often dominant than smaller males, and earlier arriving males are more often dominant than later arriving males. (4) Larger males live longer than smaller males unless they are more vulnerable to predation, and earlier arriving males live longer than those arriving later. The study was conducted in pink salmon (*O. gorbuscha*), whose reproductive behavior is similar to the other salmon species spawning at high densities (Quinn 1999). However, all pink salmon are 2 years old at maturity, thus the salmon returning on even- and odd-numbered years are genetically distinct (Heard 1991). This allowed us to essentially compare two isolated populations ("broodlines") using the same site.

Methods

Study area

The study was conducted at a small, unnamed stream on Chichagof Island in Port

Frederick, southeastern Alaska in the summers of 1997-2000. For the purposes of this study we named it Himmel Creek. A logjam limited pink salmon to the lower 330 m of the creek in 1997 and 1998. The logjam was washed out in the winter of 1998 and the fish used 800 m of creek in 1999 and 2000. Creek width varied from 3.7-11.8 m depending on location and discharge, which varied greatly with rainfall. Water velocity measured every 10 m in the middle of the creek on a single day in 1997 ranged from 0.54-1.8 m/sec, and over the season ranged from 0.31-1.33 m/sec at a single site. Chum salmon (*O. keta)* co-occur in this creek during the early part of the pink salmon run. Bear predation is a threat to the fish throughout the run; between 5 and 12 brown bears (*Ursus arctos*) were seen feeding on salmon in the creek.

Field sampling

A beach seine and dip nets were used to capture as many of the salmon in the creek as possible (1255 fish in 1997, 1092 in 1998, 1382 in 1999, and 758 in 2000). We anesthetized them using MS-222 and we recorded the body length (mid-eye to hypural plate), hump depth (from the lateral line to highest point on the dorsal hump), snout length (tip of upper jaw to mid-eye), and sex of each fish. After direct examination, spawning status of each fish was categorized as unripe (eggs or milt not expressed freely when squeezed), ripe (all eggs present in body cavity or milt present), partially spawned (for females only: some but not all of the eggs had been released), and completely spawned (no eggs or milt could be expressed). We removed, weighed, and counted a small sample of eggs (10-15 g) from females that were ripe or partially spawned to estimate average egg size. Fecundity was estimated by sacrificing 20 females prior to spawning in 1997, 50 in 1998, 49 in 1999, and 36 in 2000. Body length, hump depth, and snout length of these females were recorded and fecundity was estimated based on total egg weight divided by the average individual egg weight.

Not all fish could be captured on the day that they entered the stream so we estimated freshwater age (number of days in the stream) at the date of capture based on criteria validated by repeated observations of tagged fish. Criteria for freshwater age estimation were as follows: 1 d: sea lice (a parasitic marine copepod, *Lepeophtheirus salmon*is), present on body or slime present, few to no scratches on the flesh, no visible decay of skin or fins, 2 d: no slime or sea lice, few to no scratches, and no decay of skin or fins, 3 d: scratches present and decay beginning on belly and/or fins, 4 d: decay prominent on belly and fins but body still in good shape, color visibly fading, 5+ d: decay very prominent. All females that we examined had completed spawning within 4 d of arrival.

All pink salmon except those sacrificed to estimate fecundity were tagged with white, lettered, plastic disk tags, allowing us to identify and observe individuals from the bank without disturbing them. All pink salmon, tagged and untagged, were counted daily by walking the entire accessible length of stream. The behavior of tagged fish was observed daily in two approximately 100 m-long sections of the creek in 1997 and on all tagged fish in 1998 and 1999. We recorded presence on the spawning grounds and courtship status of males, categorized as dominant (closest to the female in the hierarchy or the sole male courting a ripe female), subdominant (actively courting a female but not closest to her) or alone (male was not involved in courting). The dominance scores (3, 2 or 1, respectively) given each male each day were averaged over his life in the stream.

When tagged fish were found dead, we recorded mode of death (bear kill or senescent) but only 20-31% of the tags were recovered in any year. We are fairly confident of the mode of death designations as we canvassed the stream for dead fish >3 times a day (before and after bear actively daily) allowing little time for scavenging to occur. The fish that were not recovered were probably washed out of the creek by high flows into the ocean, where recovery is impossible, or removed from the stream by bears (Willson et al. 1998; Quinn and Buck 2000). We estimated the longevity of such "missing" fish by adding one day to the last day the fish was observed alive on the spawning grounds (i.e., assuming it was killed or died the day it became missing) but we made no assumptions about mode of death. The study was terminated before the end of the spawning run, but after the peak, on 2 September 1997 with 635 fish remaining, on 6 September1999 with 723 fish remaining (Figure 1.1). In 1998 the study was terminated on 14 September with only 25 fish remaining on the spawning grounds, most of which were near death, and on 21 September 2000 after the last fish had died (Figure 1.1).

Analysis

A series of forward stepwise multiple regressions models were used to examine associations between reproductive opportunity, life-history traits, and breeding date with sexes investigated independently. Each model was blocked by year to remove the influence of year-to-year variation. To investigate the reproductive potential of males, two models were built, one using amount of time to court and spawn with females (longevity) as the response variable and the other using status (average dominance score) as the response variable. Two models were used to examine female reproductive potential with fecundity and nest guarding time (longevity) as response variables. The predictor variables for both sexes included body size, entry date (day when the individual entered the spawning grounds, relative to the first fish that year), year, mode of death, hump depth, and average egg weight for females. A forward stepwise logistic regression model blocked by year was used to examine the influence of mode of death, body size, and entry date on whether or not a female completed egg deposition. ANOVA was used to compare body size between the sexes and compare life-history traits (body size, longevity, hump and snout size, egg size, and fecundity) between years. Bonferroni tests were used to look for patterns of differences in life history traits between broodlines. Univariate linear regressions were used to examine the relationship between body size and entry date in males and females and between body size and dominance in males by year. Statistical package SPSS v7.5 was used for all analyses.

Results

General demographic patterns

Pink salmon first entered Himmel Creek from August 1-15 and densities of live pink salmon peaked about 2-3 weeks later (Figure 1.1). There was more variation in body length among males than females but average length did not differ between sexes within years ($t = 1.29$, $df = 4474$, $P = 0.286$; Table 1.1). There was significant variation among years in longevity (males: $F = 6.35$, $df = 644$, $P < 0.001$, females: $F = 2.97$, $df =$

673, $P = 0.031$), body length, hump depth and snout length ($P < 0.001$ for each sex and all traits). Females also varied in egg size ($F = 164.68$, $df = 953$, $P < 0.001$) but not in fecundity among years ($F = 2.04$, $df = 149$, $P = 0.111$; Table 1.1). This variability did not appear to be due to differences between broodlines; there were no consistent life history differences between the even and odd years (Table 1.1). Bear predation had a significant impact on the population. We recovered 26.4% of the tagged fish after death and 58% of those had been killed by bears (Table 1.2). Even under the most conservative assumption (that none of the missing fish were killed), bears still killed 15% of the tagged fish (Table 1.2).

Female traits

Larger females produced more ($t = 7.13$, $df = 151$, $P < 0.001$) and larger eggs ($t = 20.45$, $df = 953$, $P < 0.001$) than did smaller females (Figure 1.2), but females with large eggs for their size had fewer eggs than predicted by body size alone ($t = 3.56$, $df = 145$, $P < 0.001$). Freshwater longevity of females was best explained by entry date (females that arrived early lived longer than those that arrived later; $t = 3.6$, $df = 486$, $P < 0.001$; Figure 1.3) and cause of death (those that died of senescence lived longer than those that were killed; $t = 2.49$, $df = 486$, $P = 0.013$). There was also an interaction between body size and mode of death ($t = 2.39$, $df = 486$, $P = 0.017$; Figure 1.4). Large senescent fish did not live as long as small senescent fish but among bear-killed fish there was no relationship between body size and longevity. The logistic regression showed that predation strongly influenced whether or not females died before they spawned ($t = 3.57$, *df* = 488, *P* < 0.001; Figure 1.5). Of mortalities we were able ascertain mode of death, predation accounted for 97% of prespawning mortality, and larger females were marginally more likely to die before spawning than smaller females (*t* = 1.82, *df* = 488, *P* = 0.069). However, females killed by bears were not significantly larger than females that senesced (*t* = 0.575, *df* = 650, *P* = 0.55).

Male traits

A multiple regression model showed that larger males had higher dominance scores (*t* = 3.0, *df* = 193, *P* = 0.003), as did earlier arriving males (*t* = 1.99, *df* = 193, *P* = 0.048). There was also a significant interaction between body length and entry date; earlier arriving large males were more often dominant than later arriving larger males but later arriving small males were more often dominant than earlier arriving small males (*t* = 2.13, *df* = 193, *P* = 0.034; Figure 1.6). Longevity was reduced by bear predation (*t* = 6.63, *df* = 401, *P* < 0.001; Figure 1.5) and larger males tended to have shorter lives than smaller males (considering all causes of mortality: *t* = 1.88, *df* = 401, *P* = 0.061). However, the average length of males killed by bears did not differ from that of males dying of senescence (*t* = 0.46, *df* = 420, *P* = 0.64).

Variation in patterns among years

Although many patterns emerged when all years were examined simultaneously, when the years were examined individually some patterns were evident in one year but absent or the opposite in other years. For example, large females entered the spawning grounds earlier than smaller females in 1997 ($t = 2.76$, $df = 246$, $P = 0.006$) and 1998 ($t = 4.34$, $df = 460$, $P < 0.001$) but there was no relationship between size and entry timing in 1999 ($t = 0.57$, $df = 649$, $P = 0.57$) and smaller females arrived earlier in 2000 ($t = 3.07$, $df = 390$, $P = 0.004$). Small males arrived earlier than large males in 1997 ($t = 14.93$, $df = 458$, $P < 0.001$) and 2000 ($t = 3.01$, $df = 289$, $P = 0.003$), large males arrived earlier in 1998 ($t = 2.5$, $df = 571$, $P = 0.01$) and there was no relationship in 1999 ($t = 1.75$, $df = 720$, $P = 0.08$). When all the data were considered with "year" as a factor, large males were more often dominant than small males. However, when each year was examined individually we saw smaller (earlier arriving) males being more often dominant than larger (later arriving) males in 1997 ($t = 2.59$, $df = 118$, $P = 0.01$) but larger males were more often dominant than smaller males in 1998 ($t = 3.69$, $df = 59$, $P = 0.001$) and 1999 ($t = 2.65$, $df = 39$, $P = 0.012$).

Discussion

Although pink salmon have a strict two-year life cycle that results in two genetically distinct broodlines (Heard 1991), life history traits did not vary consistently between even and odd years so our interpretations did not consider this effect. One consistent finding was the importance of bears. Bears killed a significant proportion (no fewer than 15%) of the salmon, and the effects of predation were seen in many aspects of our findings. This very conservative estimate of predation is based on the unlikely assumption that all fish not recovered died a senescent death. Bears may drag their kills into the riparian vegetation beyond the range of our surveys so it is likely that at least some, and perhaps many, of the fish that were not recovered were killed by bears (Quinn and Buck 2000). Research elsewhere in Alaska has also indicated that a large fraction or even the majority of salmon may be killed by bears in small streams (Quinn and Kinnison 1999; Ruggerone et al. 2000).

Large body size was an important contributor to potential reproductive success in females and males. Larger females had greater fecundity and egg size than smaller females, as found in many previous studies (Beacham and Murray 1993). Thus, all other things being equal larger females can produce more and larger offspring. Larger juvenile salmon are less vulnerable than smaller ones to gape-limited predators (Parker 1971; Hargeaves and LeBrasseur 1986; West and Larkin 1987) and the larger progeny of a given cohort have higher survival rates at sea than smaller ones (Healey 1982; Koenings et al. 1993). The amount of energy a female can dedicate to egg production is limited, and resulted in a trade off between number and size of eggs produced. The optimal balance between size and number of eggs may vary with the quality of the incubation habitat (Quinn et al. 1995; Hendry et al. 2001).

Large body size was hypothesized to increase longevity and thus duration of nest defense in females because of potentially higher energy reserves in the larger fish (Van den Berghe and Gross 1986) but this was not the case. Indeed, smaller females lived longer than larger females among those dying of senescence. Longevity was primarily controlled by entry date (and predation); early females lived longer than later arrivals. This seems to be a general pattern in salmon (e.g., McPhee and Quinn 1998; Hendry et al. 1999) and all future studies of salmon reproduction should explicitly consider this factor. Once a female has spawned, she increases her fitness by guarding her nest from other females competing to use the same site. Females that arrive early need to protect their eggs as long as possible because so many females are yet to dig redds whereas the redds of females that arrive later have little chance of disturbance by other females. This results in stronger selection pressure for increased lifespan earlier in the run. Timing of arrival on the spawning grounds and maturation are highly heritable traits in salmonids (Quinn et al. 2000).

Only 13% of the females died before spawning and almost all predation occurred after egg deposition, so although predation shortened life span by 28% on average, this affected nest defense duration more strongly than egg deposition (65% of the females that completed spawning were killed by bears). Larger females were marginally more likely to die before spawning than smaller females and bear predation accounted for 97% of this prespawning mortality, yet the average lengths of females killed by bears overall (pre- and post spawning mortality) did not differ from those of senescent dead fish. It is possible that bears may have been preferentially preying upon fresher, larger females, which would provide a higher energy source for the bears than smaller fish or ones nearer death.

Larger males were more often dominant, as indicated by access to females, than smaller males, allowing them a higher chance of fertilization success (e.g., Schroder 1981; Chebanov et al. 1983). However, the benefits of large size decreased over the course of the breeding season with dominance scores of large males decreasing with entry date (Figure 1.6). Perhaps the increasingly male-biased operational sex ratio towards the end of the season typical of salmon (Quinn et al. 1996) potentially decreasing the ability of the largest males to dominate more than one female allowing the smaller males greater access. The development of large secondary sexual characters (elongated snout and humped back) may increase a male's competitive ability (Quinn and Foote 1994) but we saw no relationship between dominance and hump and snout development, after accounting for the effect of body length. Males in this population had smaller humps, for their length, than almost all of the 20 populations from British Columbia and Washington state sampled by Beacham and Murray (1985). The use of secondary sexual characters in competition may be more important in populations with more exaggerated features, and historic selective predation may already have affected the extent of development in this population.

As with females, reduction of instream life span of males was most directly related to bear predation. This predation could have a considerable impact on a male's reproductive success as it resulted in, on average, 25% fewer days for males to court females and engage in spawning than those that experienced senescent deaths. Arrival timing was predicted to influence predation risk as earlier-arriving males might contend with high risk of bear predation, due to lower densities on the spawning grounds earlier in the breeding season. However, we found no relationship between entry date and predation risk. The data did show a trend suggesting that larger males had a shorter lifespan than smaller males. However, there was no evidence of differential predation on males with respect to body size or shape, unlike other studies (Gard 1971; Quinn and Kinnison 1999; Ruggerone et al. 2000). Thus, the reduced lifespan of larger males did not appear to be a result of bear predation.

Like virtually all salmon populations (and indeed, many animal populations throughout the world), there is some human influence at this site. There are two main types of influence but we do not believe that either significantly affected the study. First, the habitat was not pristine, as some logging has taken place in the watershed. However, the very high densities (on the order of 0.5 fish/m^2) of adult pink and chum salmon indicated that the stream was not in a degraded state. Second, the population is subject to commercial fishing, which could affect the results by reducing the density of adult salmon or by selective removal of certain phenotypes. Natural variation in pink salmon abundance from one year to the next in this region is at least a factor of 10 (Rigby et al. 1991), so fishing may have less influence on density than natural processes. The fisheries for pink salmon in this area use purse seine nets and trolling gear. These methods are not size-selective so we do not think the distributions of morphology or size were affected by fishing, as might be the case with gillnets. The fishing takes place some distance from the creek and salmon are seen milling for days around the mouth before ascending. We therefore think that the effects of fishing on timing of stream entry are probably negligible.

Conclusions

Body size was an important influence on the potential reproductive success of individuals, affecting the quantity and size of eggs produced and the ability of males to access females. However, the timing of arrival on the breeding grounds in some cases overcame the positive influence of body size on reproductive success. Early-arriving females lived longer than later arrivals, and the relationship between male size and dominance depended on arrival date. Timing may co-vary with body size and needs to be more carefully examined in salmon and other animals with similar breeding systems.

In addition to the insights that our data provide for reproductive system, they also send two cautionary notes regarding the design of field studies on reproduction in salmon and other organisms. First, some patterns (e.g., the relationship between body size and entry timing) were statistically significant in one year but were not evident across all four years of data. Given the close connections between body size, arrival date, longevity and reproductive success, results from a single year, no matter how large the sample size or the level of statistical significance, cannot be considered conclusive. We also note that the vast majority of studies on reproductive behavior of salmonids (and many other organisms) have been conducted in artificial channels or in pens within streams (e.g., Schroder 1981; Keenleyside and Dupuis 1988; Foote 1990; Fleming and Gross 1994), in natural habitats where predation did not occur (Quinn and Foote 1994), or was so rare that the authors did not mention it (Van den Berghe and Gross 1986). Future studies should explicitly consider whether processes observed under such controlled conditions would operate similarly in natural systems with predation.

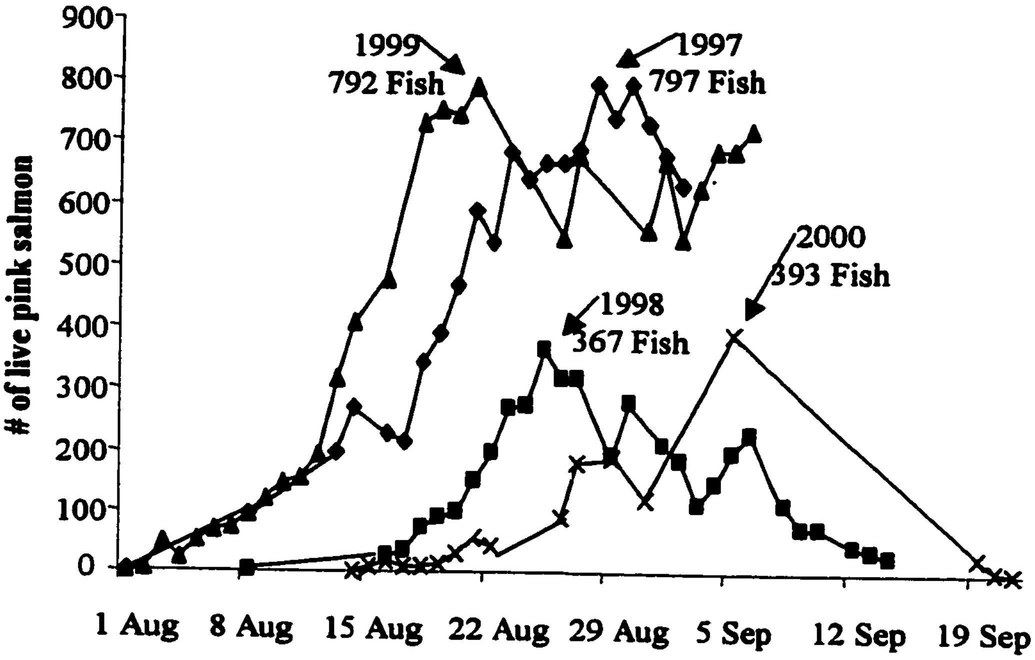


Figure 1.1. Daily total numbers (arrows indicate peaks) of live pink salmon in Himmel Creek during the spawning seasons from 1997 to 2000.

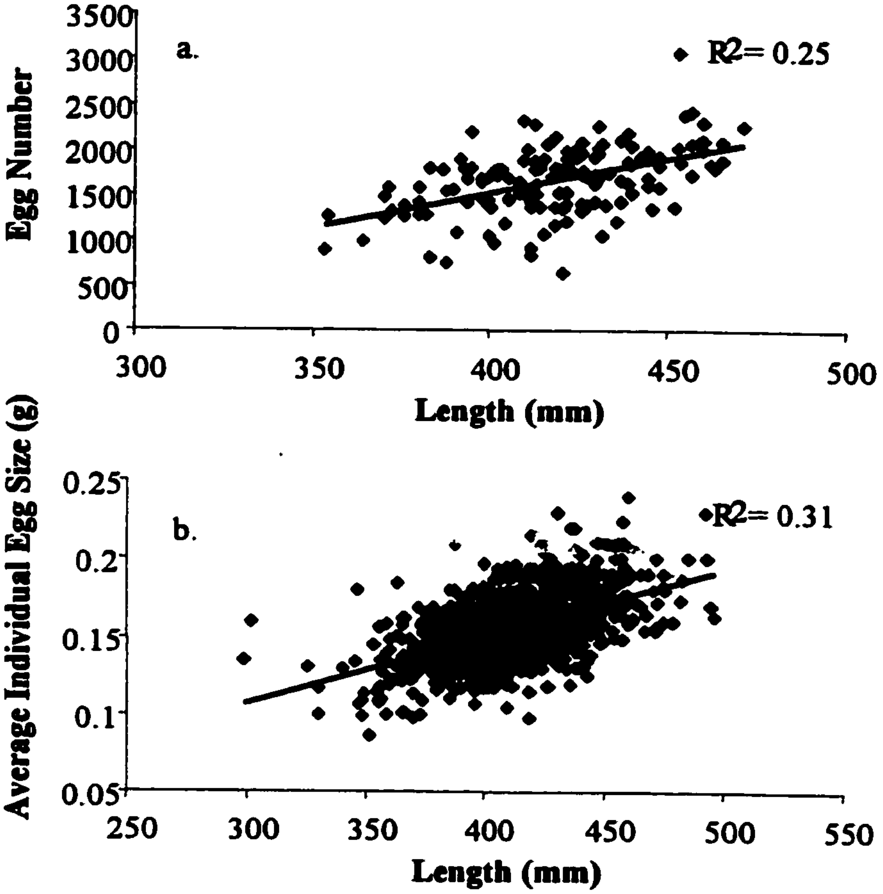


Figure 1.2. Relationships between body size and (a) the number (n = 153) and (b) the average weight (n = 954) of eggs produced by female pink salmon spawning in Himmel Creek, 1997 to 2000, with the results of linear regression analysis.

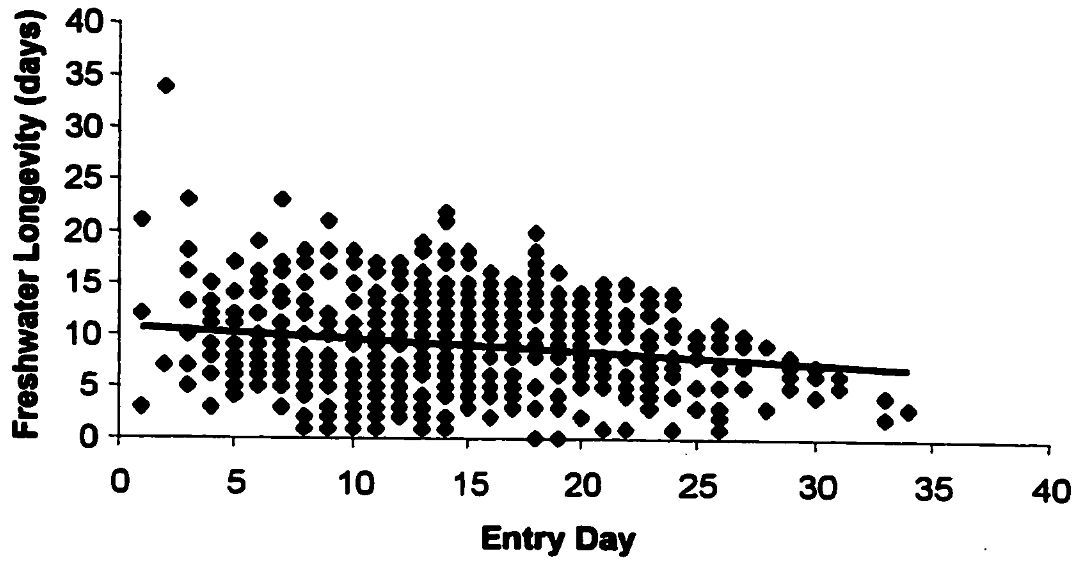


Figure 1.3. The relationship between date of entry onto the spawning grounds and longevity for female pink salmon in Himmel Creek, Chichagof Island, Southeast Alaska, during the spawning runs of 1997 - 2000. The line represents the fit of the regression model.

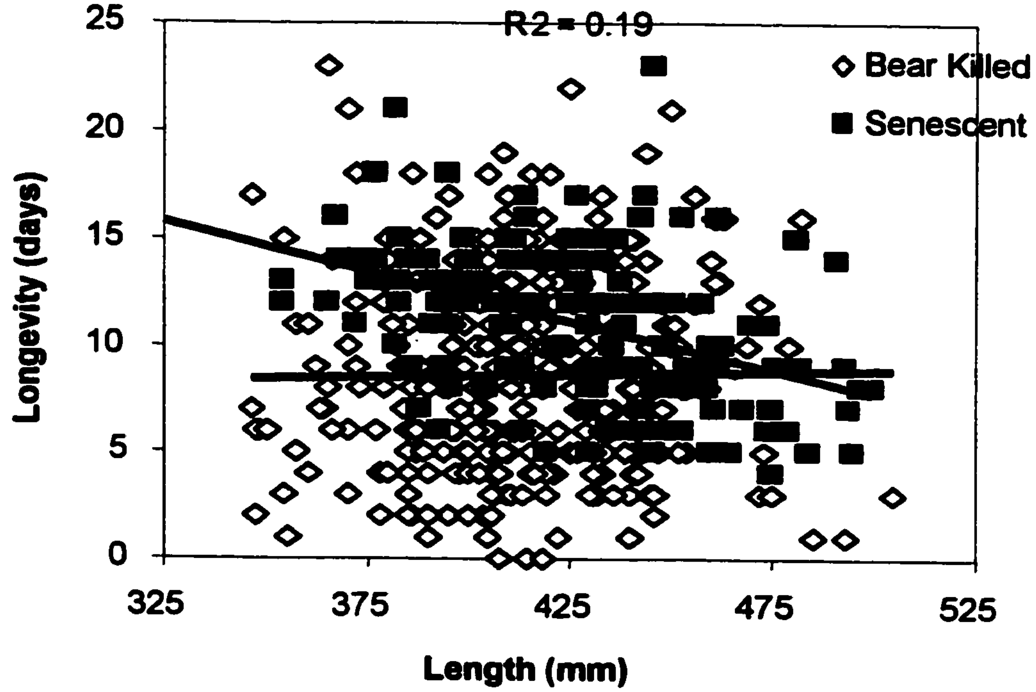
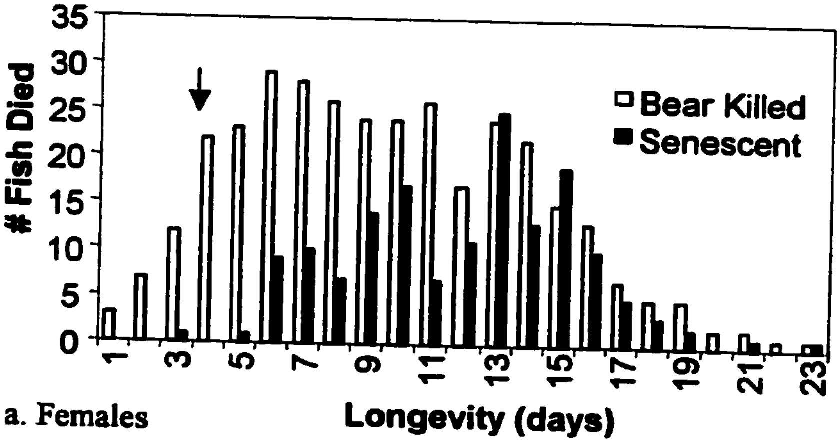
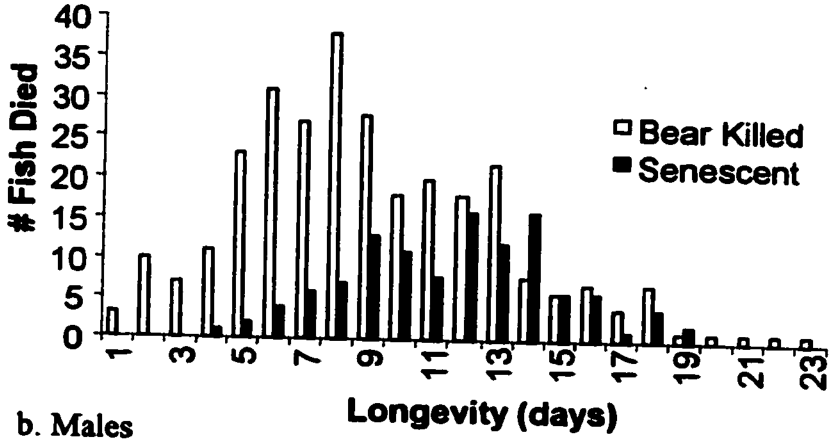


Figure 1.4. The relationships between body size and longevity of bear-killed and senescent female pink salmon spawning in Himmel Creek, Chichagof Island, Southeast Alaska, during the spawning runs of 1997 - 2000. The lines represent the fits of linear regression models. TheR^2 value is for the fish that died of senescence; the slope of the line for bear-killed salmon was not significantly different from zero.



a. Females



b. Males

Figure 1.5. Distribution of longevity of bear-killed and senescent pink salmon spawning in Himmel Creek, Chichagof Island, Southeast Alaska, during the spawning runs of 1997 - 2000. Arrow indicates day by which all females had deposited their eggs (spawning completed).

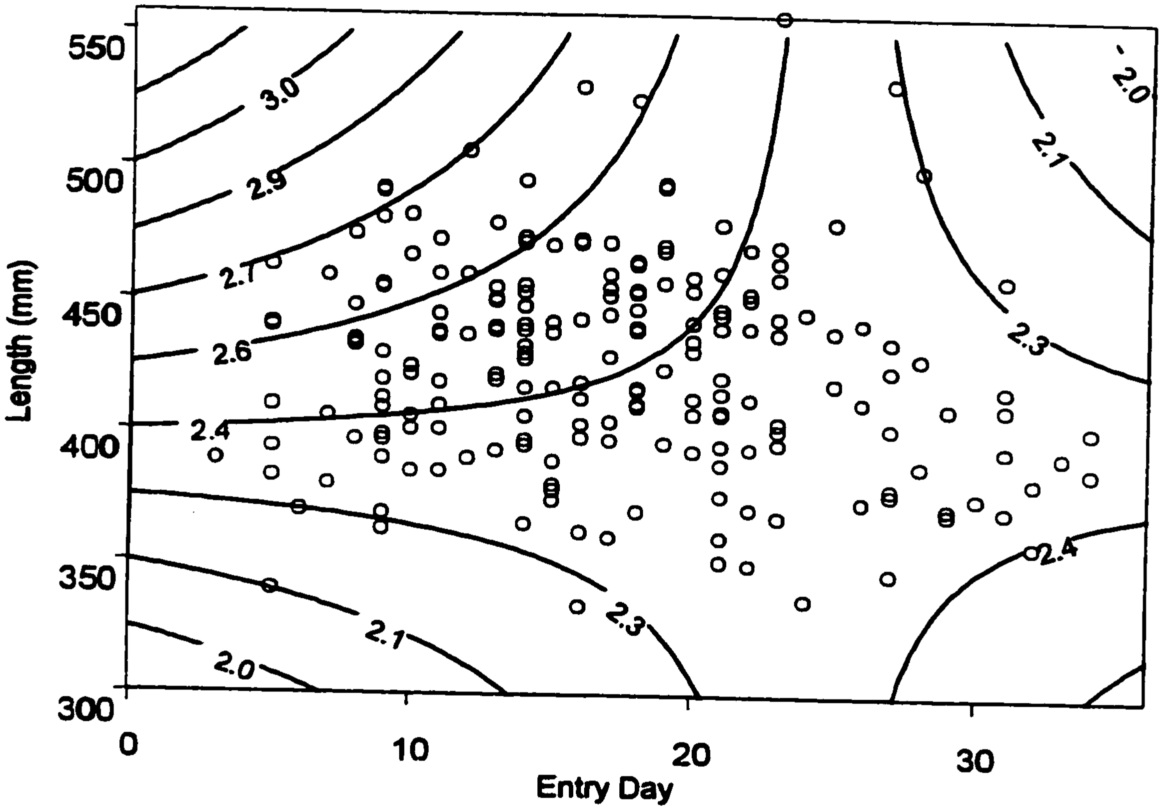


Figure 1.6. Contour plot showing the outcome of the interaction of body size and entry date on average dominance score (maximum = 3) of male pink salmon spawning in Himmel Creek, Chichagof Island, Southeast Alaska, during the spawning runs of 1997 - 2000. The contour lines show the dominance score as fitted by the model that corresponds with the entry date and length of a fish. The points show actual spread of data used for the model.

Table 1.1. Range, sample size (N), average (Ave.), and standard deviation (SD) of standard length, snout length (mideye - end of snout), hump depth (lateral line - highest point of hump), freshwater longevity, fecundity, and average egg size measured on pink salmon spawning in Himmel creek, Chichagof Island, Southeast Alaska in 1997-2000.

		Males				Females			
Trait	Year	Range	N	Ave.	SD	Range	N	Ave.	SD
Length (mm)	1997	319-560	695	439	40.9	334-504	558	439	26.4
	1998	289-508	603	425	33.9	339-483	489	426	22
	1999	304-495	726	385	30.6	299-491	652	390	21.8
	2000	312-486	318	414	29.8	302-496	440	414	21.7
Snout (mm)	1997	35-121	681	70	15.5	27-87	549	45	6.9
	1998	36-101	604	69	11.8	29-87	486	46	5
	1999	36-99	728	59	9	29-59	654	41	4.1
	2000	36-94	320	65	10.9	32-77	440	45	5.5
Hump (mm)	1997	32-121	696	69	15	28-81	558	44	6.5
	1998	34-113	604	70	13.2	32-82	489	46	5.3
	1999	32-99	728	57	9.5	28-57	654	41	4.2
	2000	39-109	320	66	11.8	28-78	440	45	5.5
Longevity (d)	1997	0-16	84	8.2	3.4	1-23	89	8.6	4.1
	1998	1-17	309	7.8	3.7	1-19	309	8.8	4.1
	1999	1-18	163	9.5	4.6	1-23	154	9.9	5.4
	2000	0-24	94	8.1	4.6	0-22	125	8.5	4.5
Fecundity	1997					906-3068	20	1797	466.4
	1998					809-2416	47	1670	334.6
	1999					625-4121	50	1577	513
	2000					973-2279	36	1760	302.7
Egg mass (g)	1997					0.12-0.24	118	0.17	0.024
	1998					0.12-0.22	242	0.17	0.018
	1999					0.09-0.19	380	0.14	0.017
	2000					0.10-0.19	237	0.15	0.016

Table 1 2. Number of pink salmon tagged in Himmel Creek, number of tagged fish found dead, and number of those found dead that had been killed by bears in 1997-2000.

	# tagged		# (%) found dead		# (%) of the dead killed	
Year	males	females	males	females	males	females
1997	696	559	176 (25%)	226 (40%)	62 (35%)	60 (27%)
1998	603	489	101 (17%)	160 (33%)	87 (86%)	47 (29%)
1999	727	655	145 (20%)	140 (21%)	103 (71%)	99 (68%)
2000	318	440	99 (31%)	140 (32%)	96 (97%)	135 (96%)
Total	2344	2143	521 (22%)	666 (31%)	348 (67%)	341 (51%)

Chapter 2: Is bigger really better: relative importance of body size and breeding date in the reproductive success of pink salmon, *Oncorhynchus gorbuscha*.

Introduction

Since the work of Darwin, variation in reproductive success among individuals has been extensively investigated in many organisms (e.g., Clutton-Brock 1988). Understanding the factors that affect this variation is key to understanding the selective pressures driving adaptation and ultimately evolution (Roff 1992; Stearns 1992; Andersson 1994). Salmonid fishes, including Pacific salmon, *Oncorhynchus* spp., Atlantic salmon and brown trout (*Salmo* spp.) and the chars (*Salvelinus* spp.) have been the focus of many reproductive success investigations (reviewed by Fleming 1998). The breeding system of Pacific salmon makes them particularly well suited for testing hypotheses regarding reproductive success. These anadromous, semelparous fishes achieve most of their adult body size while feeding at sea, do not feed or grow during the breeding season, home to their natal stream to breed at very discrete times of the year, and die upon completion of spawning, allowing researchers to estimate an individual's lifetime reproductive output in one season (see reviews in Groot and Margolis 1991). Reproductive success of salmon has typically been estimated by indirect methods using surrogate traits such as observations of courtship, spawning events, and physical attributes of the fish such as size or shape (Gross 1985; Keenleyside and Dupuis 1988; Fleming and Gross 1994; Quinn and Foote 1994; Mangel 1996). However, several males may fertilize eggs during a given spawning event, females have more than one spawning event, and the survival of the offspring cannot be inferred from observations on the adults. Thus the reproductive success of individual male and female salmon cannot be accurately measured indirectly.

Pioneering work by Schroder (1981) and Chebanov et al. (1983) used biochemical markers to demonstrate the fertilization success of different males participating in spawning events in controlled arenas. Since then, advances in molecular biology such as DNA microsatellites provide more powerful tools for kinship analysis and the direct measurement the reproductive success of salmon (Wright and Bentzen 1994; McConnell et al. 1995; O'Reilly and Wright 1995). Microsatellites and similar tools can be used to determine the parentage of individual offspring but most studies to date have been focused on determining the fertilization success of males (e.g., Jordan and Youngson 1992; Morán et al. 1996; Foote et al. 1997), neglecting the variation in reproductive success among females, and most have measured reproductive success by sampling the offspring as embryos or at juvenile life history stages, neglecting important events that may occur later in life. In contrast, our approach was to test hypotheses concerning the actual reproductive success of the parents in the most appropriate measure, the number of mature progeny.

Most of the past research on reproductive success in salmonids has emphasized the importance of adult body size. Females can benefit from large size by producing larger and more numerous eggs (Beacham and Murray 1993; Dickerson et al. 2002), successfully compete for access to high quality breeding sites (Foote 1990), and dig deeper (hence safer) redds (Steen and Quinn 1999). Males may benefit from large size by an increased competitive ability allowing greater access to spawning females (Gross

1985; Keenleyside and Dupuis 1988; Quinn and Foote 1994; Kitano 1996; Dickerson et al. 2002).

Though less thoroughly investigated in salmonids, timing of arrival on the spawning grounds may have as great or greater influence on reproductive success than body size. Dickerson et al. (2002) found that early arriving pink salmon, *O. gorbuscha*, males had higher dominance scores than later arrivals, and that later in the spawning season when few ripe females were available, the benefits of large body size on dominance were negated. Earlier-arriving fish also typically live longer than later arrivals, allowing more time for males to participate in spawning events and females a longer nest guarding period (McPhee and Quinn 1998; Hendry et al. 1999; Dickerson et al. 2002). Another consideration, which affects both the benefits of large size and early arrival, is predation. Previous studies have shown that bears can kill a large portion of the population and tend to prey on larger individuals (Quinn and Kinnison 1999; Ruggerone et al. 2000; Quinn and Buck 2001). The probability of a salmon being killed also depends on salmon density, which is lower early in the season, (Quinn et al. 2003), and hence on arrival date.

The use of microsatellites to perform a parentage analysis from a small piece of fin allows us to investigate the importance of these potential influences on reproductive success directly. The objective of this study was to examine the relationship between arrival timing, body size, and other associated traits (fecundity, egg size, dominance, and longevity) and the number of adult progeny produced in pink salmon. We hypothesized that more offspring would be produced by: (1) females that are larger, have higher fecundity and larger eggs than females that are smaller, less fecund and have smaller eggs, (2) longer lived females compared to shorter lived females (although no female's life expectancy spans the entire run thus early arriving females may see a smaller benefit from longer life than later arriving fish), (3) longer lived males compared to males that lived a shorter time on the breeding grounds, and (4) larger males and those with higher dominance scores compared to smaller and less dominant males, (5) males that arrive early in the breeding season compared to later arrivals. Finally, based on the nature of the competitive breeding system, we predicted greater variation in reproductive success among males than among females.

Methods

Field Sampling

This study was carried out on a natural population of pink salmon in a small, unnamed stream located on Chichagof Island in Port Frederick, Southeastern Alaska. We sampled adult salmon during the summers of 1997-2000 and so, because all pink salmon are two years old at maturity, the fish sampled in 1999 were the offspring of the 1997 fish, and those sampled in 2000 were the offspring of the 1998 fish. In 1997 and 1998 the lower 330 m of stream were accessible to spawning. Flooding in the winter of 1998 gave salmon access to an additional 470 m of stream in 1999 and 2000. The stream's bank full width ranged from 3.7 – 11.8 m. The discharge of the stream varied greatly with rainfall; water velocity ranged from 0.31 – 1.33 m/s at a single site over the course of the run. The early part of the pink salmon spawning period overlapped with spawning by chum salmon (*O. keta)*, and both species were subjected to predation by 5-12 brown bears (*Ursus arctos*).

Our goal was to sample all pink adult salmon that entered the creek but logistic constraints prevented 100% sampling. Sampling stopped on 2 September 1997, after the peak of spawning but with 635 fish remaining alive, on 6 September 1999 with 723 fish remaining, on 14 September 1998 with only 25 fish remaining on the spawning grounds, most of which were near death, and on 21 September 2000 after the last fish had died (Figure 1.1). Many of these living fish had been sampled but the combination of protracted spawning period, episodic floods, and bear predation made it impossible to sample every fish.

A beach seine and dip nets were used to capture as many pink salmon as possible. Upon capture, fish were tagged with white, uniquely lettered, plastic disk tags, allowing identification of individual fish from the bank without disturbing spawning behavior. The fish were identified to sex, measured for body length from mideye – hypural plate (a measurement unbiased by the greater development of jaws in males), depth of the dorsal hump (from the lateral line to highest point on the hump), snout length (tip of the upper jaw to mid-eye), and a small fin clip from the pectoral fin was taken and preserved in 95% ethanol for later DNA analysis. A small (10-15 g) sample of eggs was taken from ripe females and later weighed and counted to estimate individual egg size. Over all four years 2348 males and 2139 females were tagged (1997: 696 m, 558 f; 1998: 604 m, 489 f; 1999: 728 m, 652 f; 2000: 320 m, 440 f).

The number of eggs produced by a female (fecundity) is likely to have a large effect on her reproductive success, so we established the length - fecundity relationship for the population by sacrificing 20 females in 1997, 50 in 1998, 49 in 1999, and 36 in 2000 prior to spawning. Body length, hump depth, and snout length of these females were recorded and fecundity was estimated based on total egg weight divided by the average individual egg weight. The combination of length, which was obtained from almost all females, and egg weight, obtained from most females, explained 34% of the variation in fecundity.

Each day, all pink salmon, tagged and untagged, were counted, and tagged males were observed to determine dominance. In 1997 observations were made on tagged fish in two ~100 m long sections of the creek due to the large numbers of fish present. In 1998 observations were made on the entire accessible reach. Based on their behavior, males were categorized as 3 (dominant; closest to the female in the hierarchy or the sole male courting a ripe female), 2 (subdominant; involved in courting but not in the primary position) or 1 (alone; male was not involved in courting). Two estimates of potential reproductive success were calculated from these observations. First, the dominance scores given each male each day were averaged over his life in the stream and termed average dominance. The second score, days dominant, was the total number of days a given male was seen in the dominant or primary position with a ripe female.

We were not able to capture all fish on the first day of their entry into the spawning grounds so for fish caught later we estimated freshwater age (number of days the fish had spent in the stream) based on criteria validated by repeated observations of tagged fish. The criteria for freshwater age estimation were as follows. Fish were assumed to have entered within 1 d if they had sea lice (a parasitic marine copepod, *Lepeophtheirus salmonis*), present on body and/or slime present, few or no scratches on the flesh, no visible decay of skin or fins; 2 d if they had no slime or sea lice, few or no scratches, and no decay of skin or fins; 3 d if they had scratches and the belly or fins were beginning to show decay; 4 d if decay was prominent on the belly and fins, and color was fading but the body was still in good condition; and 5+ d if there was prominent decay present.

A fin clip was taken from all untagged fish found dead (an additional 1025 fish) and we recorded the date and mode of death (bear kill or senescent). Only 20 - 31% of the tags were recovered in any year. The fish not recovered were probably washed out of the creek by high flows into the ocean when they became too weak to resists the current, or were removed from the stream by bears (Quinn and Buck 2000; Gende 2002). We estimated the longevity of fish we did not find dead by adding one day to the last day the fish was observed alive on the spawning grounds (assuming it was killed or died the day it became missing) but we made no assumptions about the mode of death.

Molecular Techniques

Total genomic DNA was isolated from 20 - 30 mg of fin tissue using ammonium acetate extraction procedures based on those for the Gentra Systems™ (Minneapolis, MN) Puregene DNA isolation kit. Concentrations of the isolated DNA were measured using spectrophotometry and diluted to 100 ng for use in the polymerase chain reaction

(PCR). After screening ~20 microsatellite loci, six were chosen for this study based on their large allele numbers and high levels of heterozygosity (Table 2.1).

A Perkin-Elmer 9600 thermocycler was used for all PCRs in a 10 µL volume consisting of 10 mM Tris-HCL at pH 8.3, 50 mM KCL, 2 mM $MgCl_2$, 0.8 mM deoxynucleotide triphosphates (dNTPs, 0.2 mM each), 0.5 units *Taq* DNA polymerase, primers ranged from 0.09 – 0.3 µM, and 100 ng DNA template. The following PCR profile was used for amplification: 5 cycles of 94°C for 1 min, touch down technique starting at the annealing temperature plus 5° C for 30 sec and reducing one degree each cycle, and 72° C for 15 sec; 7 cycles of 94° C for 1 min, annealing temp. for 30 sec, 72° C for 15 sec; 17 cycles of 94° C for 30 sec, annealing temp. for 30 sec, 72° C for 15 sec; 72° C for 30 min. (Table 1).

PCR products were combined and then visualized using the MegaBACE 1000 capillary electrophoresis system (Amersham Pharmacia Biotech Limited, Piscataway, NJ). The forward primer of each primer pair was labeled with one of three fluorescent chemical labels (FAM, NED or HEX), and each sample was electrophoresed with a 900 base pair internal size standard labeled with ROX (ET900-R, Amersham Pharmacia). Analysis of MegaBACE output was performed with Genetic Profiler software, v1.1 (Amersham Pharmacia Biotech Limited, Piscataway, NJ).

Analysis

Tests for deviations from Hardy-Weinberg equilibrium were done using GENEPOP, v3.1 (Raymond and Rousset 1995). A simulation, run using pedigree v3.0

(Craig Busack, Washington Department of Fish and Game) to test the ability to determine parentage using the loci chosen, indicated that there was > 99.9% chance of correctly determining parentage via exclusion with the chosen loci. The reproductive success of adults was assessed by determining the number of offspring that returned two years later to spawn. Both Cervus (Marshal et al. 1998) for its exclusion application and PAPA (Duchesne et al. 2002) maximum likelihood software (which allows accounting for genotyping errors) were used to determine parentage of the returning offspring (1999 and 2000 fish).

To assess the relative importance of morphology, life history, and behavior to reproductive success, various general linear models were used with the sexes analyzed separately and with all models blocked by year to account for interannual variation (SYSTAT v7.5). Very few families were represented by many offspring so for purposes of analysis parents were placed into one of three categories: no offspring, one offspring, and greater than one offspring. The predictor variables for models of both sexes included length, instream longevity, mode of death, hump depth, snout length and arrival date. The female models also included fecundity and average egg mass, and the male models included both scores of dominance. To correct for multiple test sequential Bonferroni tests were used to further clarify relationships between number of offspring produced and longevity, dominance, and arrival timing in males. F-tests were used to compare variances in body size and arrival timing between females with and without identified offspring and differences variance in reproductive success between genders.

Results

In 1997 all six loci were out of Hardy-Weinberg equilibrium whereas in 1998 5 of the six loci were in Hardy-Weinberg equilibrium (Table 2.1). In all cases loci were out of Hardy-Weinberg equilibrium due to heterozygosity deficiencies. Of the adult offspring sampled in 1999 and 2000, 723 (34%) were assigned to at least one parent (28% from 1999 and 36% from 2000), giving us 297 male parents, and 271 female parents with identified offspring. There was no significant difference in variance in reproductive success between females and males ($F = 1.77$, $df = 2$, $p = 0.36$).

In males, there was no significant relationship found between the number of offspring produced and body size ($F = 0.002$, $df = 1297$, $p = 0.96$), shape (hump: $F = 0.013$, $df = 1299$, $p = 0.91$, snout: $F = .0121$, $df = 1284$, $p = .073$), or mode of death ($F = 0.376$, $df = 182$, $p = 0.54$). However, males who produced no offspring had the shortest instream life span (fewest days alive in freshwater), followed by those who produced one offspring, and the males that produced more than one offspring had the longest average life span ($F = 8.134$, $df = 390$, $p = 0.005$, Table 2.2). Males who produced no offspring also had the lowest average dominance score and those that produced more than one offspring had the highest ($F = 5.223$, $df = 179$, $p = 0.023$, Table 2.2). Males who produced more than one offspring arrived earliest on average, followed by those who produced one, and the males with no offspring had the latest average arrival timing ($F = 3.74$, $df = 458$, $p = 0.05$, Table 2.2). There was also a significant interaction between entry date and number of days a male spent in the primary position behind a female and the number of offspring he produced ($F = 3.941$, $df = 159$, $p = 0.049$). Early in the season, males with high dominance scores produced the most offspring but as the season progresses, the benefits of dominance were decreased (Figure 2.1).

In females, there was no significant relationship between the number of offspring produced and fecundity (F = 0.5, df = 1047, p = 0.48), egg size (F = 0.793, df = 339, p = 0.37), body shape (hump: F = 0.252, df = 1047, p = 0.62, snout: F = 0.811, df = 1038, p = 0.37), mode of death (F = 0.004, df = 378, p = 0.95) or instream longevity (F = 1.909, df = 397, p = 0.99). However, there was evidence of stabilizing selection on entry date and possibly length. In 1998 variance in arrival timing was higher in females that did not have any identified offspring than in those that had at least one (F = 1.711, df = 354, p = 0.05). It appears that females with an intermediate entry date were most successful although, this relationship was not apparent in 1997 (F = 0.601, df = 176, p = 0.31; Figure 2.2). Variance in length was significantly higher among females that had no offspring than among those with at least one offspring identified in 1998 (F = 1.299, df = 377, p = 0.04) and approaching significance in 1997 (F = 0.613, df = 64, p = 0.07). It appeared that females of intermediate size were most successful in 1998 but this pattern was not apparent in the 1997 fish (Figure 2.3).

Discussion

All loci from 1997 and one from 1998 had too many homozygous individuals and thus were out of Hardy-Weinberg equilibrium. This is probably due a combination of null alleles, large allele drop out (larger alleles are harder to amplify and thus tend to have weaker signals), and missed alleles during the scoring process. This would hinder an analysis of population structure but it does not decrease our confidence in our parentage assignments because the unidentifiable offspring had no known parents rather than too many possible parents.

Surprisingly, neither body size nor shape was a significant predictor of offspring production in males although both (and especially size) have been correlated with dominance (e.g., Fleming and Gross 1994; Quinn and Foote 1994). Larger males in this population had higher dominance scores (Dickerson et al. 2002), and males with higher dominance scores produced more fish, but the effects of arrival timing could counter-balance the effect of these traits. Perhaps as the season progresses and the operational sex ratio shifts to a larger number of males per ripe female (Quinn et al. 1996) the influence of size on dominance decreases as larger males are no longer able to defend access to ripe females. This would reduce the influence of size and/or dominance on offspring production towards the end of the season.

Longer lived males were more successful, presumably because the longer life span allowed them to participate in more spawning events than shorter lived fishes (as hypothesized in the model by Gross 1985). Males in this population who were killed by bears had a 25% reduction in life span (Dickerson et al. 2002) yet mode of death did not influence reproductive success. Predation is probably still important to a degree but not a strong enough influence to see a significant effect in this analysis.

In this population larger females had larger and more numerous eggs and there was sufficient variation in these traits for there to be a potential impact on variability of reproductive success (ranges: size 302 - 504 mm body length, 625 – 4121 egg number from sacrificed females, and 0.1 – 0.24 g average egg weight; Dickerson et al. 2002). We therefore expected to see a significant relationship between reproductive success and body size, as well as egg size and high fecundity. However, this was not the case. We are not suggesting that fecundity and egg size do not have an impact on variability in reproductive success but rather that their importance was not strong enough to be visible on the scale of this study. If we had looked at survival just to hatching or emergence we may have seen a relationship. We did see a relationship between size and reproductive success in the form of lower variances among fish that produced offspring compared to those who did not. In 1998 it appears that there was a mid-range size that was optimal (figure 2.3). This relationship is probably driven by a number of factors. Fish in the small range of sizes have fewer and smaller eggs (Dickerson et al. 2002), and are at greater risk of redd superimposition (Steen and Quinn 1999). Larger females may have to contend with higher predation rates, although there was no relationship between size and predation in this population (Dickerson et al. 2002).

As the primary focus of females on the spawning grounds after egg deposition is nest defense (Fleming 1998; McPhee and Quinn 1998) we hypothesized that females with longer instream life spans would have greater reproductive success but we found no such relationship. The average life span of a female in this population was 8.5 - 9.9 days while the duration of the run was ~35 days on low density years (1998 and 2000) and considerably longer during high density years (1997 and 1999 Figure 1.1). No one female could protect her eggs throughout the entire run and in fact at most are only guarding for ~30% of the run, thus the duration of nest guarding may not have a large impact on reproductive success. Entry date exhibited the strongest influence on reproductive success. In 1998 stabilizing selection appears to be acting on entry date with entry during the middle of the run showing the most offspring production. This relationship is logical; if directional selection were operating then the mean trait value would be moving. Stabilizing selection was not apparent in 1997 but we were unable to sample the end of the run that year and could have missed enough of the late fish to mask this influence. A mid-range entry could benefit a female by reducing risk of redd superimposition compared to earlier arrivals (van den Berghe and Gross 1989) and access to a greater choice of males than later arrivals. Early emerging fry may be vulnerable to predators (e.g., Brännäs 1995) and heavy, density-dependent predation on pink and chum salmon fry was reported in Hooknose Creek, B.C. by Hunter (1959). Both early and late fry may miss the optimal window of food availability, and the date of seaward migration can strongly affect survival in pink salmon (Taylor 1980).

Although ocean mortality was not included as a parameter in this study, its effects will influence the results because reproductive success was defined as number of offspring returning to spawn, rather than fertilization success or production of juveniles, as most studies have done. Survival from egg to adult is more strongly influenced by processes in freshwater than at sea. Total mortality from egg to adult for pink salmon is ~ 99.73% with ~ 91% of this mortality occurring during the egg to smolt period and ~ 9% occurring during the smolt to adult period (Bradford 1995). The majority of lifetime mortality thus occurs in freshwater, and the variability in mortality is also higher at this stage than at sea (geometric mean variance 0.54 vs. 0.33; Bradford 1995). However, the mortality at sea is important to reproductive success and it may counterbalance that in freshwater in some cases. In particular, research on salmon indicates that the timing of seaward migration has a strong effect on survival, exerting more influence than body size in many cases, but the optimal date for survival varies among years with the distribution of prey and predators (e.g., Taylor 1980; Holtby 1988; Holtby et al. 1990). Finally, there are family-specific patterns of survival at sea in pink salmon (Geiger et al. 1997) and chinook salmon (Unwin et al. in press), with no obvious adult phenotypic trait controlling it.

In summary, the correlations (or lack thereof) between adult traits and lifetime reproductive success result from processes on the breeding grounds that we were able to measure, and from complex physical processes during the incubation and marine periods that were not directly sampled and probably are subject to considerable variation among years. These processes will have acted on the population for countless generations, so the absence of strong linear trends (e.g., in body size or spawning date) is not surprising.

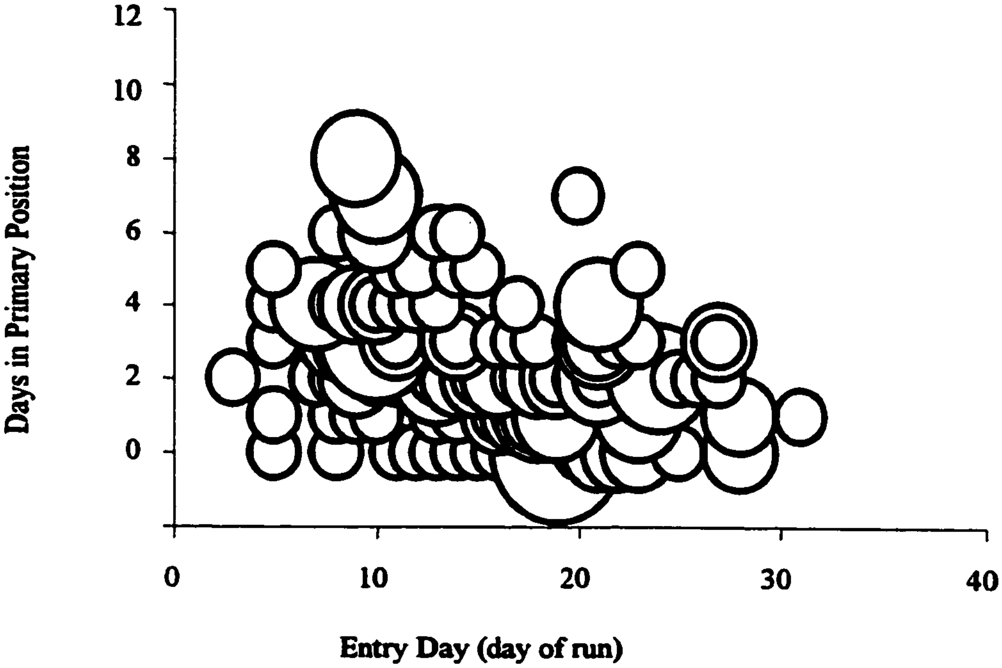


Figure 2.1. The interaction between male pink salmon dominance (days in the primary position behind a ripe female) and entry day (day from first day of run) and its affects on offspring production. Circle size represents number of offspring (smallest = 0 offspring, largest = 6 offspring).

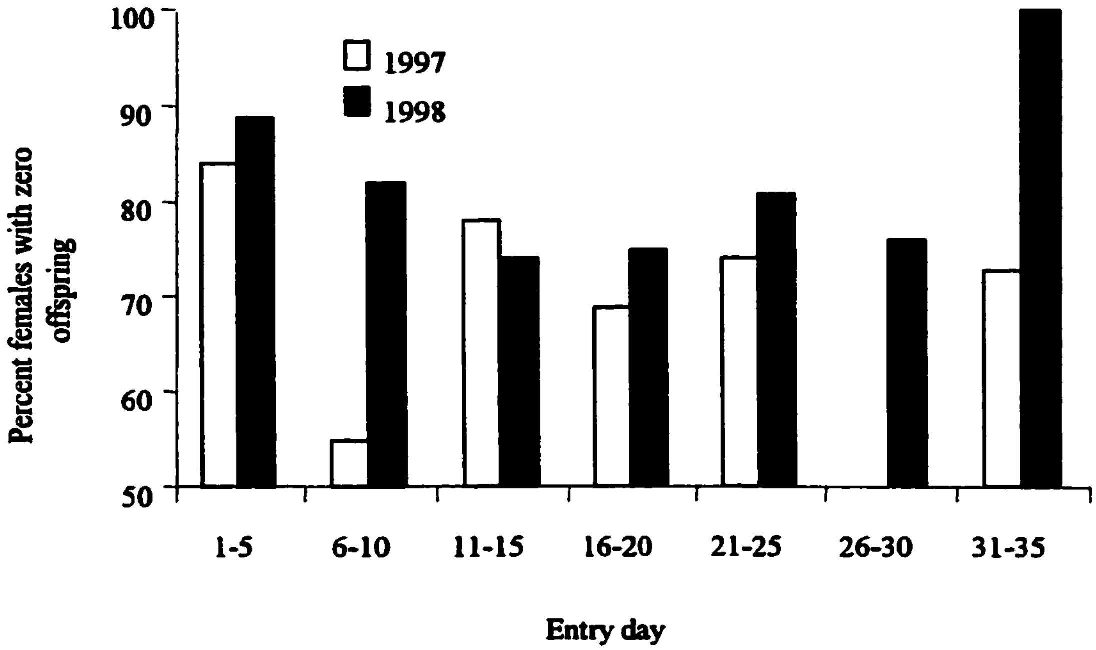


Figure 2.2. Proportion of female pink salmon that produced no identified adult offspring by entry day (day from the first day of the run) in 1997 and 1998.

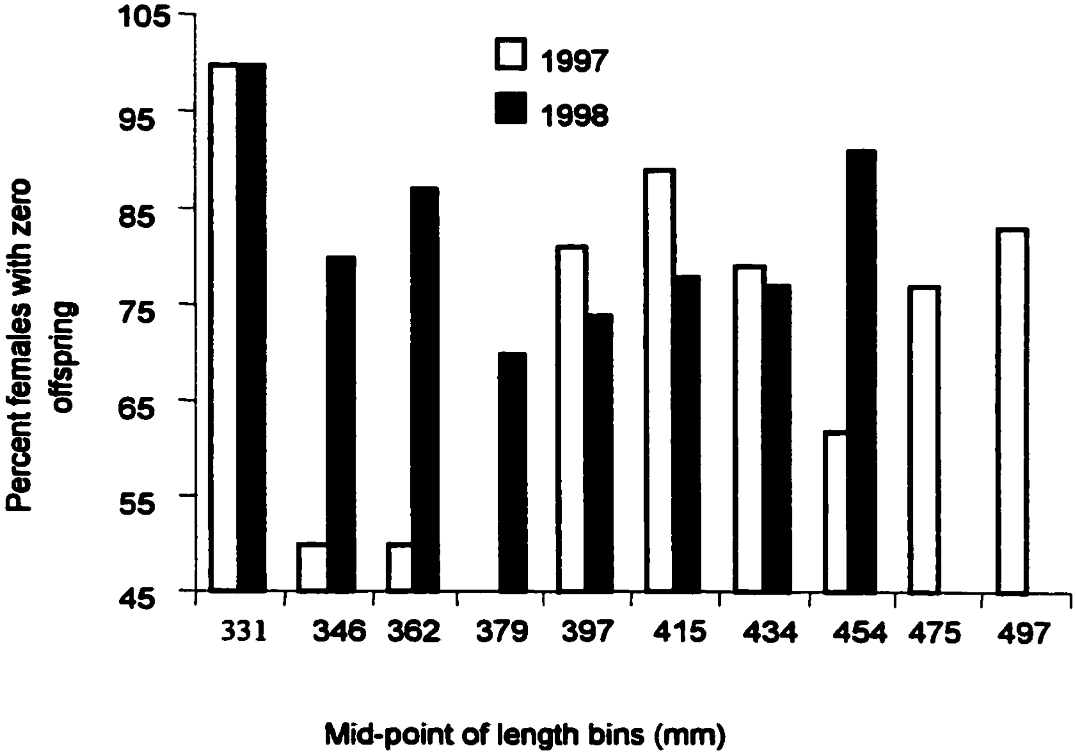


Figure 2.3. Proportion of female pink salmon that produced no identified adult offspring by length in 1997 and 1998.

Table 2.1. Number of alleles, the size range in base pairs, percent heterozygosity, the probability of departure from Hardy-Weinberg equilibrium, F_{IS} values, and annealing temperature for the loci used in parentage analysis from pink salmon spawning in 1997 and 1998.

Year	Locus	# Allele	Size range	% He	P HWE	F_{IS}	Annealing Temp.
1997	Ots101	93	220-500	84	<0.01	0.145	50
1998		91		85	0.06	0.13	
1997	Ogo1a	60	190-550	93	<0.01	0.047	59
1998		62		95	0.07	0.25	
1997	Ogo4	54	169-320	85	<0.01	0.127	59
1998		67		89	<0.01	0.09	
1997	Omy1001UW	99	210-470	96	0.02	0.025	53
1998		95		96	0.15	0.02	
1997	Omy1212	64	229-475	95	<0.01	0.019	60
1998		59		97	0.05	0.002	
1997	Oki23	42	152-340	94	0.03	0.024	54
1998		45		96	0.05	0.006	

Table 2.2. Mean and standard error of instream longevity (days in freshwater), dominance score (average over lifespan), and day of entry (day of the spawning run) of male pink salmon that produced zero, one, or more than one offspring. Brackets show significant differences ($p < 0.05$) based on Bonfferoni post hoc comparisons.

	Longevity	Dominance	Entry Day
Offspring#	Mean (SE)	Mean (SE)	Mean (SE)
0	7.56 (0.22)	2.45 (0.04)	18.12 (0.23)
1	8.63 (0.40)	2.5 (0.07)	17.98 (0.44)
>1	8.86 (0.52)	2.79 (0.07)	16.27 (0.73)

Chapter3: Size-assortative mating in salmonids: negative evidence for pink salmon in natural conditions.

Introduction

The mating systems of many animals, including fishes, are strongly affected by male and female body size. Larger males often have a competitive advantage over smaller males and thus have a greater ability to select females in mating systems dominated by male competition (Kodric-Brown 1990). Larger females produce more numerous and larger offspring, and may acquire higher quality breeding or nesting sites. These female traits would be expected to result in higher offspring survival, hence large males should both choose large females when possible (e.g., Grant et al. 1995) and be able to dominate access to them (Morris et al. 1992). Females are more receptive to large males (e.g. Schroder 1981), perhaps because size is often heritable and females that mate with larger males may have larger offspring. Females mating with large males may gain direct advantages in quality of the male's territory or parental care (e.g., Downhower & Brown 1980), or indirect benefits in his successful genotype (Kirkpatrick 1996).

In some cases, competition and choice combine to produce size-assortative mating (the tendency for individuals of similar relative sizes to mate together; e.g., lampreys: Malmqvist 1983), and this can be an important aspect of the evolutionary and behavioural ecology of the species. Size-assortative mating has been studied closely in salmonid fishes, and it has been reported in natural populations of Japanese charr, *Salvelinus leucomaenis*, (Maekawa et al. 1994), sockeye salmon, *Oncorhynchus nerka* (Hanson & Smith 1967), and coho salmon, *O. kisutch* (Chebanov 1990), and in experimental arenas in sockeye (Foote 1988, 1989), chum, *O. keta* (Chebanov 1979) and pink salmon *O. gorbuscha* (Chebanov 1980, 1989). Such non-random mating not only affects patterns of reproductive success within populations but may also contribute to isolation between anadromous and non-anadromous forms because they differ greatly in body size (e.g., Taylor et al. 1996). However, assortative mating is not always observed (Schroder 1981; Quinn and Foote 1994), and many reported examples of size-assortative mating involved a large dichotomy in size classes or were conducted in artificial areas where other factors influencing mate choice and competition were controlled (e.g., Foote 1988, 1989; Maekawa et al. 1994). Under natural conditions, choice and competition may be influenced by the ripeness of available females, vigor of the males, threat of predation, and other factors that might reduce the importance of size.

In addition to the diverse factors that influence mate pairings under natural conditions, the study of size assortative mating in salmonids is complicated by methodological issues. Male dominance is often assessed by the number observations or length of time that the male is in closest proximity to a ripe female (e.g., Gross 1985; Quinn and Foote 1994; Dickerson et al. 2002). However, one of the main mechanisms of female choice is to delay spawning when courted by a small or otherwise undesirable male (Foote 1989; Berejikian et al. 2000; de Gaudemar et al. 2000). Thus one might question whether a male seen in an apparently dominant position with a female is favored by her or is actually being subjected to this delaying tactic and so more likely to be observed with her.

This study tested the hypothesis that mating is size-assortative with three years of observations in a naturally reproducing population of pink salmon, a species with normal rather than bimodal distributions of male and female body sizes. The reproductive behaviour of pink salmon is similar to that of other salmon species (Quinn 1999) but they do not display the alternative male life history patterns based on age at maturity or a mixture of anadromous and non-anadromous fish seen in many other salmonid species. Despite the uniform age distribution and absence of non-anadromous individuals, pink salmon show great variation in male size (Beacham and Murray 1985) and therefore the scope for potential size-assortative mating. We also determined the extent to which apparent dominance based on observations corresponded to reproductive success, assessed by parentage analysis of the offspring produced by the matings in the first two years of the study.

Methods

The study was conducted on adult pink salmon in the summers of 1997 – 2000 at a small, unnamed stream (named Himmel Creek for the purposes of this study) on Chichagof Island in Port Frederick, southeastern Alaska. A logjam limited pink salmon to the lower 330 m of the creek in 1997 and 1998 but the fish were able to use 800 m of creek in subsequent years. The creek varied from 3.7 – 11.8 m wide, depending on location and discharge. Pink salmon densities were high in the creek; peak daily counts of live fish were 797 in 1997, 367 in 1998, and 792 in 1999. Within the reach of stream used by the fish, local densities varied depending on habitat type. Chum salmon (*O. keta*) co-occur in this creek during the early part of the pink salmon run. Bear predation is a threat to the fish throughout the run; between five and 12 brown bears (*Ursus arctos*) were seen feeding on salmon in the creek.

A beach seine, dip nets, and stick seines were used to capture as many of the salmon in the creek as possible. They were anesthetized in MS-222 and we recorded the body length (mid-eye to hypural plate) and sex of each fish. All fish were tagged with white, lettered, plastic disk tags, allowing us to identify and observe individuals from the bank without disturbing them. The behavior of tagged fish was observed in two approximately 100 m long sections of the creek in 1997 and in the entire reach in 1998 and 1999. We categorized each male's courtship status as "3" if the male was dominant (closest to the female in the hierarchy or the sole male courting a ripe female), "2" if he was in a subdominant position or "1" if he was not involved in courting. The identity of the female being courted was recorded.

As part of a larger study of reproductive success, fin clips were taken from all fish when tagged from 1997 – 2000 for use in an analysis of parentage. DNA was extracted from these clips using ammonium acetate. PCR was used to amplify 6 microsatellite loci (Ogo1a, Ogo4, Ots101, Omy1001UW, Omy 1212, and Oki23) and the resulting product was visualized using the MegaBACE 1000 capillary electrophoresis system (Amersham Pharmacia Biotech Limited, Piscataway, NJ). Using a combination of CERVUS, exclusion software (Marshal et al. 1998) and PAPA, maximum likelihood software (Duchesne et al. 2002) the adult offspring (fish from 1999 and 2000) were matched with their parents (fish from 1997 and 1998). All pink salmon mature at two years of age, so sampling in 1999 and 2000 was sufficient to obtain all adult offspring produced in 1997 and 1998, respectively.

Possible size-assortative mating was examined from both female and male perspectives, because salmonid mating systems involve male competition and female choice. Univariate linear regressions were used to investigate the following relationships (SPSS v7.5). From the female perspective we looked for a relationship between the body size of a given female and (1) the average body size of the males in the dominant position with her over all observations, (2) the average body size of all of the males courting her, regardless of their status, and (3) the average body size of all males with whom she produced identified offspring. From the male perspective we looked for a relationship between the body size of a given male and (1) the average length of all females he courted, (2) the average size of the females he was in the dominant position with, and (3) the average body size of all females with whom he produced identified offspring. Each of the three years was analyzed separately because fish from different years were physically separated and the average body size of both females and males differed among years. An ANOVA, blocked by year to remove year-to-year variation was used to investigate the relationship between the males average dominance score and the number of identified offspring produced. Offspring production was categorized as zero, one, and more than one offspring identified.

Results

There was no relationship between the average length of males courting a given female and her length in any year (1997: $F_{1,44} = 0.557$, $P = 0.50$, $r^2 = -0.01$, 1998: $F_{1,25} = 0.173$, $P = 0.68$, $r^2 = -0.042$, or 1999: $F_{1,71} = 0.395$, $P = 0.53$, $r^2 = -0.009$), nor was female length related to the length of the primary male courting her (1997: $F_{1,38} = 2.086$, $P =$

0.16, $r^2 = 0.028$, 1998: $F_{1,25} = 0.713$, $P = 0.41$, $r^2 = -0.03$, or 1999: $F_{1,59} = 1.267$, $P = 0.27$, $r^2 = 0.005$; Figure 3.1). There was also no relationship between the length of a female and the average length of the males that sired her offspring (1997: $F_{1,30} = 2.209$, $P = 0.15$, $r^2 = 0.039$, 1998: $F_{1,32} = 0.022$, $P = 0.88$, $r^2 = 0.032$; Figure 3.2).

The length of a given male was unrelated to the average length of females he courted in any year (1997: $F_{1,38} = 0.85$, $P = 0.36$, $r^2 = -0.004$, 1998: $F_{1,30} = 0.005$, $P = 0.94$, $r^2 = 0.034$, or 1999: $F_{1,121} = 0.284$, $P = 0.60$, $r^2 = -0.006$; Figure 3.3), nor was it related to the average length of the females with whom he was dominant (1997: $F_{1,30} = 0.361$, $P = 0.55$, $r^2 = -0.012$, 1998: $F_{1,22} = 1.427$, $P = 0.25$, $r^2 = 0.019$, or 1999: $F_{1,67} = 0.524$, $P = 0.47$, $r^2 = -0.007$). There was also no relationship between the length of a male and the average length of the females with whom he produced adult offspring (1997: $F_{1,31} = 1.69$, $P = 0.20$, $r^2 = 0.022$, 1998: $F_{1,35} = 0.016$, $P = 0.90$, $r^2 = 0.029$; Figure 3.4). Not only were there no linear relationships but there were no significant parabolic relationships in any of the above comparisons for either sex in any year (NS in all cases).

To assess the validity of our observational data on dominance we compared our measure of dominance with the number of offspring produced. Males that produced two or more detected adult offspring had the highest average dominance scores, followed by males that produced one offspring, and males that produced no detected adult offspring had the lowest average scores ($F = 5.223_{1,179}$, $P = 0.023$).

Discussion

Within this population, larger males were more often dominant and thus were better able to select a female to court, and larger females produced larger and more numerous eggs than smaller females (Dickerson et al. 2002). Thus, large males should choose large females when possible. Large females should be more receptive to large males because size is heritable and females that mate with larger males may have larger offspring (Beacham and Murray 1988; Smoker et al. 1994). Despite this logic, there was no evidence of size assortative mating for either sex, in any year, with any index of mating. This negative result is consistent with the field study by Quinn and Foote (1994) but inconsistent with other fieldwork (Hanson and Smith 1967) and several studies in experimental arenas (e.g., Foote 1988).

In any study reporting negative evidence, one must consider the power of the study to falsify the null hypothesis of no effect. Given the levels of variation observed (power ranged from 0.05 – 0.29), only very large sample sizes (~700,000 - 560 million) could have detected significant effects. There was also no evidence of parabolic relationships (large males courting large and small females rather than females of intermediate sizes) as was seen in pink salmon in experimental arenas by Chebanov (1980). Thus the parsimonious conclusion is that the hypothesized patterns simply were not present, rather than that we were unable to detect them.

Pairings may not be determined by body size or choice as much as arrival timing, ripeness of available females, and availability of males. Schroder (1981) found that male chum salmon chose females based on nest-digging activity indicating proximity of the spawning event rather than size. Thus males may try to minimize the time delay between spawnings (hence maximize number of possible mating events) rather than maximize the number of potential eggs fertilized (i.e., by female size) in any given event.

Schroder (1981) also found that females preferred males of their own size or larger but choice was expressed by delaying nest construction when courted by small males. An observer might mistakenly categorize the inferior male as dominant on several occasions when in fact the female was delaying the release of her eggs (presumably to maximize the chances that a higher quality male would arrive). In this case, apparent dominance and realized reproductive success might be uncorrelated or even inversely related if females bred so quickly with desirable mates that they were seldom seen together. However, our parentage analysis revealed that males with high observer-based dominance scores indeed produced more offspring. We conclude that the males categorized as dominant with females were in fact dominant, and they participated in more numerous or more successful spawning events than those with lower scores. Most convincing, there was no size assortative tendency in the successful matings, as indicated by the lack of relationship between the size of males and females and the size of the mate with whom they produced offspring.

It is not our view that size-assortative mating does not exist. It clearly can operate and may be an important factor in both general breeding dynamics and separation between large, anadromous and smaller, nonanadromous forms of salmonids. However, the strength of the phenomenon in controlled environments should not mislead us into forgetting the many factors (e.g., female ripeness and male availability, predation threat, changing environmental conditions) that may minimize its importance (or mask its occurrence) in natural situations.

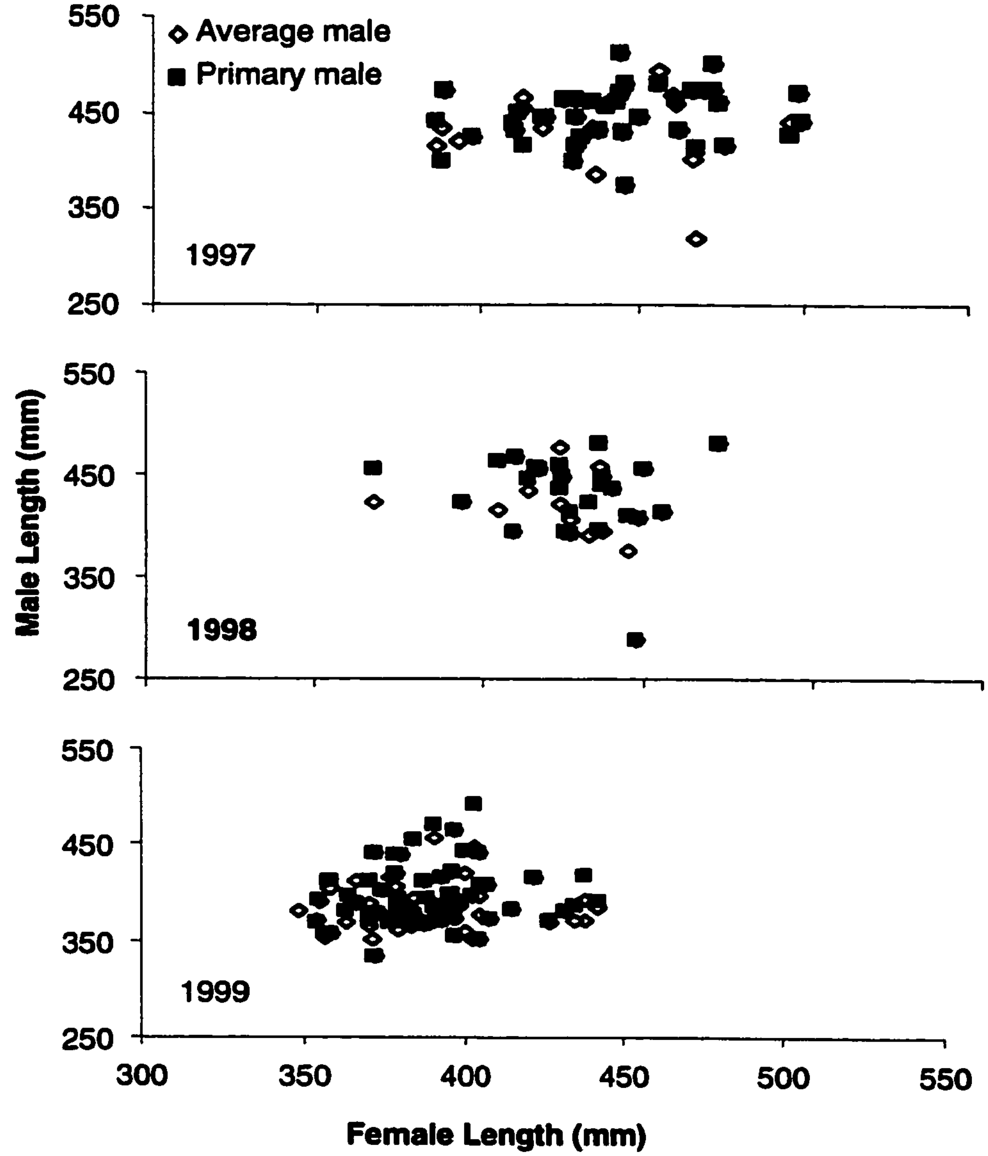


Figure 3.1. Relationship between the body length (mm) of female pink salmon and both the average body size of the male pink salmon courting her, and the average body size of the primary male pink salmon (male closest to her in the courtship hierarchy) observed courting her. Each graph represents the results from one year from 1997-1999.

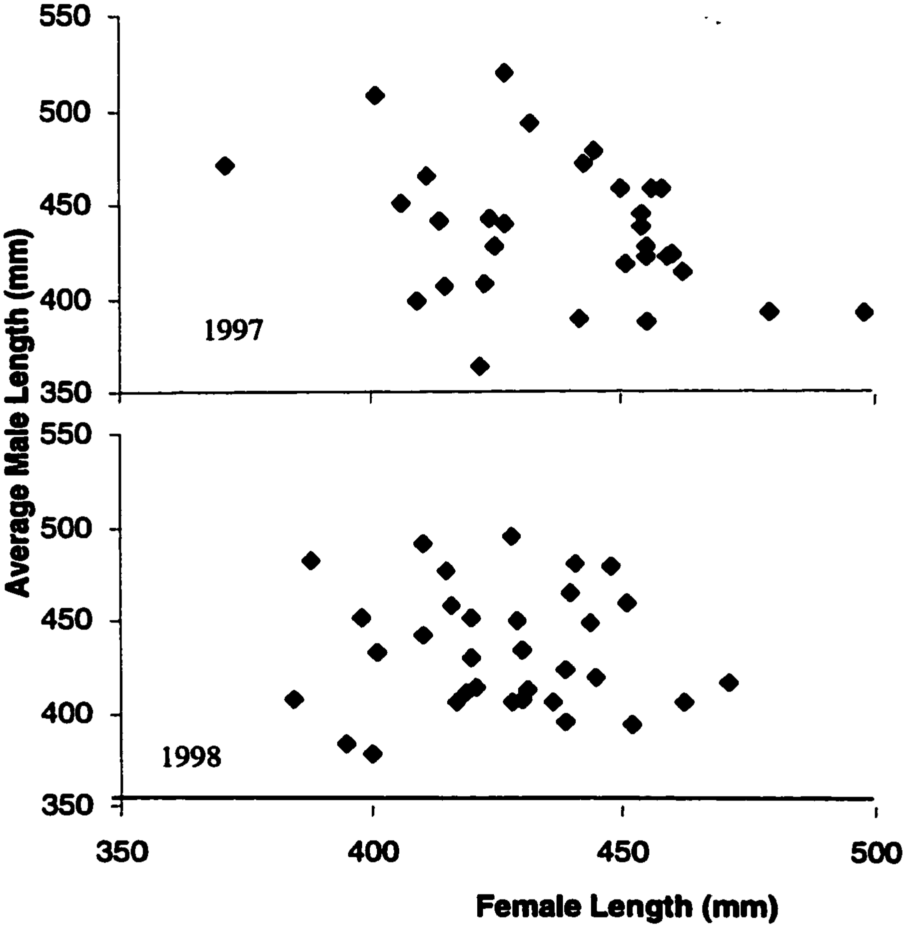


Figure 3.2. Relationship between body length of female pink salmon and the average length of the male pink salmon with whom she produced adult offspring (brood year 1997 and 1998).

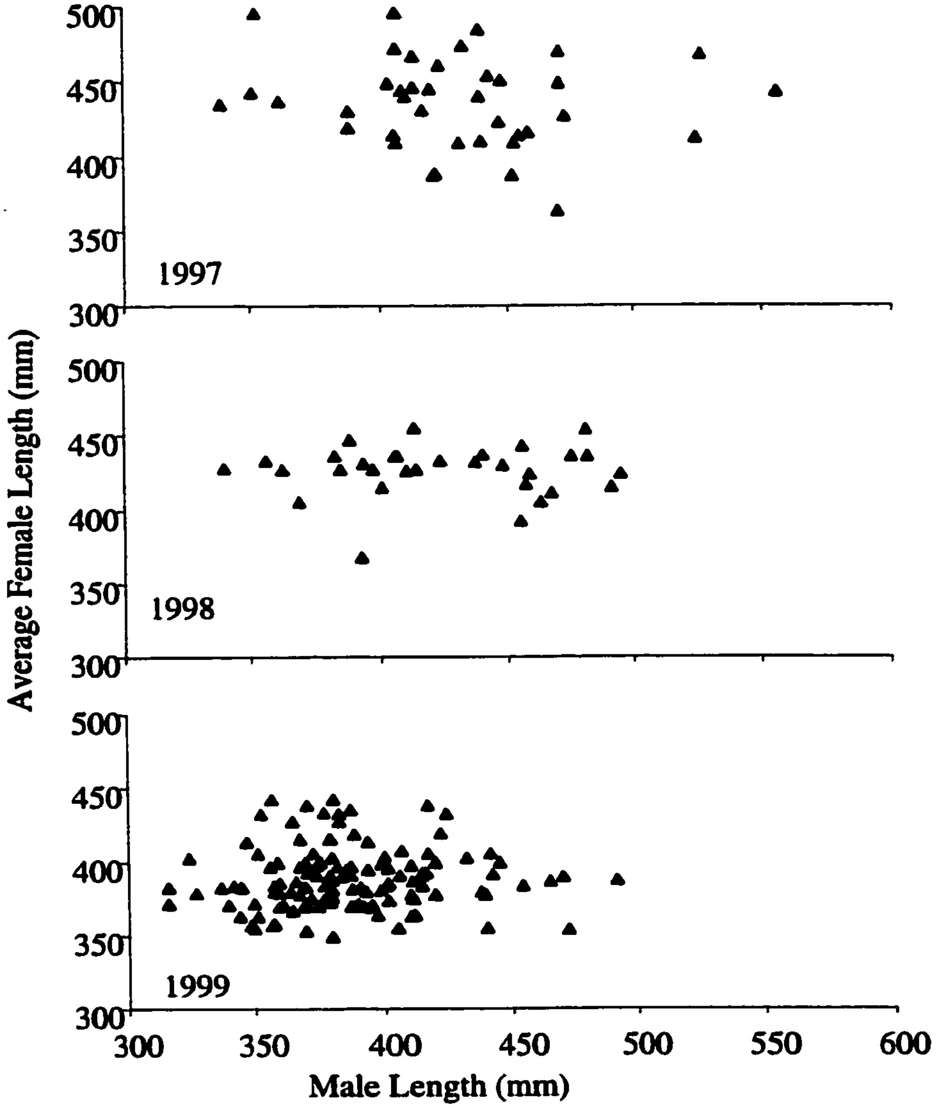


Figure 3.3. Relationship between body length of male pink salmon and the average length of the female pink salmon he was observed courting. Each graph represents the results from one year from 1997-1999.

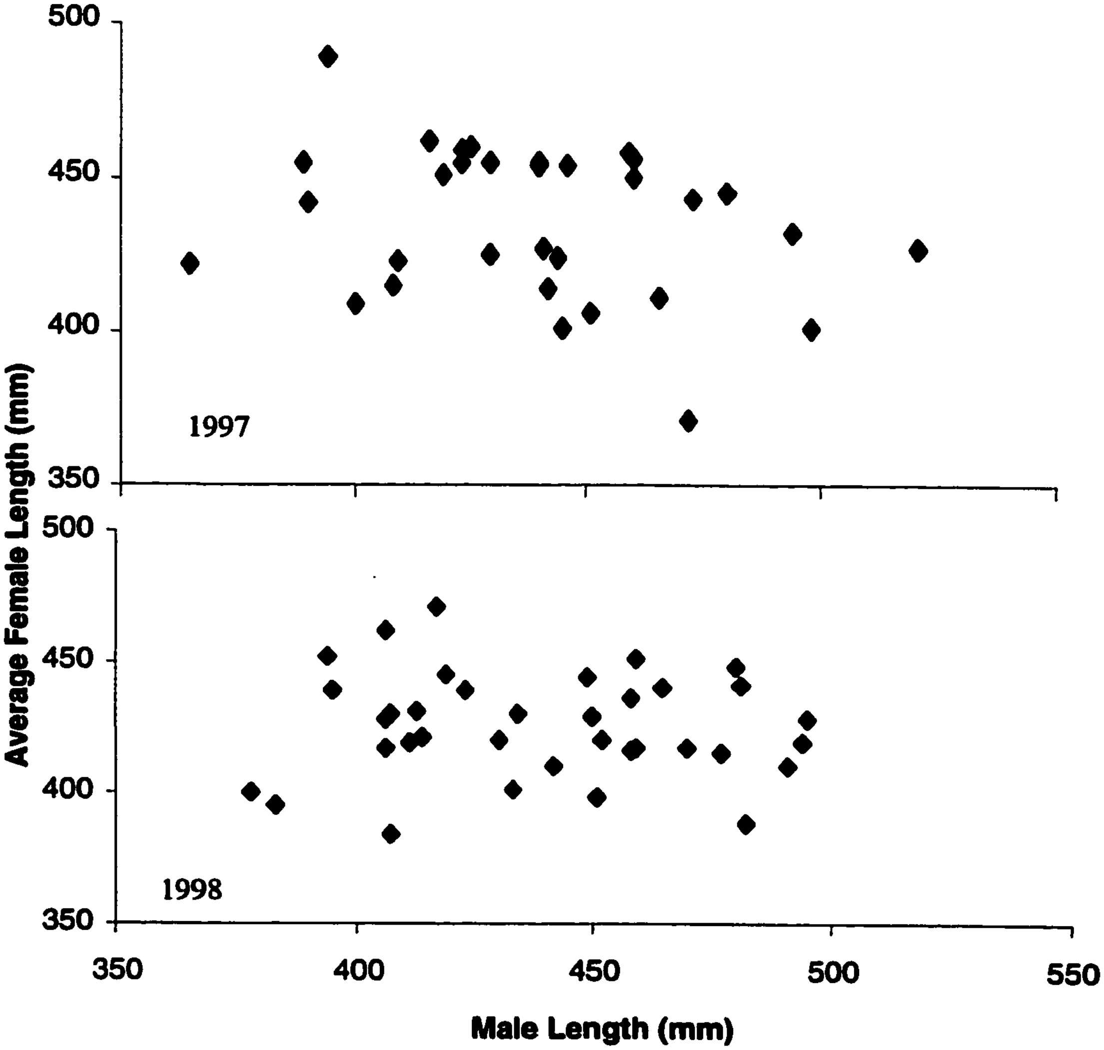


Figure 3.4. Relationship between body length of male pink salmon and the average length of the female pink salmon with whom he produced adult offspring.

Chapter 4: Use of DNA microsatellite parentage analysis to assess the heritability of life history and morphological traits in a wild population of pink salmon, *Oncorhynchus gorbuscha*.

Introduction

The theory behind evolutionary biology relies heavily on the assumption that variation within most morphological, life history and behavioral traits has some genetic basis, allowing species to evolve in changing environments. The genotype contributes to the overall phenotypic variation, acting in combination with diverse environmental factors. The level of heritability among traits reflects the balance between genetic and environmental controls, and so indicates the extent to which selection affects the population. Therefore, estimates of heritability provide us with valuable information as to the current selective pressures acting on a population, the potential responses of a species to ecological changes, and also clues to past selective pressures (Prout and Barker 1989).

Many studies have examined the heritability of morphological and life history traits of salmonid fishes, in the genera *Oncorhynchus, Salmo and Salvelinus*, in part because these species are important in aquaculture. Consequently, most of these investigations were conducted on fish that were spawned or raised in captivity (e.g. rainbow trout, *O. mykiss*: Gall and Gross 1978; Gall and Huang 1988; Siitonen and Gall 1989; Su et al 1997, coho, *O. kisutch*: Mousseau et al 1998, chinook, *O. tshawytscha*: Quinn et al 2000; Kinnison et al 2001, pink salmon, *O. gorbuscha*: Beacham and Murray

1988; Smoker et al 1994, 1998, 2000, arctic char, *Salvelinus alpinus*: Nilsson 1994; De March 1995, Atlantic salmon, *Salmo salar*: Refstie et al. 1978). These studies have been valuable in demonstrating the genetic control over various traits, and they have the advantage of large sample size and statistically balanced design (i.e., half-sibs with many replicates within families), providing more power to detect genetic effects. However, the methods often eliminate the selective forces of mate choice and competition among adults, and they also control many of the environmental factors such as food availability and temperature acting on traits like growth, egg production and maturation. Unfortunately, the differences in selective pressures between laboratory and natural condition can result in biased estimates of heritabilities (Service and Rose 1985; Coyne and Beecham 1987: Prout and Barker 1989) and the controlled environmental conditions can inflate estimates of heritability (Coyne and Beecham 1987; Price and Schulter 1991).

In general, the heritabilities of traits related to fitness tend to be low (Price and Schluter 1991). If directional selection is acting on a trait over a long period of time the genetic variance of that trait can be driven down, possible even to zero (Fisher 1958). However, the complex interactions and trade-offs between and within morphological and life history traits can keep genetic variance of fitness-related traits high even at equilibrium (Price and Schulter 1991). Body size is theorized to have many important influences on the fitness of salmonids (reviewed by Fleming 1998) and is heritable in salmonids based on work with captively reared fish (Beacham and Murray 1988) and in those spawned and incubated in captivity but released to rear at sea (Smoker 1994, 2000). The timing of arrival on the breeding grounds may actually have greater influence on fitness than size because it has strong effects on reproductive opportunity and success (Chapters 1, 2). Arrival timing and related indices (migration, spawning, and maturation timing) have been show to have the highest heritabilities among traits measured (Siitonen and Gall 1989; Su et al 1997; Quinn et al 2000).

It is important to obtain estimates of the relative level of genetic control over life history traits in wild populations, subject to the natural variation in conditions that the environment presents to the fish. Recent advancements in molecular biology such as DNA microsatellites allow us to determine parentage from small non-destructive samples of fin. Parentage analysis based on such samples allows us to investigate relationships between parents and offspring in populations living under natural conditions throughout their entire life cycle. The objective of this study was to examine relationship in body size and shape, longevity, egg size, and arrival timing between parents and offspring from wild spawning and rearing pink salmon and to calculate heritabilities based on these relationships.

Methods

Field Sampling

This study was carried out on a natural population of pink salmon in a small, unnamed stream located on Chichagof Island in Port Frederick, Southeastern Alaska. The length of the creek accessible to anadromous fish ranged from 330 – 800 m and width from 3.7 – 11.8 m (Chapter 2). We sampled adult salmon during the summers of 1997-2000 and so, because all pink salmon are two years old at maturity (Heard 1991), the fish sampled in 1999 were the offspring of the 1997 fish, and those sampled in 2000 were the offspring of the 1998 fish (Chapter 2).

Upon capture, fish were individually tagged, identified to sex, measured for body length from mideye – hypural plate (a measurement unbiased by the greater development of jaws in males), depth of the dorsal hump (from the lateral line to highest point on the hump), snout length (tip of the upper jaw to mid-eye), and a small fin clip for DNA analysis was taken and preserved in 95% ethanol. We estimated freshwater age (number of days spent in freshwater) for all fish not captured on their day of entry into the spawning grounds based on criteria validated by repeated observations of tagged fish (Chapter 2). This information was used to estimate both arrival date and longevity (instream life). To estimate each female's average egg size, a small (10-15 g) sample of eggs was taken from ripe females and weighed and counted. Over all four years 2348 males and 2139 females were captured (1997: 696 m, 558 f; 1998: 604 m, 489 f; 1999: 728 m, 652 f; 2000: 320 m, 440 f). These fish constituted about 75% of the populations among all four years.

Molecular Methods

DNA was extracted from the fin clips using ammonium acetate. PCR was used to amplify 6 microsatellite loci (Ogo1a, Ogo4, Ots101, Omy1001UW, Omy 1212, and Oki23) and the resulting product was visualized using the MegaBACE 1000 capillary electrophoresis system (Amersham Pharmacia Biotech Limited, Piscataway, NJ; Chapter 2). Using a combination of CERVUS, to perform an exclusion analysis (Marshal et al.

1998) and PAPA, to perform a maximum likelihood analysis to account for mutation and genotyping error (Duchesne et al. 2002) the adult offspring (fish from 1999 and 2000) were matched with their parents (fish from 1997 and 1998). Resulting in 723 (34%) adult offspring assigned to at least one parent, giving us 297 male parents, and 271 female parents with identified offspring and 77 adult offspring assigned to both parents giving us 72 pairs of parents.

Analysis

Because of there were few offspring for whom we could identify both parents we did not use mid-parent values, all analyses were conducted for single parents to offspring. Linear regressions were used to examine the relationship between body length, body shape, longevity, and arrival timing of both dams and sires and these traits in their male and female offspring, and between egg size of dams and their daughters (SPSS v 7.0). Year was incorporated into all models to correct for year-to-year variation. Heritabilities were calculated as 2*slope of the regression line.

Results

We did not detect a significant effect (at $\alpha = 0.05$) of dam body length, snout length, hump depth, or longevity on those traits in either her female or male offspring. There was also no significant influence of dam egg size or day of entry onto the spawning grounds on those traits in her female offspring. However, there was a significant effect of dam's day of entry on the day of entry of her male offspring ($F = 7.535$, $df = 102$, $P = 0.001$, $r^2 = 0.131$; Figure 4.1). The resulting estimated heritability was 0.72 (SE = 0.08).

There was also a significant sire effect on male offspring's day of entry (F = 6.777, df = 120, P = 0.001, $r^2 = 0.103$; Figure 4.1) with a slightly lower estimate of heritability (0.58 (SE = 0.08)). Sire hump depth, snout length, and longevity did not significantly influence those traits in either his male or female offspring ($P > 0.05$ in all cases). Sire body length did not significantly affect length in his male offspring but it had a positive influence on the length of his female offspring (F = 5.953, df = 186, P = 0.029, r^2 = 0.061; Figure 4.2). The estimated heritability was 0.48 (SE = 0.11).

Discussion

As was found in previous investigations of salmonids, arrival timing had the highest heritabilities of the traits examined in this study (e.g. Siitonen and Gall 1989; Su et al 1997; Quinn et al 2000). We did not detect heritabilities for egg size or longevity in this population although there was sufficient variability in these traits to detect it (Table 1.1). It is possible that this population is at equilibrium and so has very low heritabilities for life history traits (Fisher 1958). However, Price and Schulter (1991) found that it is very difficult to predict this, as it is possible to see low heritabilities in populations out of equilibrium and vice versa. These estimates of heritability are probably lower than we would have found if our family groups had been large enough to investigate half-sib relationships (Smoker et al 1994).

In addition the issue of whether or not there is an underlying heritability for the traits, there is also the issue of environmental effects. One would expect that traits related to growth such as length, shape (related to weight at length; Quinn and Foote 1994), egg size and in-stream longevity would have a low estimated heritability in natural situations because the environmental effects would be so large. Not only are the environmental effects large but, they are probably quite random with respect to genotype. For example, fish with a inherently high metabolic rates and growth potential would not necessarily find the most dense aggregations of food on the open ocean, and so might not express their full potential. The effect of environmental conditions of migration timing in salmon is smaller than in many other kinds of fishes (Quinn and Adams 1996) and the environment is likely to affect the entire population but not affect the relative expression of the trait. That is, if water temperatures cause the migration to be early as a whole, the fish tending to be late may still be late, relative to the rest of the population.

Consistent with this explanation, we estimated significant heritabilities for arrival timing for males (dam 0.72 and sire 0.58). These values were on the high end of the range of estimated heritabilities of timing found by other studies in salmonids (0.18-1.26; Siitonen and Gall 1989; Su et al 1997; Smoker et al 1998; Quinn et al 2000). Our estimates may be lower than would have been seen in captive breed or reared fish because heritabilities calculated from captive organisms are often higher than those calculated from organisms in their natural environment (Coyne and Beecham 1987; Prout and Baker 1989). However, our estimates were higher than the 0.18 value calculated for male pink salmon from fish breed in captivity but released as fry and to rear at sea (Smoker et al. 1998). Smoker et al. (1998) spawned their pink salmon on only two days (one early and one late) and it is possible that if they had spawned fish across a range of dates their estimates would have been higher. Unlike previous work (Smoker et al. 1998; Quinn et al. 2000) we did not see a relationship between dam arrival timing and female offspring. Males in this population appear to be undergoing directional selection for timing whereas females appear to be undergoing stabilizing selection (Chapter 2). It is possible that the differences in selective forces are driving the differences in estimated heritabilities.

We saw a significant sire effect on female length, but there was no perceivable maternal effect and neither a sire nor maternal effect on male length. Our estimate of heritabilities for females was higher than found in previous studies (0.3 captive fish and 0.2 released as fry) whereas heritabilities of length in male pink salmon have been quite high in previous studies of captive pink salmon (Beacham and Murray 1988; Smoker et al. 1994). When compared to pink salmon released as fry, the heritabilities of male length were much lower (0.8 captive and 0.4 released; Smoker et al. 1994). In this population, male size affected dominance on the spawning grounds and dominance had a significant effect on male reproductive success whereas there no direct link was seen between body length and reproductive success in females (Chapter1, 2). Genetic variation in body size in males could be lower due to the close association with fitness resulting in very low heritabilities (Fisher 1958).

In summary, there is a great deal of variability in estimated heritabilities both between populations as well as within across different rearing regimes, and through time as selective forces change. Examining these relationships in wild populations provides valuable information aiding us in understanding evolutionary biology. Our study is the first report of heritability estimates for entire generations of salmon spawning and rearing under fully natural conditions. Studies such as ours that sample adult offspring will inevitably suffer from a small sample size and low statistical power. On average there will be only one offspring per parent even if all can be identified, and many parents will have no detected offspring. However, these natural studies are an important counterpart to more controlled studies, and in this case the relative levels of genetic control over traits were consistent with those from controlled studies.

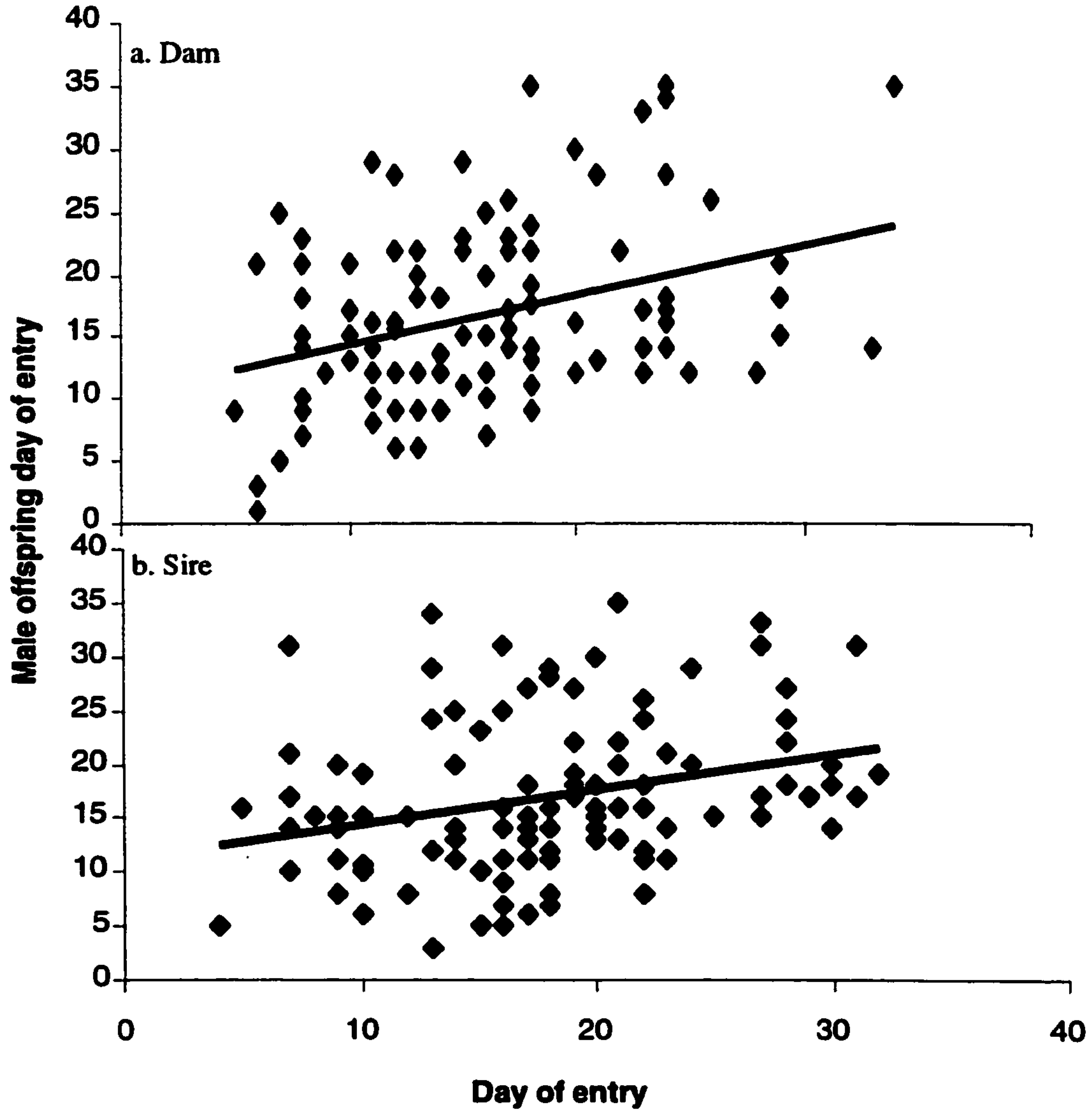


Figure 4.1. Relationship between day of entry (relative to the first day of the run) of dam and sire on day of entry of adult male offspring in pink salmon from the 1997 and 1998 brood years.

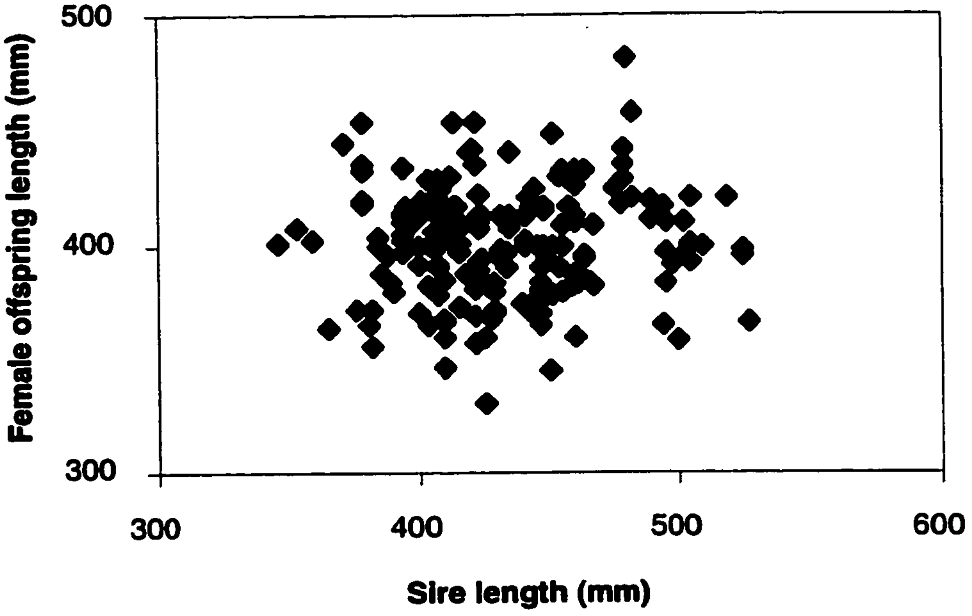


Figure 4.2. Relationship between sire length and the average length of his adult female offspring in pink salmon from the 1997 and 1998 brood years.

Chapter 5: Conclusions

When we examined the relationship between the traits thought to influence reproductive success one consistent finding was the importance of bears. Bears killed a significant proportion of the population and decreased the life span of both males and females. Although, among females the majority of this predation occurred after egg deposition thus, primarily influencing nest defense. Among males predation may have a greater impact. Males killed by bears had on average 25% fewer days to court females and engage in spawning than those that experienced senescent deaths. There was no relationship between body size and predation in either gender.

Body size was found to have an important influence on the potential reproductive success of both females and males, with larger females producing more and larger eggs and larger males having greater access to females. However, the timing of arrival on the breeding grounds in some cases overcame the positive influence of body size on reproductive success. Early-arriving females lived longer than later arrivals, and early arriving males were more dominant than later arrivals even when the later arrivals were larger. Timing may co-vary with body size and needs to be more carefully examined in salmon and other animals with similar breeding systems.

When we investigated the influence of these traits on realized reproductive success we found that body size not was a significant predictor of offspring production in males. Larger males had higher dominance scores and males with higher dominance scores produced more fish, but the effects of arrival timing appear to counter-balance the effect of these traits. As the season progresses, the influence of size on dominance decreases as the number of ripe males to ripe females increases and larger males are no longer able to defend access to ripe females. Longer lived males produced more offspring (presumably more time to participate in spawning events) yet, surprisingly, bear predation did not influence offspring production.

We did not see a significant relationship between reproductive success and egg size and fecundity. We would probably have seen a relationship if we had sampled at hatching or emergence. Among females, in 1998, it appears that there was a mid-range size that was optimal (figure 2.3). The smaller fish are probably hampered by having fewer and smaller eggs, and are at greater risk of redd superimposition (Steen and Quinn 1999). Larger females may have to contend with higher predation rates, although there was no relationship between size and predation in this population.

We did not see a relationship between offspring production and longevity in females. The primary concern of females after egg deposition is nest defense and since the run timing exceeds life span by 2/3 or more no one female could protect her eggs throughout the entire run, thus the duration of nest guarding may not have a large impact on reproductive success.

As with males, entry date exhibited the strongest influence on reproductive success. Females with mid run entry timing in 1998 produced the most offspring. A mid-range entry could benefit a female by reducing risk of redd superimposition compared to earlier arrivals (van den Berghe and Gross 1989) and access to a greater choice of males than later arrivals. Early emerging fry may be vulnerable to predators (e.g., Brännäs 1995) and heavy, density-dependent predation on pink and chum salmon fry was reported in Hooknose Creek, B.C. by Hunter (1959). Both early and late fry may miss the optimal window of food availability, and the date of seaward migration can strongly affect survival in pink salmon (Taylor 1980).

Directional selection cannot be maintained as a population will be driven by this selection to an optimum. Therefore, it is not surprising that entry timing showed the greatest influence. It is possible that the optimal entry timing shifts due to environmental factors (predation pressure, temperature, food availability, etc.) and thus is not as easily driven to an optimum as traits such as egg size may be.

There was no evidence of size assortative mating for either sex, in any year, with any index of mating. We do not conclude from this that size-assortative mating does not exist. It clearly can operate and may be an important factor in both general breeding dynamics and separation between large, anadromous and smaller, nonanadromous forms of salmonids. However, the strength of the phenomenon in controlled environments should not mislead us into forgetting the many factors (e.g., female ripeness and male availability, predation threat, changing environmental conditions) that may minimize its importance (or mask its occurrence) in natural situations.

Most of the traits examined did not show detectable heritabilities but, as was found in previous investigations of salmonids, arrival timing had the highest heritabilities of the traits examined in this study (e.g. Siitonen and Gall 1989; Su et al 1997; Quinn et al 2000). It is possible that this population is at equilibrium and so has very low heritabilities for life history traits (Fisher 1958). Our estimates of heritabilities for arrival timing for males (dam 0.72 and sire 0.58) were on the high end of the range of estimated heritabilities of timing found by other studies in salmonids (0.18-1.26; Siitonen and Gall 1989; Su et al 1997; Smoker et al 1998; Quinn et al 2000). Our estimates may be lower than would have been seen in captive breed or reared fish because heritabilities calculated from captive organisms are often higher than those calculated from organisms in their natural environment (Coyne and Beecham 1987; Prout and Baker 1989). These natural studies are an important counterpart to more controlled studies, and in this case the relative levels of genetic control over traits were consistent with those from controlled studies.

Overall, we found entry timing to be the most influential factor affecting variation in reproductive success among individuals. Studies such as this are vital to understanding the nature and force of selection on nature populations and thus furthering our ability to restore and preserve this important natural resource.

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