AN ABSTRACT OF THE THESIS OF

Haley Ohms for the degree of Master of Science in Fisheries Science presented on December 10, 2012

Title: The Influence of Sex, Migration Distance, and Latitude on Expression of Anadromy in *Oncorhynchus mykiss*

Abstract approved:

Gordon H. Reeves

    The evolution and maintenance of multiple phenotypes within populations is an interesting evolutionary and ecological question, and is becoming increasingly important for the restoration and conservation of partially migratory species. In particular, why individuals adopt a particular phenotype has been the focus of numerous research efforts. In the partially migratory species *Oncorhynchys mykiss* (commonly known as steelhead and rainbow trout), I found that sex plays a strong role in whether an individual adopts an anadromous or resident life history. I observed a higher frequency of females adopting the anadromous tactic throughout the species range, and I attributed this to the relationship between body size and reproductive success in females that is not present in males. I did not observe differences in female bias along gradients of latitude or migration distance, and I hypothesize that while the frequency of tactics among populations may change along these gradients, either the change is equal between males and females, or an interaction of latitude and migration distance may control changes. I did not observe a corresponding male bias in the one resident population I sampled, and suggest that this is the result of a high frequency of residents in that population. I propose that sex ratios could be a valuable metric for measuring frequency of life-history tactics

in populations and could facilitate the investigation into the controls of life-history tactics across broad scales.

The Influence of Sex, Migration Distance, and Latitude on Expression of Anadromy in
Oncorhynchus mykiss

by
Haley Ohms

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APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Haley Ohms, Author

ACKNOWLEDGEMENTS

To my Mom, Jeanne Sponsel, for teaching me to stand up for myself, and for showing me how to persevere. You are far and away the strongest woman I know.

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CONTRIBUTION OF AUTHORS

Dr. Gordon Reeves was involved with study design and editing all sections of this manuscript. Dr. Chris Jordan was involved with study design and editing all sections of this manuscript. Dr. Jason Dunham was involved with editing all sections of this manuscript. Matt Sloat was involved with study design, data analysis, and editing all sections of this manuscript. Kelly Christiansen provided the map of sample populations in Chapter 2 and Kathryn Ronnenberg formatted all figures and tables in the manuscript.

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The Influence of Sex, Migration Distance, and Latitude on Expression of Anadromy in *Oncorhynchus mykiss*

CHAPTER 1 - INTRODUCTION

It is often assumed that natural selection will favor a single optimum phenotype, yet there are numerous examples where multiple distinct phenotypes (i.e., polyphenism) coexist within a single population (Smith and Skúlason 1996). A common example is partial migration, where a portion of the population is migratory or resident (Chapman et al. 2011). One framework for understanding how partial migration is maintained is the conditional strategy (Lundberg 1988, Gross 1996). Under the conditional strategy, individuals adopt either a migratory or residency tactic (i.e., life history), but these tactics do not compete with each other in an evolutionary sense because they belong to the same strategy. Tactics are adopted based on an individual's condition, leading to higher fitness for that individual (Gross 1996, Gross and Repka 1998). The fitness of each tactic varies with individual condition, and fitness is equal where the relative fitness of the two tactics cross over, or at the 'switch-point' (Figure 1a). Individuals with a state greater than the switch-point adopt the resident tactic, and individuals with a state less than the switch-point adopt the migratory tactic (Figure 1a).

The location of the switch-point is determined by environmental constraints on the fitness of either tactic. For example, as freshwater growth increases, fish are more likely to acquire the resources they need for reproduction as residents, which results in higher fitness of that tactic and adjusts the switch-point so that more fish adopt the resident tactic (Figure 1b). Similarly, as migration cost increases, relative fitness of migrants decreases, and the switch-point adjusts so a lower proportion of the population adopts the migrant tactic (Figure 1c). However, the influence of these environmental constraints depends on the mating system of a species, and typically differs for males and females.

As with many fishes, the mating system of salmonids is fundamentally derived from the difference between the energy devoted to gametes in males and females. Female gametes are more energetically costly to produce than male gametes, and as a result, female reproductive success is limited by fecundity, while male reproductive success is limited by access to mates (Fleming 1998). In female fishes, potential reproductive success is primarily limited by fecundity, which is closely linked to body size (Quinn et

al. 2011, Schill et al. 2010). In contrast, males can have high reproductive success either by adopting a "fighter" tactic, through which they attempt to secure matings by aggressively excluding other males, or by adopting a sub-dominant "sneaking" tactic, through which they sneak access to females during their active spawning with a dominant male (Hutchings and Myers 1988, Thériault et al. 2007). Consequently, males are less dependent on body size for reproductive success. In partially migratory species where the main benefit of migration is greater body size, females should have a much stronger and more consistent benefit from migration than males do, and as a result, the condition-dependent switch-point for females should generally favor increased frequency of the migratory tactic compared with males (Figure 1b).

To test whether frequency of migration is greater in females and how environmental constraints influence the switch-point differently in the sexes, I chose *Oncorhynchus mykiss* as a model species. *O. mykiss* is a partially migratory species that expresses a resident tactic, commonly known as rainbow trout, and an anadromous tactic, commonly known as steelhead. Adult steelhead obtain much greater body size than rainbow trout, but generally have lower survival to reproduction and older age at reproduction (Fleming and Reynolds 2004, Jonsson and Jonsson 1993). Both males and females exhibit considerable plasticity in tactics over a wide range of habitats, have high variation in size at maturity as a result of indeterminate growth (Schill et al. 2010, Quinn et al. 2011), and are the focus of many restoration efforts that would benefit from increased understanding of environmental constraints on tactic expression (Busby et al. 1996).

My first objective was to measure the sex ratios in age-0 juveniles, smolts, and residents in one population of *O. mykiss* to test the general predictions that age-0 fish have equal numbers of males and females (a 1:1 sex ratio), that smolts are female-biased, and that residents are male-biased. My second objective was to evaluate the influences of latitude and migration distance on sex ratios, using latitude as a proxy for freshwater growth potential and migration distance as a proxy for migration cost. I predicted that sex ratios at southern latitudes would have greater female bias, reflecting increased residency

among males at southern latitudes. I also predicted that sex ratios in populations with long migration distances would have greater female bias, reflecting increased residency among males.   To address these objectives, I quantified sex ratios of smolts across a broad geographic gradient within the species' range along the eastern Pacific Rim.
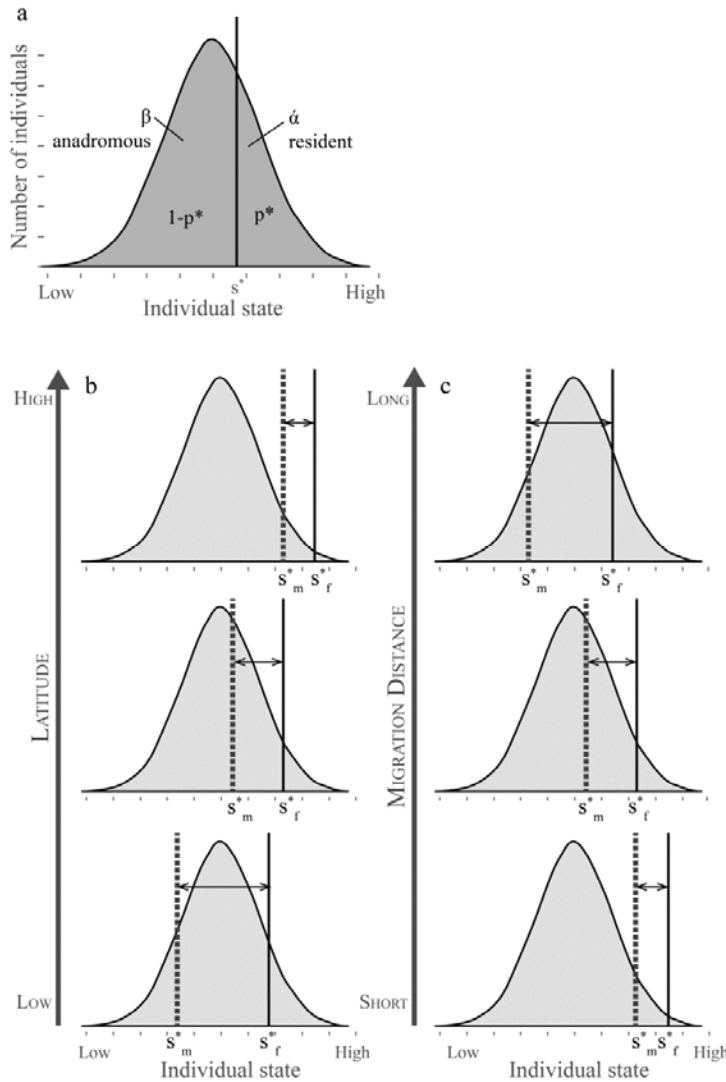
Figure 1. A population distribution of alternative tactics α and β (i.e., residency and anadromy) across a range of individual states, such as length or condition factor (a) (adapted from Gross and Repka 1998). The fitness of α and β is equal at the switch-point, s*. Differences in the relative benefits of anadromy between the sexes result in differences between s_m^*, the switch-point for males, and s_f^*, the switch-point for females in a population. s_m^* and s_f^* are differentially influenced by environmental conditions, resulting in skewed sex ratios at different environmental conditions (b, c). For example, at northern latitudes sex ratios will be only slightly female-biased, but will increase to highly female-biased in southern latitudes where residency among males is common (b). Similarly, at short migration distances sex ratios will be slightly female-biased, but will increase to highly female-biased at long migration distances where residency among males is common (c). Dotted line signifies males, solid line signifies females.

CHAPTER 2 - MATERIALS AND METHODS

Study Species

North American populations of partially migratory *O. mykiss* are distributed along the eastern Pacific Rim from southern California to south-central Alaska. Populations inhabit coastal stream systems in relatively close proximity to the ocean, as well as interior streams requiring migrations as long as 1500 km to reach the ocean (Busby et al. 1996). Prior to migration, anadromous individuals may spend between 1 and 5 years in freshwater. Freshwater residents reach maturity after 1 - 8 years (Schill et al. 2010). Steelhead and rainbow trout are sympatric and can produce offspring with a life history different from their own (i.e., steelhead parents can produce rainbow trout offspring and vice versa) (Zimmerman and Reeves 2000, Christie et al. 2011). The timing of spawning migrations for steelhead varies with both latitude and migration distance (Busby et al. 1996). Generally, interior populations migrate upstream during the summer and complete maturation prior to their winter – spring spawning period, while coastal populations enter freshwater as mature adults just before spawning. Relative to steelhead, little is known about freshwater-maturing forms of *O. mykiss* in partially migratory populations (Busby et al. 2006).

Given the potential confusion associated with the common names, and because anadromous and resident individuals are indistinguishable as juveniles, I will use the term juvenile to mean an individual with an undetermined life-history tactic, anadromous or smolt to indicate an individual that has adopted an anadromous migration, and freshwater resident to indicate an individual that matures in freshwater. In all cases, these names apply to individuals regardless of parental origin (i.e., either anadromous or resident parents).

Study Areas and Capture Methods

I used the *O. mykiss* population in the South Fork John Day (Oregon, USA) as a case study to measure the age-0, smolt, and resident sex ratios (Figure 2). The South Fork John Day has a waterfall at river kilometer 45 that acts as a barrier for upstream migration of anadromous adults and therefore makes the population above the barrier

effectively an allopatric resident population. To collect age-0 and resident *O. mykiss*, I selected 16 sampling locations in the South Fork John Day River using a generalized random tessellation stratified (GRTS) survey design (Stevens and Olsen 2004). I stratified sites by Strahler stream order (Strahler 1957) as well as their occurrence above or below the waterfall, which is a known migration barrier to anadromous adults. Sites above the waterfall were chosen to contrast sex ratios in an allopatric resident population with sex ratios in a partially migratory population below the waterfall. I assigned half of the sampling locations to above the falls and half below, and then divided sites among second-, third-, and fourth-order streams based on the relative proportion of these streams in the watershed. Based on this site selection, I sampled four sites in second-order streams, two sites in third-order streams, and two sites in fourth-order streams above the waterfall, with the same pattern below the waterfall (Table 1).

I attempted to collect at least three fish in each of five categories (<65 mm, 65-100 mm, 100-150 mm, 150-200 mm, >200 mm) (Table 1). I captured fish using a combination of electrofishing, seining, and hook-and-line sampling. Collected fish were euthanized with an overdose of MS-222, measured to fork length (mm), and scales and tissue were collected and stored on Whatman™ chromatography paper. Scales were removed in an area a few rows above the lateral line, between the anterior insertion of the anal fin and the posterior insertion of the dorsal fin (DeVries and Frie 1996). In addition, I removed gonads and extracted otoliths from euthanized fish to determine maturity status and age. Gonads were fixed in 10% buffered formalin, then transferred to vials with ethyl alcohol for storage. I visually assessed the maturity status of each fish, and did not collect gonads from fish that were clearly undeveloped.

To test the prediction that smolts are female-biased and that migration distance and latitude influence that bias (Figure 1b, c), I sampled nine populations of partially migratory steelhead located from 55.9º to 39.5º North latitude on the west coast of North America in the spring of 2011 and 2012 (Table 2) (Figure 2). All these river systems have established monitoring programs of self-sustaining wild populations with no hatchery supplementation. I measured sex ratios in the smolt life stage because they are not

subjected to possible sex-specific mortality in the ocean resulting from increased aggression by females in the ocean (Tamate and Maekawa 2004, Spindle et al. 1998), or sex-biased iteroparity (Keefer et al. 2008) and provide the earliest life stage at which to measure the anadromous component of a population. I used migration distance as a proxy for migration difficulty (Crossin et al. 2004) and latitude as a proxy for freshwater growth conditions (Metcalfe and Thorpe 1990, Morita and Nagasawa 2010).

Smolts were captured using rotary screw traps operated from March through June, 2011 and 2012, to determine the sex ratios of anadromous fish during their outmigration. Dates of operation were adjusted to local outmigration timing and river conditions (Table 3). Prior to data collection, I performed a statistical power analysis, which indicated that a sample size of approximately 200 fish would be necessary to detect a 10% difference between the sex ratios of two populations. I then developed a systematic sampling scheme based on previous years of capture data by calculating the proportion of the total catch at each site that would be required to yield approximately 200 smolt samples (Table 3). Smolt samples were then collected proportionally to the daily catch (e.g., 1 in 6 smolts captured).

Smolts collected in the smolt traps were anesthetized (MS-222), then measured for fork length (mm), and sampled for fin tissue and scales. Scales and fin tissue were collected following the same methods described for the South Fork John Day Oregon, except that at Big Ratz Creek, Alaska, tissue samples were placed in vials containing 90% ethanol, and later transferred to Whatman™ chromatography paper in the lab.

Maturity Status

I assessed the maturation state of gonad samples to classify the South Fork John Day fish as freshwater resident or undifferentiated immature individuals. I classified males as freshwater resident if spermatids were present within the testis, and females as freshwater resident if oocytes in the ovary had advanced to oil-drop stage (Nagahama 1983). Fish were classified as immature with an undetermined life history if an individual did not have these gonadal characteristics. Fixed ovaries and testes were dehydrated

through a graded series of ethanol and embedded in paraffin wax, sectioned (thickness, 5 μm), and stained with hematoxylin-eosin. Stages of development (oogenesis and spermatogenesis) were determined by light microscopy, using the protocol of Nagahama (1983) as a guide.

Sex Determination

To determine genotypic sex of all sampled fish (age-0, residents, and smolts), DNA was extracted using DNeasy 96 Tissue Kits with a BioRobot 8000 (QIAGEN). For the 2011 samples, polymerase chain reaction (PCR) was performed on approximately 25ng of DNA to amplify OmyY-1 (the Y-linked sex marker described by Brunelli et al. (2008)). I used ND3 as the internal control (5 pmol each, Domanico and Phillips 1995) and Omy-Y primer (12 pmol each), which differed slightly from the methods described by Brunelli et al. (2008). PCR products were electrophoresed through 2% agarose gels in TAE buffer and stained with GelRed nucleic acid stain (Phenix). The male band (792-bp) was clearly distinguishable from the ND3 control band (368-bp). For the 2012 samples (only smolts), DNA was extracted using DNeasy 96 Tissue Kits with a BioRobot 8000 (QIAGEN). Approximately 10-20ng of DNA was used as a template in the TaqMan 5' nuclease assay. All PCRs were done in a 5 μl volume containing GT Express Master Mix, TaqMan * SNP Gentoyping primers and minor groove binding (MGB) probes for Omy-2SEXY based on the Omy-Y1 locus. The amplification consisted of 95°C for 10 min and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The 7900 Real-Time PCR System (ABI) was used for SNP genotyping and the data were analyzed using the Sequence Detection System 2.4 (ABI). Positive and negative controls were used on each plate and the assay was tested on 150 samples from 2011 to ensure the quality of allele discrimination. These samples had 100% concordance of sex determination using both methods.

I compared the genotypic sex results with the phenotypic sex determination made using histology to assess the error rate associated with the genotypic sex determination for the freshwater residents collected in the South Fork John Day. This comparison was

not made for age-0 fish because I did not determine their phenotypic sex. These samples had 100% concordance between genotypic and phenotypic sex (n = 115).

Age Determination

I estimated ages of freshwater residents collected from the South Fork John Day using otoliths and scales. I mounted one sagittal otolith from each fish on a glass slide and polished the primordium with fine sandpaper and alumina (DeVries and Frie 1996, Zimmerman and Reeves 2000). I magnified the otoliths with a Leica compound microscope and used Image Pro® software (Media Cybernetics Inc., Bethesda, Maryland) to adjust the focus and contrast of the image. Age was estimated by counting the number of annuli, which were identified as opaque white rings (DeVries and Frie 1996).

To estimate ages of smolts and freshwater residents, I mounted scales on a glass microscope slide and examined them under 10x magnification using a Micron 780® microfiche (Bell and Howell Company). I selected at least one non-regenerated scale from each fish for ageing. Age was estimated by identifying and counting the number of annuli, which are defined as a series of circuli that are closely spaced or crossed over (Ericksen 1999). Any wider growth at the edge of the scale beyond the annuli was considered spring or summer growth and was denoted with a "+".

Four scale readers trained together and each reader aged all of the scales from a population. Readers aged the scales twice and the ages were compared between the two reads, then any discrepancies were aged a third time. One of the four scale readers reviewed a subset of the scales from each population to bring consistency between multiple readers and sites. Scales from Big Ratz Creek, Alaska and Cummins Creek and Trask River, Oregon were exceptions, and they were aged by a trained set of readers at their respective state agencies (Alaska Department of Fish and Game and Oregon Department of Fish and Wildlife, respectively).

Data Analysis

I performed exact binomial tests to evaluate whether the sex ratios of age-0 juveniles, freshwater residents, and smolts differed from 1:1. I performed an odds ratio test to evaluate whether the sex ratio in freshwater residents above and below the waterfall differed. To evaluate whether migration distance or latitude influenced the proportion of female smolts in each of the nine populations, I constructed separate logistic regression models for migration distance and latitude. Because of potential differences between the sexes in age, length, and migration date, these variables were also included as predictors in each model. Prior to analysis I transformed several predictor variables. To control for the increase in smolt age with latitude (Metcalfe and Thorpe 1990), I normalized age by assigning a relative age of zero to the modal value within each population. I standardized fish length by subtracting the mean value for each population from each individual length (Zuur et al. 2008). These data transformations also eliminated the collinearity between age and other predictor variables. For migration date, I normalized the date of capture relative to the start of trap operation at each site to remove the between-site variation in run timing. I natural log transformed migration distance because of the extreme range of migration distances (Table 2) and because these values caused poorly conditioned model matrices which resulted in errors.

To assess variation among smolts in the size at outmigration, I modeled fish length as a function of sex, age, and outmigration date using generalized linear mixed effects model. I used a similar approach to assess variation in the outmigration timing as a function of sex, age, and length, as well as variation in age as a function of sex, outmigration date, and length. I used a subset of the Trask River data (n = 262) in the logistic regression and the generalized linear mixed effects models. I performed all analyses in R version 2.14.1.

This work was conducted under ACUP permit number 4185 authorized by Oregon State University and under NOAA permit number 16576. Support for this work was provided by the Bonneville Power Administration (Project 2003-017 to Dr. Chris
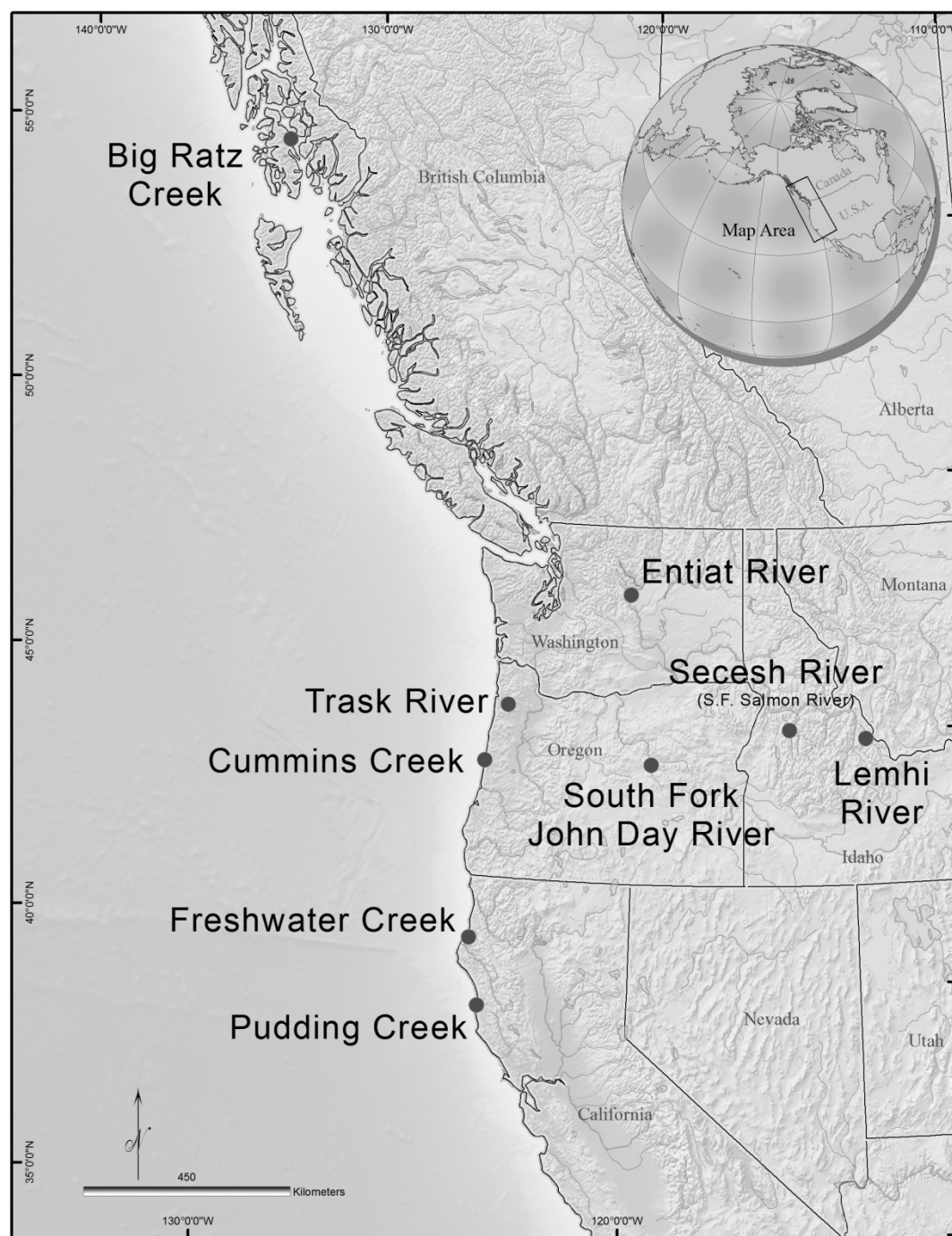
Figure 2. Location of South Fork John Day resident population (Oregon, USA) and all sampled smolt populations.

Table 1. Number of mature resident rainbow trout and juvenile *O. mykiss* in different size classes collected from the South Fork John Day River,Oregon, from 2011.

	Stream order	Number of Fish Collected by Size Class (mm)				
		≤ 65	66-100	101-150	151-200	>200
Above barrier waterfall	2	21	25	29	5	1
	3	15	6	12	8	4
	4	0	0	3	10	15
Below barrier waterfall	2	32	17	15	12	1
	3	11	10	10	3	1
	4	0	1	18	21	1

Table 2. Geographic locations and physical/biological characteristics of streams where smolts were collected in Alaska, Washington, Oregon, Idaho, and California (Figure 2).

River	Trap location (state)	Latitude (°N)	Longitude (°W)	Migration distance (km)	Drainage area (km^2)	Elevation (m)	Climate type (precipitation as rain)	Stream conditions	Other salmonids[b] present
Short Migration									
Big Ratz	AK	55.8900	-132.6334	1	41	296	Pacific maritime, 152-559 cm/y	Lake- and rainwater-fed.	Sockeye, coho, pink, chum, Dolly Varden, cutthroat
Trask River	OR	45.4156	-123.6023	45	75	499	Pacific maritime, 318-508 cm/y	Flashy stream, high flows Nov. to March.	Coho, cutthroat, Chinook, chum
Cummins Creek	OR	44.2670	-124.0890	2	21	332	Pacific maritime, 180-230 cm/y	High-gradient stream, peak flows Nov. to March, no estuary[a].	Cutthroat, coho, occasional Chinook
Freshwater Creek	CA	40.7837	-124.0831	14.5	77	281	Pacific maritime, 100-150 cm/y	Lower 6 km has cattle grazing, is low-gradient, and confined by levees.	Chinook, coho, steelhead, cutthroat, occasional chum
Pudding Creek	CA	39.4541	-123.7576	7	39	156	Mediterranean, average 98 cm/y	Impoundment 1 km upstream of ocean mouth; resulting reservoir inundates 4.3 km of stream. Reservoir actively managed for fish.	Coho

River	Trap location (state)	Latitude (°N)	Longitude (°W)	Migration distance (km)	Drainage area (km²)	Elevation (m)	Climate type (precipitation as rain)	Stream conditions	Other salmonids[b] present
Long Migration									
Entiat River	WA	47.6636	-120.2505	770	816	1298	Semi-arid high desert, 25-254 cm/y	Originates from 11 glaciers and snowfields in the Cascade Range. Limit to anadromy is Entiat Falls at rkm 54.4.	Chinook, sockeye
Lemhi River	ID	45.1526	-113.8135	1090	3160	2182	Semi-arid high desert, average 51 cm/y	Fed by snowmelt runoff and ground-water springs. Significant irrigation diversion.	Chinook, introduced brook trout, Dolly Varden
Secesh River	ID	45.0382	-115.7421	1240	626	2055	Semi-arid high desert, 51-152 cm/y	Peak discharge results from snowmelt runoff in late May and June.	Westslope cutthroat trout, coho, bull trout, introduced brook trout
South Fork John Day River	OR	44.4189	-119.5394	690	1441	1466	Semi-arid high desert, 25-51 cm/y	Fed by snowmelt runoff and ground-water springs. Waterfall at rkm 45 restricts upstream movement of steelhead. Some irrigation diversion.	Steelhead/ rainbow trout only

[a] Stream empties directly into the ocean.

[b] Sockeye salmon – *Oncorhynchus nerka*; Coho salmon – *O. kisutch*; Pink salmon – *O. gorbuscha*; Chum salmon – *O. keta*; Dolly Varden – *Salvelinus malma*; Cutthroat trout – *O. clarkii*; Chinook salmon – *O. tshawytscha*; Westslope cutthroat trout – *O. clarkii lewisi*; Bull trout – *Salvelinus confluentus*; Brook trout – *S. fontinalis* (introduced from eastern U.S.).

Table 3. Details of sampling methods, sample size, and dates of trap operation at each trapping location for steelhead smolts.

River	Sampling Methods	Sample Size	Trap Operation	Trap Operator
Short Migration				
Big Ratz	1 in 7 smolts captured	200	April 17 to June 14, 2011	Alaska Dept. of Fish and Game
Trask River	All smolts captured	431	March 7 to June 18, 2012	Oregon Dept. of Fish and Wildlife
Cummins Creek	1 in 3 smolts captured	163	March 3 to June 4, 2012	Oregon Dept. of Fish and Wildlife
Freshwater Creek	All smolts captured	125	April 12 to June 1, 2011	California Dept. of Fish and Game
Pudding Creek	1 in 3 smolts captured	193	April 10 to June 13, 2011	Campbell Timberland Management Group
Long Migration				
Entiat River	1 in 10 smolts captured	166	March 17 to May 14, 2011	U.S. Fish and Wildlife Service
Lemhi River	All smolts captured	222	April 19 to May 24, 2011	Idaho Dept. of Fish and Game
Secesh River	All smolts captured	121	May 1 to July 5, 2011	Nez Perce Tribe
South Fork John Day River	1 in 7 smolts captured	120	April 12 to June 17, 2011	Oregon Dept. of Fish and Wildlife

CHAPTER 3 - RESULTS

To measure the age-0 sex ratio in the South Fork John Day, I combined samples from above and below Izee Falls to increase the sample size (n = 80). The age-0 sex ratio did not differ from 1:1 (exact binomial test, 95% confidence interval 33% to 55%, p = 0.31; Figure 3). Of the 74 fish captured in the resident population above Izee Falls, 49 were mature, and for these the sex ratio was equal between males and females (exact binomial test, 95% confidence interval 34% to 64%, p > 0.5). Thirty-one of the 116 fish captured in the partially migratory population below Izee Falls were mature, and for these the sex ratio was also equal between males and females (exact binomial test, 95% confidence interval 25% to 61%, p = 0.47). Further, the resident sex ratios above and below Izee Falls did not differ from one another (odds ratio test, p > 0.5).

The number of smolts captured at all sites ranged from 120 to 431 in 2011 and 2012 (Table 3). Smolt sex ratios were all female-biased (exact binomial tests, Table 4) and ranged from 56% to 76% female. Latitude did not have an effect on female bias (logistic regression, drop in deviance test, $\lambda^2 = 0.105$, p = 0.75) (Figure 4), nor did migration distance (logistic regression, drop in deviance test, $\lambda^2 = 0.108$, p = 0.74) (Figure 5).

Mean length did not differ between males and females after accounting for age and outmigration date (95% confidence interval: -2.692 to 2.209, t = -0.193, p = 0.847), and range of length values was equal for males and females (Figure 6). Mean length decreased by 1.5 mm for every successive week of the outmigration period across all sites (95% confidence interval: -2.131 mm to -0.826 mm, t = -4.444, p < 0.001). Mean length was generally similar among the sites, except for Pudding Creek and the Secesh River, where mean lengths were smaller than the other sites.

Mean smolt age did not differ between males and females after accounting for length and outmigration date (logistic regression, point estimate: -0.047, 95% confidence interval: -0.104 to 0.009, t = -1.637, p = 0.102). However, mean age decreased over the course of the outmigration such that for every increase in week there was a -0.025-year decrease in mean smolt age (logistic regression, 95% confidence interval: -0.041 to -0.010 , t = -3.284, p = 0.001). Mean smolt age also increased with latitude, and for every

degree increase in latitude there was an increase in age of 0.086 years (logistic regression, 95% confidence interval: 0.029 to 0.143, t = 2.966, p = 0.025) (Figure 7). Mean outmigration date did not differ between males and females after accounting for length and age (logistic regression, point estimate: -0.174, 95% confidence interval: -0.393 to 0.044, t = -1.568, p = 0.117).
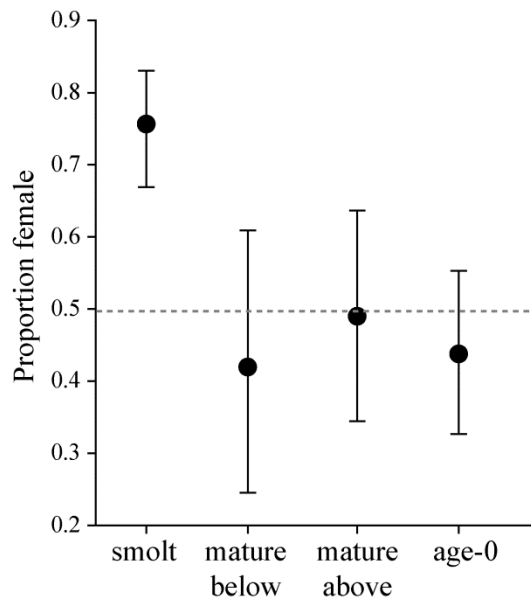
Figure 3. Proportion of females in samples of *O. mykiss* collected from the South Fork John Day River in summer of 2011. The proportion of females in the sample of migrating smolts was significantly greater than 0.50, whereas sex ratios for maturing residents below the waterfall, maturing residents above the waterfall, and age-0 fish combined from below and above the barrier waterfall were not different from 0.50.

Table 4. Smolt sex ratios from all populations sampled in 2011 and 2012. Binomial test results and p-values indicate all populations differ from a 1:1 sex ratio and are female biased.

River	Proportion of Females	Sample Size	95% Confidence Interval	p-value
Short Migration				
Big Ratz	71%	198	64-77%	< 0.001
Trask River	56%	366	51-61%	0.02
Cummins Creek	58%	154	50-66%	0.04
Freshwater Creek	71%	122	62-79%	< 0.001
Pudding Creek	63%	193	56-70%	< 0.001
Long Migration				
Entiat River	62%	147	54-70%	0.005
Lemhi River	60%	218	53-67%	0.003
Secesh River	65%	99	54-74%	0.005
South Fork John Day River	76%	119	67-83%	< 0.001
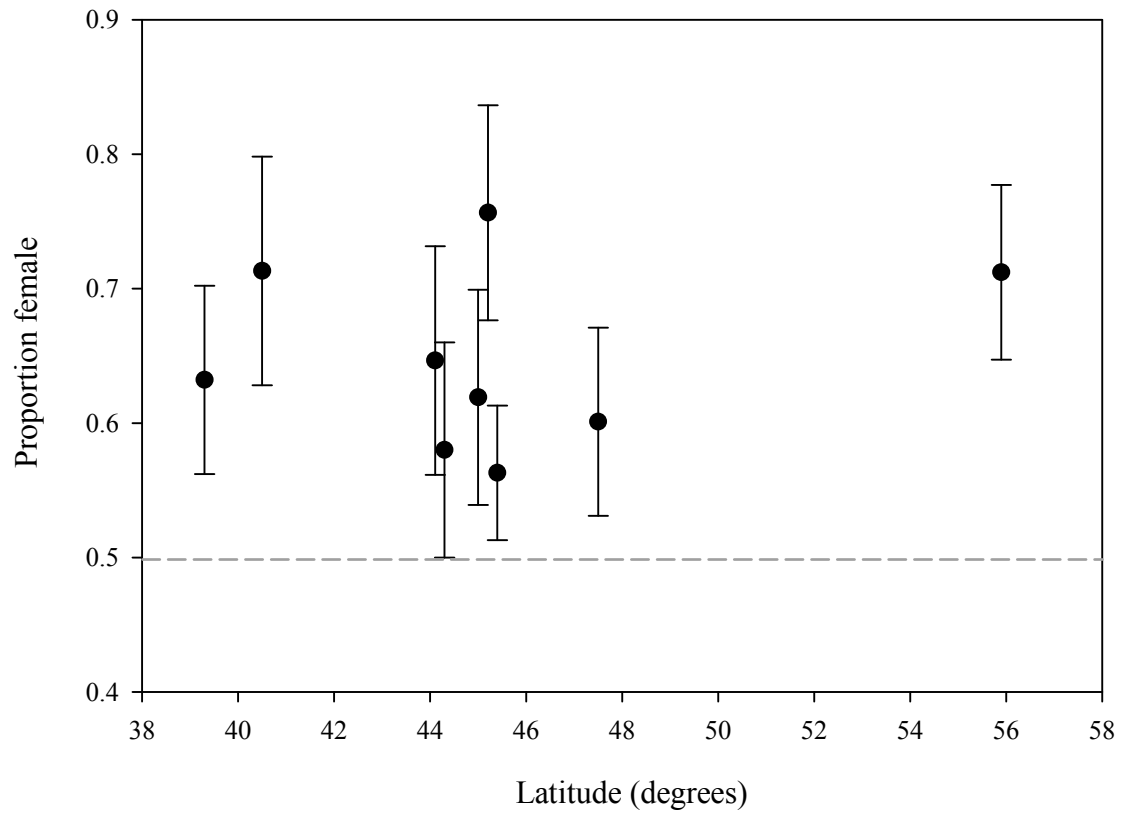
Figure 4. Estimated proportion of female steelhead smolts and 95% confidence intervals from all populations arranged by latitude. Order of populations is: Pudding Creek, California; Freshwater Creek, California; Secesh River Idaho; Cummins Creek, Oregon; Entiat River, Washington; South Fork John Day River, Oregon; Trask River, Oregon; Lemhi River, Idaho; and Big Ratz Creek, Alaska.
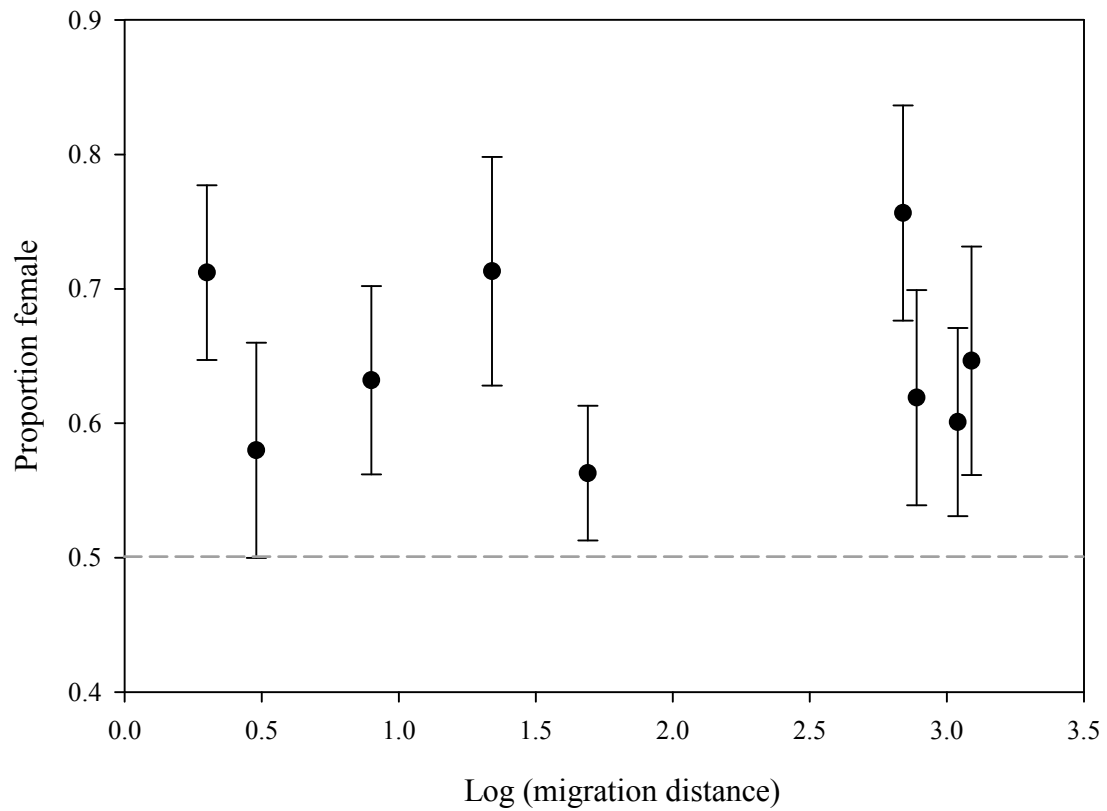
Figure 5. Proportion and 95% confidence intervals of female steelhead smolts along gradient of migration distance (natural log, km). Order of populations is: Big Ratz Creek, Alaksa; Cummins Creek, Oregon; Pudding Creek, California; Freshwater Creek, California; Trask River, Oregon; South Fork John Day River, Oregon; Entiat River, Washington; Lemhi River, Idaho; and Secesh River, Idaho.
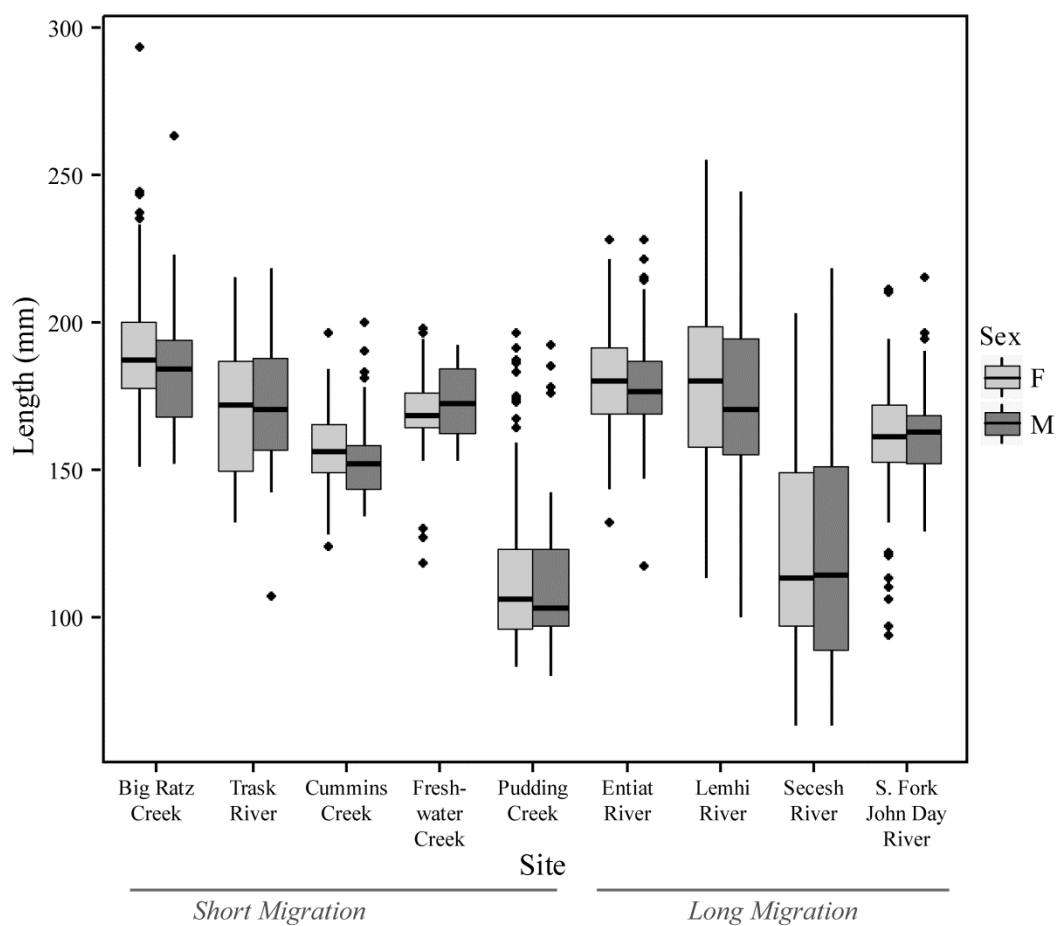
Figure 6. Distributions of fork lengths of steelhead smolts from all sampled populations (Figure 1). Mean lengths and variance do not differ between males and females in any population.
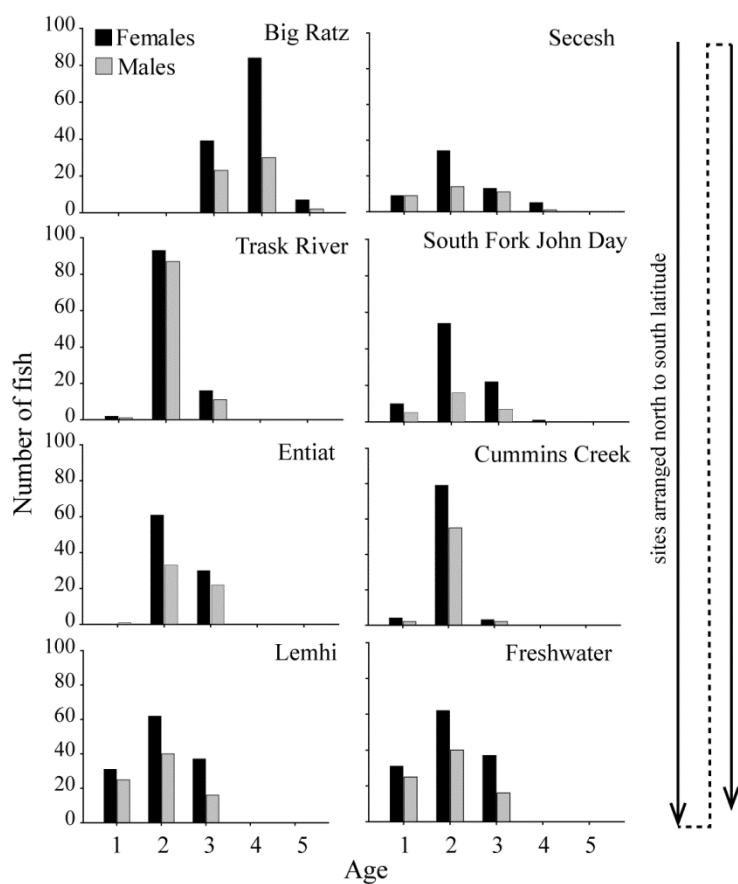
Figure 7. Frequency of smolt ages in each population. Note that mean smolt age increases with increasing latitude.

CHAPTER 4 - DISCUSSION

In the South Fork John Day River, I found that *O. mykiss* had an even ratio of males to females in their first year of life. This result is consistent with other studies that have measured age-0 sex ratios of *O. mykiss* in nature (Rundio et al. 2012, Schill et al. 2010) and under artificial propagation (Magerhans and Hörstgen-Schwark 2010). Because the age-0 sex ratio was not different from 1:1, I assumed that any change in sex ratios at later life stages indicated a difference between sexes in the adoption of life-history tactics.

In contrast to the age-0 sex ratio, I found strong evidence that anadromous *O. mykiss* are more likely to be female throughout their range, confirming my hypothesis that andromy should confer greater benefits to females than males. Contrary to my findings, Ward and Slaney (1988) found that smolt sex ratios did not differ from 1:1 in a coastal population in British Columbia during a three year study. However, the relatively smaller sample sizes used by Ward and Slaney (1988) (n = 54, 126, 91) may have limited their power to detect a significant difference for a smaller deviation from 1:1, at least in some years. In contrast, Peven et al. (1993) reported that 63% of smolts in a mid-Columbia River population were female. Varying degrees of female bias have been consistently observed in adult anadromous *O. mykiss* (e.g., Ricker 2002, Campbell Timberland Management 2008, Love and Harding 2009). However, female bias at this life stage can be confounded by differential ocean mortality, differential age at maturity (Busby et al. 1996), and differential rates of iteroparity between the sexes (Keefer et al. 2008; Wertheimer and Evans 2005). Finally, female-biased smolt (or non-spawning migrant) sex ratios are not unique to *O. mykiss*; they have been reported in other partially migratory salmonids such as Atlantic salmon (*Salmo salar*; Jonsson et al. 1998), masu salmon (*O. masou*; Tamate and Maekawa 2004), and Dolly Varden (*Salvelinus malma*; Koizumi et al. 2006).

The observed differences between males and females in the frequency of anadromy are indirect evidence of differences in the locations of the switch-points (s_m* and s_f*), which are related to individual condition. I investigated differences between males and females in smolt length, age, and outmigration date and found both sexes to be

similar. There is some indication from other studies that the switch-point based on freshwater growth differs for males and females (Morita and Nagasawa 2010), however those differences take place in an earlier life stage than when I measured. There may also be other factors, such as lipids (McMillan et al. 2012), not considered here, that may be important indicators of individual condition. This highlights the importance of investigating proximate cues and mechanisms of tactic choice separately in males and females.

I did not observe changes in sex ratios associated with latitude or migration distance, and I have two possible explanations for this. The first is that the overall frequency of anadromy and residency changed in response to migration distance, latitude, or both, but that the change was similar between males and females across these gradients, resulting in no change in the sex ratios (Figure 8d). To my knowledge, there is no available data on changes in sex ratios (i.e., differences in males and females) in response to environmental constraints. However, there is evidence of population-level changes in tactics (not sex-specific) along the environmental gradients of latitude and migration distance. Morita and Nagasawa (2010) found a correlation between increased residency associated with increased body size and water temperatures at lower latitudes in masu salmon. They also cite decreases in degree of migratory tactic (i.e., anadromy or freshwater migrations) with decreasing latitudes in brown trout (*Salmo trutta*) (Elliott 1994), white-spotted charr (*Salvelinus leucomanenis*) (Yamamoto et al. 1999), Dolly Varden (*Salvelinus malma*) (Maekawa and Nakano 2002), and masu salmon (*O. masou*) (Malyutina et al. 2009). Migration distance has been shown to have an effect on frequency of anadromy (sockeye salmon [*O. nerka*], Kristoffersen et al. 1994; brown trout [*S. trutta*], Bohlin et al. 2001), although the results are slightly less consistent (brown trout [*S. trutta*], Jonsson and Jonsson 2006). These positive correlations lead me to believe that changes in the frequency of anadromy or residency could be taking place over the latitude and migration distance gradients, but that the change was equal between males and females. If this is the case, it implies that females are equally as plastic as males in their ability to change tactics. It is generally thought that female tactics are less

plastic than males because of the high energy requirements for gamete production and the relationship between body size and fecundity. However, if they are in fact equally as plastic as males this could indicate that age at maturity, survival to maturity, and parity may play larger roles in their fitness than previously expected.

My second explanation is that latitude and migration distance have an interactive effect on sex ratios such that the effect of one depends on the other. For example, if migration difficulty is high and freshwater growth is low, the overall effect of migration difficulty is less and populations are more likely to be anadromous. In contrast, if migration difficulty is high but freshwater growth is also high, the overall effect of migration difficulty is higher and individuals are more likely to be resident. Findstad and Hein (2012) found this to be true in populations of Arctic charr (*Salvelinus alpinus*) in Norway. They were not able to detect a pattern with migration difficulty or freshwater productivity alone, but the interaction between them was a significant predictor of anadromy. Given that I only had nine observations of migration distance and latitude, I could not investigate this statistical interaction (Zuur et al. 2008). This interaction may be especially important in my study because if there is poor growth at higher elevations (which is not accounted for in the latitude metric), then this could lessen the effect of a long migration distance and would obscure any pattern. This alternative underscores the importance of looking at environmental constraints in terms of both costs and benefits and that not accounting for both could lead to inconclusive or misleading results.

Finally, my failure to detect a male-biased sex ratio in resident fish in the South Fork John Day partially migratory population, despite a high female bias measured in the smolts, deserves some discussion. The lack of male bias is counter to recently reported results of very high male bias in a partially migratory resident population in California (Rundio et al. 2012), as well as observations made in a coastal Washington population by McMillan (2007). I hypothesize that lack of resident male bias in the South Fork John Day was due to a high abundance of resident *O. mykiss* in that river that overwhelmed any male bias associated with the female-biased smolts. To investigate this idea further, I simulated populations of 10,000 fish and adjusted the overall proportion of residents in

the populations (Figure 9). I used three different scenarios of female-biased smolts (51%, 60%, and 80%), and found that in populations with an overall high proportion of residents there was no male bias in residents, even under high (80%) female bias (Figure 9). In populations with a low proportion of residents, resident sex ratios were highly male- biased, even under low (51%) female-biased smolts. This demonstrates that the relative proportion of a tactic in a population is an important influence on the sex ratio in that tactic. Taken a step further, this may be a valuable tool for estimating the proportion of residents in a population using sex ratios of residents and smolts. It could be a relatively simple way to infer a population-level dynamic that can be difficult to measure, and has the benefit of being non-lethal, which is becoming increasingly important as many species are protected under the Endangered Species Act.
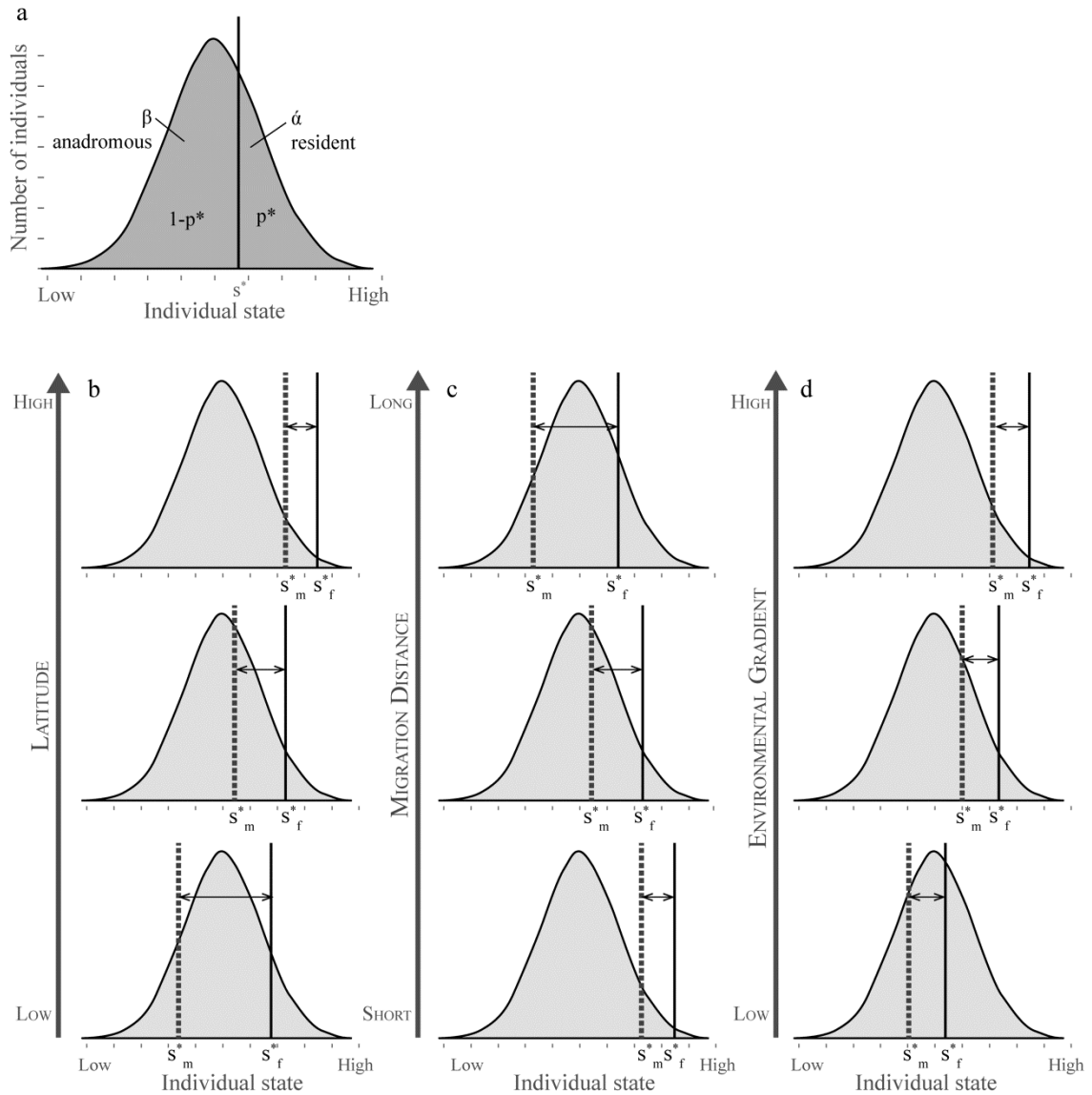
Figure 8. The conditional strategy (a) and my original predicted changes in sex ratios along gradients of latitude (b) and migration distance (c). One alternative explanation for why I did not observe these hypothesized patterns is displayed in (d). It is possible that the relative frequency of resident and anadromous tactics changed in response to environmental gradients, but that males and females responded equally, resulting in no observable patterns in sex ratios (d). Dotted line signifies males, solid line signifies females.
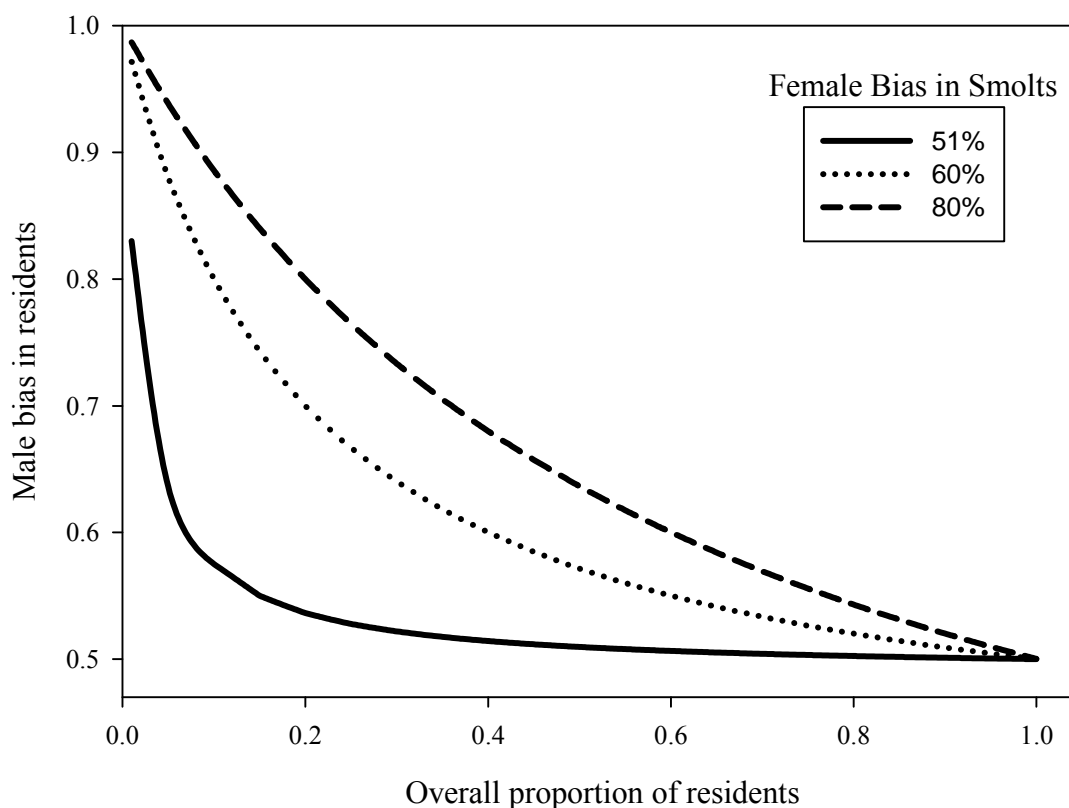
Figure 9. Data simulation to investigate how the overall proportion of residents in a population influences the sex ratio of residents. I simulated populations of 10,000 fish with differing proportions of residents. Under three different scenarios of female-biased smolts (51%, 60%, and 80%), I found that in populations with a low proportion of residents, resident sex ratios were highly male-biased, even under low (51%) female-biased smolts. In populations with a high overall proportion of residents, resident sex ratios were not male-biased, even under high (80%) female-biased smolts. This emphasizes the importance of the overall proportion of residents when estimating a sex ratio, but also demonstrates how sex ratios can be used as a tool to estimate the overall proportion of residents.

CHAPTER 5 - CONCLUSION

Proximate and ultimate controls on alternative life-history tactics are an interesting evolutionary question, but are coming to the forefront as an important question for conservation and restoration of threatened and endangered species. I demonstrated that sex is an important predictor of an individual's tactic, likely due to differences in fitness determinants between males and females. I found that sex ratios did not vary along environmental gradients of latitude and migration distance, possibly due to an interaction between these environmental conditions, or because males and females respond equally to these conditions. Whether the sexes respond equally to environmental conditions is an important question for understanding the overall resilience of the species, as well as predicting future effects of climate change. I emphasize the importance of considering sex when investigating proximate and ultimate controls on life-history tactics, and propose that sex ratios can be a powerful tool for investigating frequencies of tactics within populations.

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