GENETIC VARIABILITY WITHIN BULL TROUT (*Salvelinus confluentus*) POPULATIONS IN THE YAKIMA RIVER BASIN

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ABSTRACT

GENETIC VARIABILITY WITHIN BULL TROUT (*Salvelinus confluentus*) POPULATIONS IN THE YAKIMA RIVER BASIN

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Stream connectivity in the Yakima River basin has been fragmented by the construction of reservoirs with no fish passage. Bull trout (*Salvelinus confluentus*) exist in the basin in adfluvial and fluvial forms, as well as resident populations. The genetic structure of twelve spawning populations of bull trout was examined for this study, using six polymorphic microsatellite loci. Overall expected heterozygosities were low (0.2252-0.4544) and variability among populations was high ($F_{ST} = 0.217$). Pair-wise comparisons of genotype frequencies show gene flow between four fluvial populations with no migration barriers. In addition, one population from Rimrock reservoir had similar gene frequencies, possibly reflecting historical connectivity with fluvial populations. In the two reservoirs sampled that contained more than one population, each spawning stream was genetically distinct. These results show that without migration barriers, there is potential for gene flow, however there seem to be other factors contributing to levels of genetic exchange.

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CHAPTER I

INTRODUCTION

Bull trout, *Salvelinus confluentus*, are a member of the char family with a core range in the northwestern United States and southwest Canada (Cavender 1978; McPhail and Baxter 1996). The species overall is in decline due to habitat degradation and fragmentation, competition from introduced species, and water quality conditions (Fraley and Shepard 1989; Rieman and McIntyre 1993; Kanda et al. 2002). In 1998, bull trout were officially listed as "threatened" throughout their range under the U.S. Federal Endangered Species Act of 1973 (ESA). Conservation efforts to maintain existing populations and protect habitat are ongoing throughout their range in the Pacific Northwest and southwestern Canada.

Protection and management of bull trout populations is complex due to highly variable life history patterns. Bull trout are iteroparous fall spawners, with most spawning occurring in high elevation headwater streams. After two to four years rearing in their natal streams, juveniles migrate to a larger body of water; fluvial populations to mainstem rivers, and adfluvial populations to lakes or reservoirs. Some coastal populations have anadromous life history forms that utilize the near-shore ocean environment. Further, an alternate strategy occurs, where juveniles mature in their natal streams and remain as resident adults (Fraley and Shepard 1989; Pratt 1992). In the Yakima River basin fluvial, adfluvial and resident forms are present (WDFW 1997).

Bull trout populations in the Pacific Northwest of the United States have been subdivided into several levels of differentiation range-wide. The U.S. Fish and Wildlife

Service, in listing bull trout under the ESA, determined five Distinct Population Segments (DPS): Klamath River in Oregon, Columbia River Basin, Jarbridge River in Nevada, Coastal/Puget Sound, and St. Mary/Belly River in Montana (Waples 1991; USFWS 1998). These delineations were made based on geographical location and connectivity alone. A phylogenetic study by Taylor et al. (1999), using mitochondrial DNA markers, found genetic subdivision into "coastal and interior lineages," with the Cascade mountain crest as the geographical separator. Additionally, Spruell et al. (2003), using microsatellite nuclear DNA markers, proposed recognition of three genetically differentiated groups: Coastal, Snake River and nearby tributaries, and the Upper Columbia River. However, no samples from central Washington state were included in this study. Further, an early study by Leary et al. (1993) compared bull trout in the Klamath River and Columbia River drainages using allozyme markers, and found them to be distinct enough to qualify as separate species under ESA criteria.

Genetic analysis of bull trout has thus far primarily focused on questions of overall genetic variation, and relationships among populations on a large geographical scale (Leary et al. 1993; Taylor et al. 1999; Taylor et al. 2001; Spruell et al. 2003). Individual watersheds have been studied in Idaho and Montana (Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001), but there have been no genetic studies of bull trout populations in central Washington State. Habitat throughout the range of bull trout has been fragmented by stream quality conditions and physical barriers (Rieman and McIntyre 1993). The Yakima River basin is an excellent example of this phenomenon. Irrigation dams without fish passage were constructed throughout the basin

in the early 1900s; however, one large stream system remains free flowing. Therefore studying bull trout in this watershed provides an opportunity to contrast genetically isolated bull trout populations with those in a barrier free system.

An overall pattern throughout genetics studies of bull trout has been high levels of variation among populations, and low variation within populations (Leary et al. 1993; Kanda and Allendorf 2001; Spruell et al. 1999). Declining population size, combined with population isolation, could lead to loss of genetic diversity and evolutionary potential (Rieman and Allendorf 2001). A small number of effective breeders within a population increases the rate of genetic drift, and leads to fixation of alleles, increasing the vulnerability of populations to extinction via stochastic natural events or human impacts (Rieman and McIntyre 1993). Genetic data, and knowledge of local population structure among bull trout within specific watersheds, in conjunction with demographic and historical information, can provide valuable insight for conservation efforts.

Several large data sets exist for information on bull trout populations in the Yakima River basin. The Washington State Department of Fish and Wildlife (WDFW) has been conducting annual redd count surveys on spawning tributaries from 1984 to the present, each year including more streams and making index areas more precise (E. Anderson, personal communication 2002). These counts show yearly fluctuations in spawning escapement, and provide overall estimations of population sizes (Table 1; Dunham et al. 2001; Rieman and Allendorf 2001).

Demographic studies of bull trout reservoir populations have been carried out since 1996 by Dr. Paul James of Central Washington University, under funding

Table 1 Bull trout populations sampled in the Yakima River basin[a]

Stock	Populations	Identifier	Yr of dam construction	Life history	Nr of redds	Nr of years[b]
Rimrock Lake	South Fork Tieton	SFT	1925	Adfluvial	158	9
	Indian Creek	IN		Adfluvial	169	12
American-Naches	American River	AM	NA	Fluvial	6	7
	Union Creek	UN		Fluvial	13	7
	Rattlesnake Creek	RT		Fluvial	50	7
	Crow Creek	CR		Fluvial	15	4
Kachess Lake	Box Canyon Creek	BX	1912	Adfluvial	8	13
	Mineral Creek	ML		Adfluvial	15	
Bumping Lake	Deep Creek	DP	1910	Adfluvial	85	12
Keechelus Lake	Gold Creek	GL	1917	Adfluvial	19	19
Ahtanum	Ahtanum	AH	NA	Resident	32	3
North Fork Teanaway	NF Teanaway	TN	NA	Resident	0[c]	5

[a] Based on 1997 Washington State Salmonid Stock Inventory (WDFW 1997) and unpublished data provided by E. Anderson of Washington State Department of Fish and Wildlife, Yakima, WA.

[b] Number of years of surveys from which average number of redds is derived.

[c] No survey index area established, though surveys have been conducted for 5 years.

provided by the Bureau of Reclamation (BOR). Fish tagging studies have provided information about number of spawning runs undergone by individual fish, average size and weight of fish within populations, and spawning site fidelity. This information and several studies by graduate students working in the basin (Sexauer 1994; James 1997; Meyer 2002; James 2002; Polachek and James 2003) have contributed to the bulk of the knowledge about local bull trout populations in reservoirs. Bull trout populations in non-regulated waters have been largely unstudied, though a radio telemetry project due to start in 2003 will add to the knowledge base (E. Anderson, personal communication 2002).

Currently, the U.S. Fish and Wildlife Service (USFWS) is coordinating the creation of a bull trout recovery plan, and the Yakima River basin is part of the Middle Columbia River Recovery Unit. USFWS, the agency responsible for implementing ESA policy on listed freshwater species, is working with federal, state and tribal agencies to determine the actions needed to protect and maintain bull trout populations. Isolation due to dams and fragmentation of habitat from irrigation practices is considered by USFWS to be "the major threat" to bull trout in this unit (USFWS 2002). One of the four main objectives listed is the preservation of genetic diversity, and creating the potential for gene flow connectivity (USFWS 2002).

Thus, this study of population substructure and genetic diversity of bull trout in the Yakima River basin is timely. Information about genetic relationships between spawning populations in the basin can provide insight for decisions about recovery plans. Issues of the impacts of irrigation dams and other migration barriers can be examined in

light of these results.

The objective of this study was to determine genetic population substructure of bull trout in the Yakima River basin where irrigation practices have fragmented habitat and limited potential for gene flow. The goal was to address two main questions: 1) Do Yakima River bull trout populations show low levels of within population variation? 2) Are spawning populations of bull trout genetically distinct from one another, and what is the genetic relationship among these populations? With knowledge of the genetic relationships within this watershed, it is then possible to use existing demographic data to further our understanding of the possibilities for conservation efforts and recovery of this threatened species.

CHAPTER II

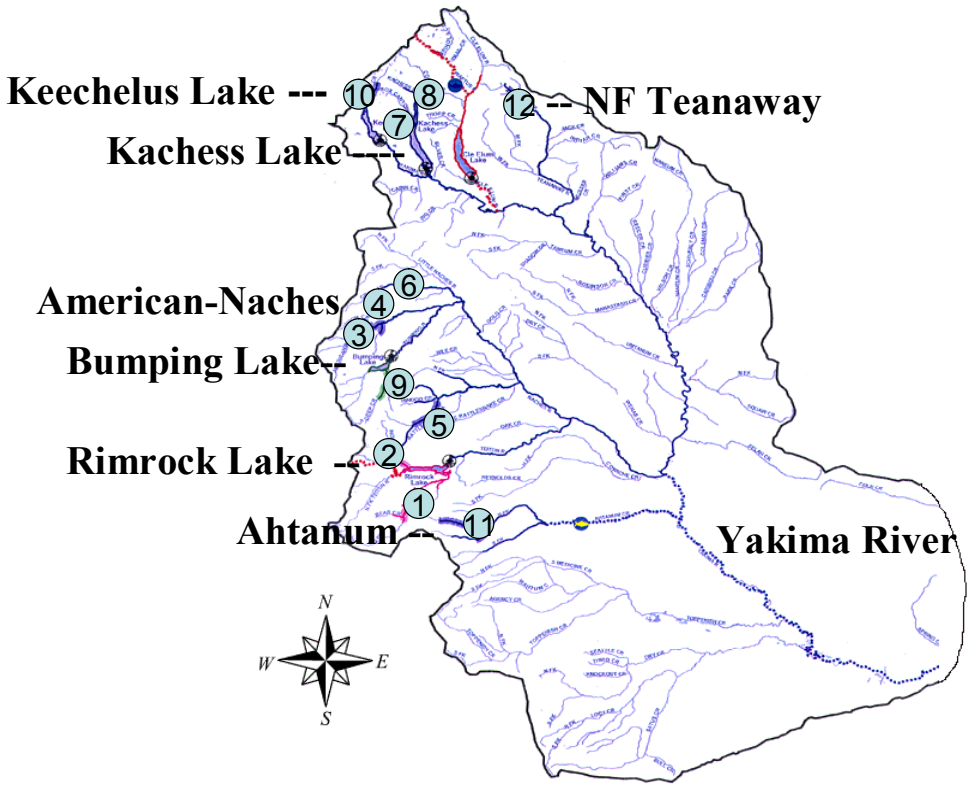
MATERIALS AND METHODS

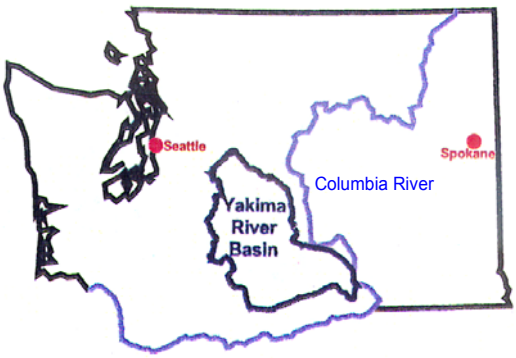
Study Area: Yakima River Basin

The Yakima River is a main tributary to the Columbia River in Washington State (Figure 1). The river drains 15,941 square kilometers in a 346 kilometer journey from the Keechelus reservoir to the mouth. Elevations range from 2494 meters in the Cascade Range, to 104 meters at the mouth of the Columbia (YVCG 1993). Rainfall is variable, as the river runs through high mountains with 356 centimeter mean-annual precipitation, to dry shrub steppe with less than 25 centimeters (YVCG 1993). Streams sampled for this study are all found at river kilometer 172 and above, in the cooler and wetter mountain zones.

At the turn of the last century, agriculture became a major economic factor in the Yakima River basin. In the early 1900s, the federally managed Bureau of Reclamation began the construction of a series of dams in the Yakima Basin to take advantage of the abundant snow falls in the Cascade Mountains. Water for irrigation became available to the arid, but fertile valleys of central Washington. Mid-elevation lakes (900-1200 meters) were converted to holding reservoirs, so that water could be allocated to farmers and other water users in the basin on a regulated basis. None of the reservoirs were built to allow for fish passage, thus bull trout and other fish populations have become physically isolated.

Bull trout in the Yakima River basin have been delineated into “stocks” based primarily on presumed life history traits and geographic location. Each stock may contain





1. South Fork Tieton River
2. Indian Creek
3. American River
4. Union Creek
5. Rattlesnake Creek
6. Crow Creek
7. Box Canyon Creek
8. Mineral Creek
9. Deep Creek
10. Gold Creek
11. Ahtanum drainage
12. North Fork Teanaway

Figure 1 Location of bull trout populations sampled in the Yakima River basin of central Washington state

one or more known spawning tributaries (see Table 1). For this study, bull trout "populations" are defined as a group of interbreeding individuals in a specific stream. Thus each stock may have multiple populations of bull trout. Stocks are described here as they are currently recognized by the Washington Department of Fish and Wildlife, and these delineations are used for management purposes (WDFW 1997). Five of the recognized bull trout stocks are adfluvial, residing in reservoirs and spawning in tributaries to these lake systems. Two river systems, the American-Naches and the Yakima River are considered to have stocks of fluvial bull trout, with various spawning tributaries. There are also two resident populations, delineated as such for their small adult size and the presence of thermal and water quality barriers.

Of the five reservoirs with adfluvial bull trout, four were sampled for this study (see Table 1). Bumping Lake, where the first dam was built in 1910, has only one spawning tributary, Deep Creek. The two reservoirs in the Upper Yakima River system, Kachess Lake and Keechelus Lake, were dammed in 1912 and 1917 respectively. Kachess Lake has two known spawning tributaries, Mineral Creek and Box Canyon Creek, while Keechelus Lake has only one, Gold Creek. Rimrock Lake, above the Tieton River, is the only reservoir that was not a natural lake prior to impoundment in 1925 and has two spawning tributaries, Indian Creek and South Fork Tieton River (WPRS 1981). The Cle Elum-Wapatus Lake stock was excluded due to lack of information about bull trout spawning areas and basic stock attributes (WDFW 1997). Thus, six adfluvial populations were sampled overall (see Table 1).

Only one of the two fluvial stocks was sampled, the American-Naches stock.

There is little information available about the remnant Yakima stock found in the Upper Yakima River, and therefore it was not sampled. Four known spawning tributaries within the American-Naches river system were sampled for this study: American River, Union Creek, Rattlesnake Creek, and Crow Creek. Though there is high recreational use in this area, a large part of the drainage area is within designated wilderness areas, and much of the bull trout spawning occurs in these areas of high water quality. There are no known migration barriers.

The remaining two populations that were sampled are classified as resident stocks. In the Ahtanum drainage, which supports the furthest downstream population of bull trout in the Yakima River, the North Fork, Middle Fork and South Fork were all sampled, and analyzed as one population. The other resident population included in this study was the North Fork Teanaway stock. Both of these river systems are impacted by irrigation practices in the lower stretches. During the summer months, the lower stretches are largely de-watered, and inadequate screening on irrigation diversions has adversely affected bull trout (WDFW 1997). The timing of agricultural water needs coincides with bull trout spawning migrations, and limits the movement of adult fish into these systems. It is uncertain whether migration occurs during winter months when irrigation practices are not drawing water from the rivers.

In total, twelve spawning populations were sampled for this study, six adfluvial, four fluvial and two resident populations. The fluvial populations are the only bull trout in the Yakima basin with no known barriers to migration between spawning tributaries. The adfluvial populations have been isolated for 78-93 years behind irrigation dams with

no fish passage. A mean generation interval for bull trout has been estimated at 5 years (Pratt 1992). Thus, this period of isolation represents 15-19 bull trout generations. The migratory potential of resident populations has been impacted by irrigation practices as well, though physical barriers have primarily been seasonal (WSDOE 2003).

Field Methods

The genetic samples used for this study were collected over a period of time from 1996-2001. At Rimrock Lake, samples were collected at both Indian Creek and South Fork Tieton River during the fall of 1996. A weir and box trap were set at the mouth of these rivers to capture adult bull trout as they returned to the reservoir after spawning. Fish were measured and weighed, and genetic samples were taken from the anal fin. Samples from Deep Creek, the single spawning tributary to Bumping Lake, were obtained in the same manner in 1997. Tissue samples were stored in 95% ethanol at room temperature until DNA was extracted in 2001.

All other samples were obtained from juvenile/resident fish by netting during night snorkeling surveys, adapted from methods of Thurow (1994). Distribution of juveniles in the spawning/rearing streams was largely unknown, but most sampling was done in spawning index areas during the summer months, before redds and spawning fish were present. Fish were netted using 10, 15, or 20 centimeter aquarium nets, and held in a storage container until processing. Using a plastic bag marked with five millimeter increments, fork length was measured for each fish, and a tissue sample was taken from the caudal fin. No anesthetic was necessary using this method, thus reducing possible

long term effects to the fish sampled. Fin tissue was stored in 95% ethanol at 4° C.

Samples from Mineral Creek, a tributary to Kachess Reservoir, were collected in 1997. All other samples for this study were collected in the summer of 2001 by the author, with field assistance and equipment furnished by the Okanogan-Wenatchee National Forest. For most surveys, two snorkelers were in the water capturing fish, and one or more people were on the banks carrying equipment, recording data and taking genetic samples. The author was present for all snorkeling surveys, along with other volunteers of varying experience. Stream reaches were sampled by one-pass surveys, with several reaches sampled for each stream, to avoid re-sampling fish, and to minimize number of siblings.

Sampling Sites

The following is a specific description of sampling sites for each stream where bull trout genetic samples were collected. This information is included as it may be useful in future studies seeking to locate juvenile bull trout habitat, or for local biologists interested in general densities and conditions.

South Fork Tieton River

South Fork Tieton River is one of two tributaries to Rimrock Lake reservoir. Twenty samples from this stream were collected in September 1996, and 10 in September 2000 by Dr. Paul James and students from Central Washington University, as part of a long term demographic study for the Bureau of Reclamation (BOR). Genetics samples were collected for future analysis from out-migrating adult bull trout. The weir and box

trap for this tributary were placed upstream from the mouth of the river, at the primitive South Fork campground. Fish collected in 1996 were tagged, thus preventing sampling of the same individual in 2000.

Indian Creek

Indian Creek is the other tributary to Rimrock Lake with a population of spawning bull trout. Post-spawning adult fish were sampled in this stream in September 1996. Sampling location was a box trap and weir constructed 100 meters upstream from the Highway 12 bridge crossing.

American River

The American River is a mainstem river flowing into the Naches River, and is one of the only unregulated subbasins in the Yakima River basin. There is a population of fluvial bull trout that spawn in upper sections of this river, and are part of the American-Naches bull trout stock. Thirty fish were sampled here in two nights. On the first night, the snorkel survey was started at the Hell's Crossing campground (river km 9.5), just below the Hwy 410 bridge crossing. Eighteen juvenile bull trout were captured and sampled within 200 meters. The remaining fish were sampled further upstream, in a 300 meter section paralleling the Pleasant Valley campground (river km 16.2). We began at the large pool at the lower end of the campground, and ended below the mouth of Kettle Creek. During the day, large fluvial adults were seen in this stretch, but during genetic sampling only juveniles were seen and captured.

Union Creek

Union Creek is a short tributary to the American River, and is part of the American-Naches stock. Less than one kilometer from the mouth there is a large waterfall which is a migration barrier. However, this stream accounts for the majority of the bull trout redds found in the American River system (E. Anderson, personal communication). Genetic samples from this stream were collected separately due to this concentration of redds. Sampling for this stream was completed in two nights of snorkeling, covering the lower half of the stream in the first night, and the upper half the second night, starting 50 meters below the Hwy 410 bridge. Approximately 500 meters of the stream were snorkeled. A total of 11 bull trout were seen and captured, but it was not possible to sample further without risking re-sampling individuals. Only juvenile bull trout were sampled.

Rattlesnake Creek

Rattlesnake Creek is a tributary to the mainstem Naches River, and the majority of bull trout spawning takes place above the William O. Douglas wilderness boundary, 18 kilometers from the mouth. Due to difficult access to this stream, genetic sampling was done in conjunction with redd surveys in September. Thirty samples of juvenile fish were collected in two nights of snorkeling, and an additional three samples were collected from adult carcasses found along the stream. Though adult spawning bull trout were encountered during snorkel surveys, they were deliberately excluded from sampling to avoid handling pre-spawning, sensitive fish. In total, 800 meters of stream was sampled, ending at the MJB trail crossing.

Crow Creek

Crow Creek is a tributary to the Little Naches River, and in this stream the bull trout spawning index area begins five miles from the mouth, in the Norse Peak wilderness. Thirty-one juvenile fish were sampled in two nights of snorkeling. The first night's sample encompassed 1000 meters of stream, from the 1922 trail crossing to the wilderness boundary. Ten fish were caught in this distance. The second night was begun at the wilderness boundary (river km 18.3), and snorkeled through 2000 meters of patchy habitat, finding only 6 bull trout. However, in the confined area of the stream, 15 bull trout were located easily within the next 200 meters of snorkeling. Densities were much higher in this area, and fish were concentrated in flat terraces and in the shallow margins of bedrock pools.

Box Canyon Creek

Box Canyon Creek is one of two spawning tributaries to Kachess Lake reservoir. Thirty-one samples from this stream were collected in two nights in August of 2001. On the first night, snorkel surveys started approximately 400 meters below the barrier falls (Peek-a-Boo Falls), where the washout from an old road intersects the creek, and continued to the falls. The second night, snorkeling began 300-400 meters below the first starting site, and included the wide, gravelly area where most of the spawning in this stream occurs. Both reaches are upstream of the confined "box" canyon. Juvenile fish and possible resident adults were sampled.

Mineral Creek/Kachess River

Mineral Creek is the other tributary to Kachess Lake with a population of spawning bull trout. There is some confusion in local and published references to this spawning stream. The stream that flows in to the reservoir is the upper Kachess River, and the confluence with Mineral Creek is approximately two kilometers from the mouth. Both streams have large barrier falls within one kilometer from this confluence, thus the habitat available for juvenile/resident bull trout is limited. Most of the spawning takes place on the Kachess River fork, but genetic samples for this study were collected from Kachess River below the confluence of the two streams, and up Mineral Creek to the falls. The samples are listed in the Washington State Department of Fish and Wildlife (WDFW) database as Mineral Creek, thus the name of the creek remains the same in this study. Other publications and studies may refer to this spawning area as Mineral Creek, or alternately, Kachess River (WDFW 1997; Meyer 2002; Spruell and Maxwell 2002).

This site was sampled in the fall of 1997 by Paul and Brenda James, plus additional snorkelers from the WDFW Olympia office. All 30 samples were collected in one night of snorkeling. Primarily juvenile fish were sampled and possibly resident adults. Snorkeling survey started below the confluence of Mineral Creek and Kachess River, where Trail #1331 crosses the stream, and continued up along Mineral Creek to the barrier falls. Tissue samples were taken to the WDFW genetics lab in Olympia for long term storage, and sub-samples from this source were obtained for this study. All associated data collected with the samples are archived by WDFW as well.

Deep Creek

Deep Creek is the sole bull trout spawning tributary to Bumping Lake reservoir. As part of the study of bull trout in BOR managed reservoirs conducted by Dr. Paul James, fish in Deep Creek were sampled in August 1997. A box trap and weir were placed approximately 100 meters below the double-culvert road crossing (USFS Road 1800), adjacent to a left bank gravel bar. All fish captured were post-spawning adults.

Gold Creek

Gold Creek is the only spawning tributary to Keechelus Lake reservoir, at the upper most end of the Yakima Basin. This stream was extremely difficult to sample, due to low densities of bull trout and flashy, erratic water conditions. In the fall of 2000, a weir dam and box trap was constructed to capture outgoing adfluvial adults. Only one fish was caught and sampled before the weir dam was washed out. The remaining 20 samples were collected during three separate nights snorkeling, twice in August and once in October of 2001.

The first area sampled was 6.8 kilometers upstream from the I-90 bridge crossing, at the first stream crossing of Trail #1314. Water flows were very low, and there was very little available habitat. Three juvenile bull trout were caught and sampled in approximately 300 meters. The second night was begun at the large pool below the I-90 bridge and snorkeled up to the confluence of the main channel with the artificial kokanee spawning channel. The sampling effort then moved upstream to river kilometer 3.1, where there was no water flowing, only isolated pools. In all, six juvenile bull trout were sampled that night and 1000 meters were snorkeled. The final night of snorkeling was in

early October. The sampling began at river kilometer 4.6, where Trail #1314 drops down to the stream. Water flows were high due to fall rains, and most of the bull trout were concentrated in slack water refugia, thus making locating and capturing them more feasible. Eleven juvenile bull trout were sampled in 300 meters. Due to the high elevation of the site, and early snow, it was not possible to return for more samples prior to lab analysis.

Ahtanum Creek

In this stream, three forks (North, Middle and South) were sampled for a combined total of 30 fish, which were analyzed as one population. In total, six nights were spent snorkeling these reaches. South Fork Ahtanum appeared to have the lowest densities. On the first night, sampling was begun 4.8 kilometers from the end of the paved road. Stream habitat appeared adequate, and bull trout had been recorded in this stretch in previous years (K. Gullet, personal communication 2001), but no bull trout were found in 600 meters of survey. The second night, sampling began approximately 8 kilometers further upstream, where the road crosses the stream. In 300 meters, 6 bull trout were captured.

Middle Fork Ahtanum had the highest densities of bull trout. Two nights of snorkeling, one in August and one in October, yielded 16 samples. The first night, sampling began at the lower end of Tree Phones campground, and continued to 50 meters past the first road culvert (300 total meters). Seven bull trout were captured, and at least that many more were seen, but not captured due to stream turbidity. The second night, in October, water temperatures were low and fish were slower and easier to capture. Nine

fish were caught in a 300 meter reach 1.6 kilometers downstream of Tree Phones campground.

North Fork Ahtanum was sampled in two nights as well. The first night, snorkeling was along the stream parallel to Snow Cabin campground, covering 300 meters. Five juvenile/resident bull trout were sampled. The second night, 3 fish were sampled in 400 meters of stream starting at the Grey Rock Trail crossing.

North Fork Teanaway River

The North Fork Teanaway River is an upper basin tributary to the Yakima River. There is little information about bull trout populations in this system, but numbers appear to be historically low (WDFW 1997) and recent flooding events in the winter of 1995-1996 have further degraded the available habitat. It is unknown whether adults spawning in this system are primarily resident (as delineated by WDFW) or possibly fluvial (P. James, personal communication 2003). The stream was sampled in one night, by four experienced snorkelers. Ten fish were captured in approximately 3000 meters of snorkeling, starting below Camp Wahoo and moving upstream in the North Fork Teanaway mainstem.

Laboratory Methods

Microsatellites are short (2-9 base pair) repeating segments of the nuclear DNA which follow patterns of Mendelian inheritance (Wirgin and Waldman 1994; Avise and Hamrick 1996). This is a qualitative marker, meaning that it is not an actual gene which affects the phenotype of the individual. Qualitative markers such as microsatellites are

often used in studies of genetic structure in place of quantitative markers which are generally more difficult to isolate and are influenced by selection pressures (Merila and Crnokrak 2001; Moran 2002). Microsatellites are located in non-coding regions of the DNA and are therefore assumed to be under neutral selection. Mutation rates are relatively high, on the order of 10^3-10^5 mutations per generation, though these rates appear to be variable across taxa and between loci (Schug et al. 1997; Brohede 2003). These factors make microsatellites a useful molecular marker for looking at allele frequencies changes within and among populations over a short evolutionary time scale, as differences accumulate rapidly and they are highly polymorphic (Phillips and Pleyte 1991; Balloux and Lugon-Moulin 2001). Another important factor in the use of microsatellites is they are readily amplifiable from small pieces of animal tissue. Unlike allozyme analysis, which requires liver tissue and, consequently, the sacrifice of the animal, microsatellites can be amplified from small or damaged tissue, using polymerase chain reaction (PCR) techniques.

Tissue samples from this study were brought into the lab in various conditions. Samples collected in 1996 were stored at room temperature from the time of collection, and in some samples the alcohol had evaporated. Samples from the WDFW lab were stored at 4 °C, but then shipped at ambient temperatures to the laboratory at Central Washington University (CWU). All samples collected in 2001 were kept at 4 °C throughout the entire process. In the beginning stages of DNA extraction, some experimentation was done to determine whether DNA concentrations would vary with conditions of tissue storage, but all samples yielded sufficient DNA for successful PCR.

DNA was extracted from fin tissue with a Puregene® DNA purification kit by Gentra Systems, using a protocol slightly modified for fish tissues with a high polysaccharide content. Sub-samples of all extractions were electrophoresed through 2% agarose gels at 100 volts for 20-30 minutes to determine quality and concentration. DNA concentrations overall were high and, for most samples, 1:25 dilutions with de-ionized water yielded optimal results during PCR.

Microsatellite loci were amplified by PCR. Each reaction requires certain components: individual DNA, a specially designed primer to anneal to the segment of DNA flanking the locus, deoxynucleotide triphosphates or dNTPS (adenine, cytosine, thymine and guanine), magnesium chloride ($MgCl_2$), a buffer solution, and *Taq* polymerase in a 10 micro-liter (µL) reaction. The reactions for the primers used contained 1-2 µL DNA, 0.25-0.30 µL each of 5' and 3' primers, 0.08-0.10 µL of *Taq* polymerase, and 1 µL each of 2 mM dNTP mix, 25 mM $MgCl_2$, and 10x reaction buffer, plus sufficient de-ionized water to bring the reaction to 10-µL. During the PCR process, the reaction is first heated to separate the double strands of DNA (denaturing). The mixture is then cooled to allow the primer to anneal at a designated location, flanking the desired sequence within the DNA. New double strands are built, using free nucleotides in the mixture, during the process of extension. The process is repeated (19-30 times) until a large quantity (millions of replicates) of the desired product has been made, or "amplified." In the case of microsatellites, this product is a fragment of nuclear DNA with a certain number of base pair repeats. An MJ Research PTC-100 thermocycler was used to amplify DNA, following primer profiles described by the original laboratories,

and then modified for maximum product (Table 2). Changes to the protocol involved increasing the number of cycles.

Table 2 Microsatellite loci used, with number of alleles, size range for bull trout in the Yakima River basin populations and original reference

Locus	Number of alleles	Size range	Reference
FGT-3	6	157-175	Sakamoto et al. 1994
Oneμ7	2	218-244	Scribner et al. 1996
BT73	2	140-144	Estoup et al. 1993
SSA456	2	157-159	Slettan et al. 1995
SSA311	2	112-120	Slettan et al. 1995
OTS101	2	100-112	Small et al. 1998
SFO18	1	150[a]	Angers et al. 1995

[a] SFO18 amplified in Ahtanum Creek samples only.

Originally, seven microsatellite loci known to be polymorphic in bull trout were chosen for this study. Of these, one locus, SFO18, is primarily useful in determining groupings of coastal versus inland populations (Spruell et al. 2003). It was expected that all of the populations in the Yakima River basin would be monomorphic at the SFO*150 inland allele. However, one of the streams in this study, Ahtanum Creek, has headwaters within a few miles geographically of the headwaters of the Klickitat River. Spruell, in examining a selection of samples from the Columbia River, found bull trout from the Klickitat River to be monomorphic for the coastal allele SFO*156 (P. Spruell, personal

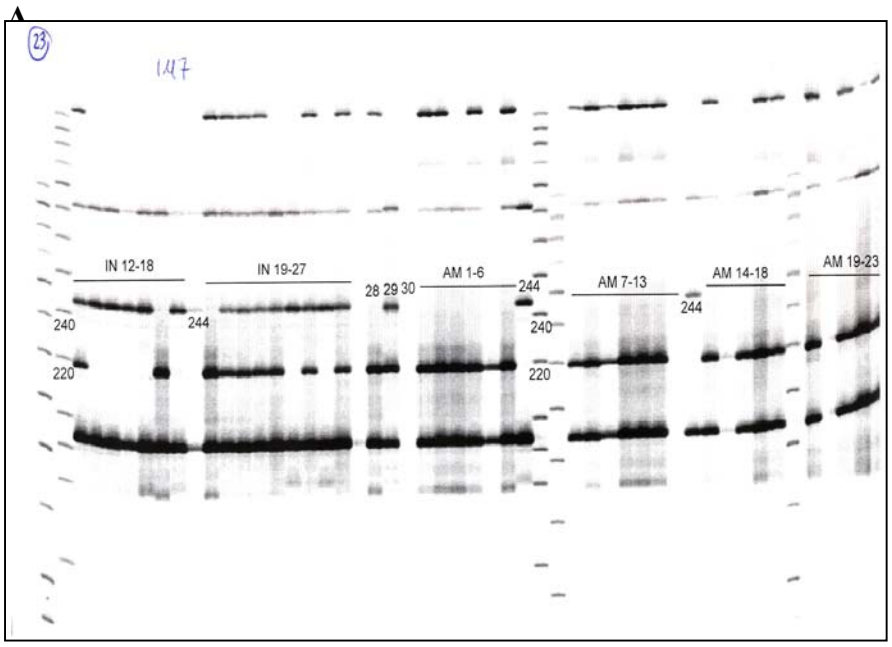
communication 2002). To test for possible connectivity between these systems, due to human intervention or changes in flow regimes, Ahtanum samples were amplified at this locus. All other populations were amplified only with six microsatellite loci (see Table 2).

All of the above lab work was conducted at Central Washington University. Amplified samples were then taken to the Wild Trout and Salmon Genetics Lab (WTSGL) at the University of Montana and run on 7% denaturing polyacrylimide gels to separate the differently sized fragments. Six µL of a formamide/loading buffer mixture was added to each 10-µL reaction, and the DNA mixture was denatured in an 80 °C waterbath for 5-10 minutes immediately prior to loading the gel. Gels were preheated for 20-30 minutes, and run at 1600-1800 volts for 2-3 hours. This allowed sufficient time for the product to separate on the gel. Each gel was loaded with Yakima River samples, other amplified samples at the same locus of known sizes, and a standard base pair DNA ladder (MapMarkerLow, Bioadventures ®). The ladder is designed to help in locating samples on the gel, and contains fragments of DNA in various sizes, in this case bands 70-400 bases long. Gel images were captured using Hitachi FMBIO-100 fluorescent imager to determine allele size for each sample (Figure 2).

Data Analysis

FSTAT 2.9.3 (Goudet 2001) was used to calculate descriptive genetic statistics which included: observed and expected heterozygosities, Weir and Cockerman's (1984) F_{ST} and F_{IS} values per population and per locus. Pair-wise comparisons were made using

A



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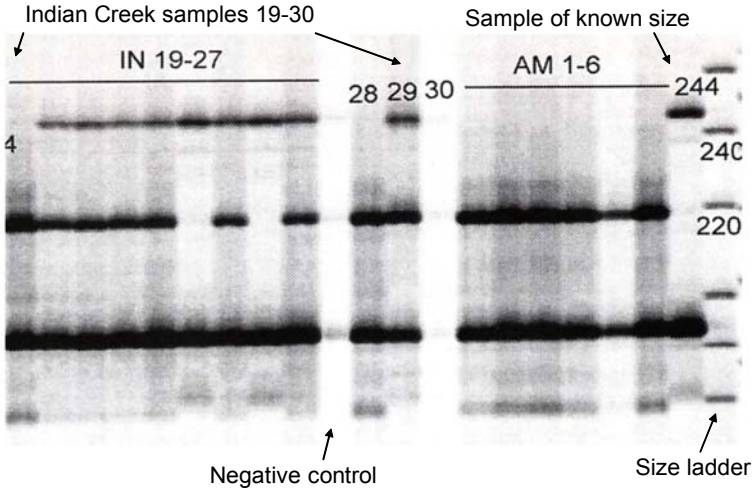


Figure 2 Polyacrylimide gel with amplified samples from Yakima River basin at the Oneμ7 locus. A) Image of completed gel. B) Detail from gel.

the log likelihood ratio of exact tests of genotypic differences, and strict Bonferonni corrections were applied to the nominal values needed for significance (Goudet et al. 1996). Deviations from Hardy-Weinberg equilibrium, allele frequencies and genotypic linkage disequilibrium were calculated using GENEPOP (Raymond and Rousset 1995). Two Hardy Weinberg global tests were run, for heterozygosity excess and deficiency, using the Markov chain method to estimate p-values (dememorization, number of batches and iterations per batch were set to recommended default values of 1000, 100 and 1000 respectively).

PHYLIP version 3.5 (Felsenstein 1993) was used to construct phylogenetic trees. For the consensus tree, allele frequency data was input into SEQBOOT using 1000 bootstrap replicates and a Cavalli-Sforza Edwards (CSE) chord distance matrix was generated for each replicate using GENDIST. These matrices were entered into the program NEIGHBOR and trees were created with the Unweighted Pair-Group Method Average (UPGMA). The program CONSENSE was then used to determine the best fit tree and this was visualized using TreeView (Page 1996). This method of tree construction creates a rooted tree with "true" branch lengths and assumes an evolutionary clock is valid. CSE chord distances were used instead of Nei's distance measures due to the inability to assume a constant population size, and to account for possible homoplasy within allele sizes. Neighbor joining, Fitch-Margoliash, and maximum likelihood trees were also created using PHYLIP.

Principal component analysis of complete genotypes within populations was also used to examine clustering patterns. PCA-GEN (Goudet 1999) for genetic data was used

to create the graph and to calculate per axis inertia and p-values. For regression data, MINITAB version 13.32 was used. Mantel nonparametric test calculator for Windows, version 2.0 (Liedloff 1999) was used to analyze significance of distance matrices for isolation by distance regressions.

CHAPTER III

RESULTS

Field Sampling Results

Genetic samples were collected from twelve streams in the Yakima Basin. The goal was to collect a minimum of 30 samples from each population, and this was achieved in nine of the 12 streams (Table 3). The smallest number of samples collected was 10 in the Teanaway River, where bull trout densities are extremely low, and sampling effort was only one night of snorkeling. Both adult and juvenile bull trout were sampled for this study.

Within each population, multiple age classes were sampled (Figure 3). There have been a variety of studies done on length/age class relationships in bull trout (Fraley and Shepard 1989; Pratt 1992; Rieman and McIntyre 1993) and Goetz (1989) shows a compilation of this data from throughout the Pacific Northwest. As with any growth parameter, there are local differences based on stream temperatures, life history traits, food availability and location. For the age class frequency data, an average of all possible lengths were estimated at each year class, based on a table of lengths from various studies provided in Goetz (1989; Table 4).

No zero class fish (< 50 millimeters) were sampled for this study due to the difficulty of obtaining a sufficient amount of tissue without damaging effects to the fish. In nine of the 12 populations, snorkel sampling was done in the stream, and all fish sampled were age class I-IV or resident adults. Bull trout in the 200+ millimeter size

Table 3 Summary of genetic sampling efforts for Yakima River basin bull trout

Population	Samples	Age class	Date of sampling	Stream temp (°C)	Length of stream (m)
Rimrock Lake					
South Fork Tieton	30	Adult	September 1996		Stationary trap
Indian Creek	30	Adult	September 1996/2000		Stationary trap
American-Naches					
American River	30	Juvenile/resident	July 2001		500
Union Creek	11	Juvenile/resident	July 2001	11	600
Rattlesnake Creek	33	Juvenile/resident	September 2001	11	800
Crow Creek	31	Juvenile/resident	August 2001	11	3200
Kachess Lake					
Box Canyon Creek	31	Juvenile/resident	August 2001	13	500
Mineral Creek	30	Juvenile/resident	October 1997		700
Bumping Lake					
Deep Creek	30	Adult	September 1997		Stationary trap
Keechelus Lake					
Gold Creek	21	Juvenile/resident	August/October 2001	9/8/6	1600
Ahtanum drainage					
South Fork	6	Juvenile/resident	August 2001	16/11	900
Middle Fork	16	Juvenile/resident	August/October 2001	9/4	600
North Fork	8	Juvenile/resident	September 2001	11/13	700
NF Teanaway	10	Juvenile/resident	October 2001		3000+

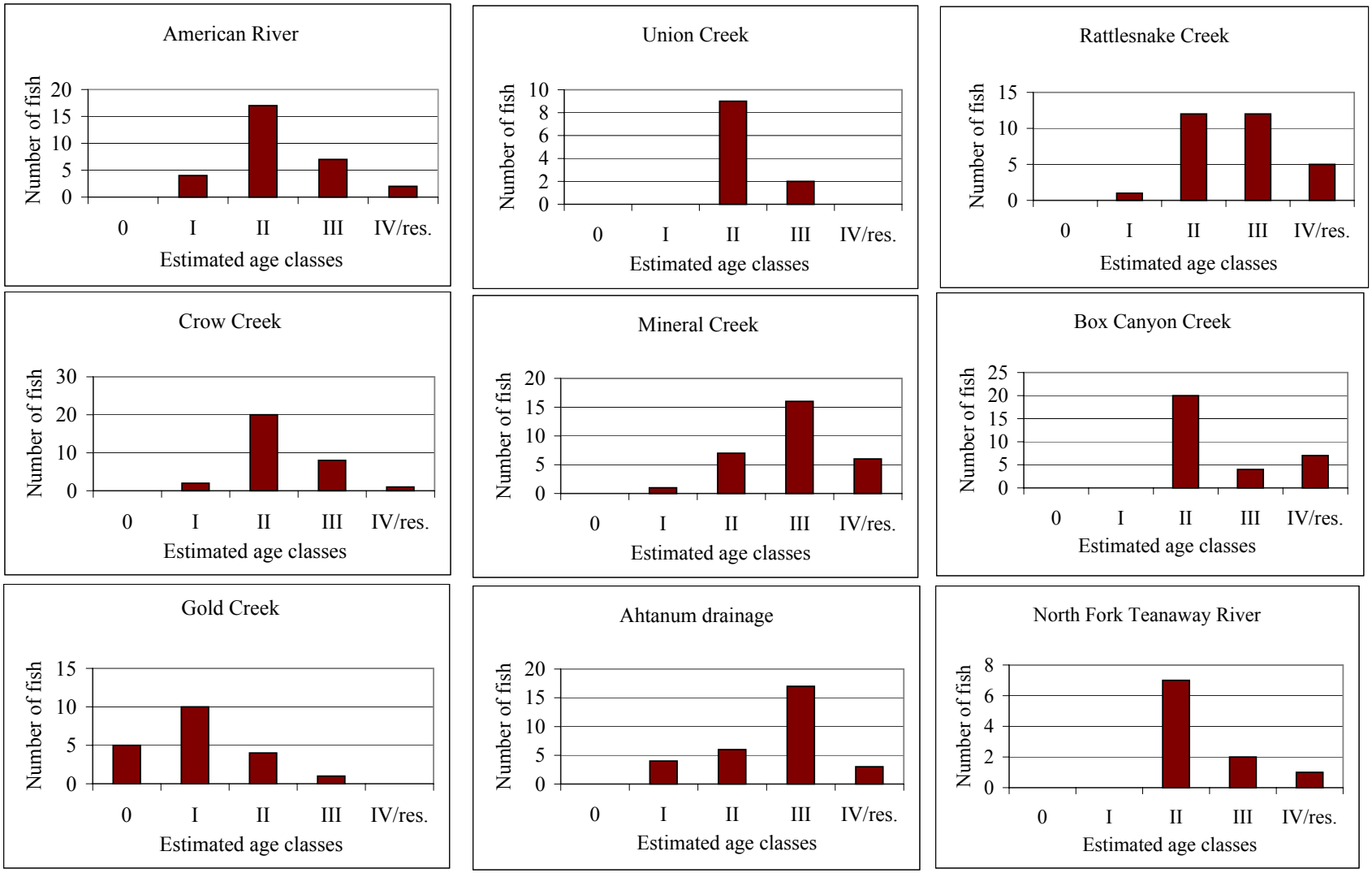


Figure 3 Age class frequencies for Yakima River basin bull trout populations sampled via snorkeling . See Table 4 for age class sizes.

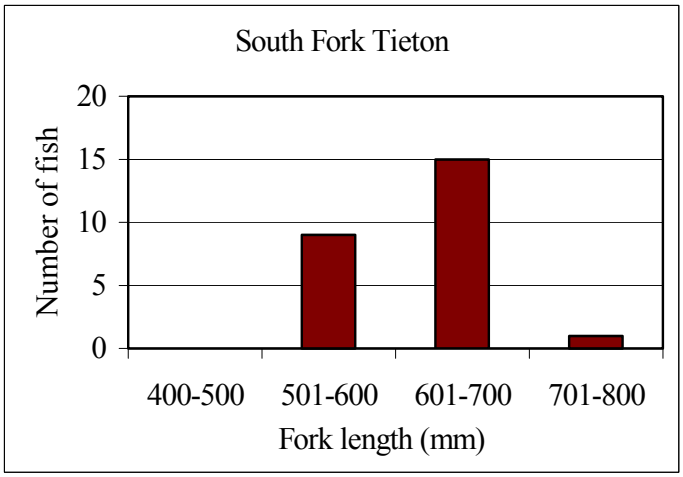
Table 4 Age class estimates for various size classes of juvenile bull trout[a]

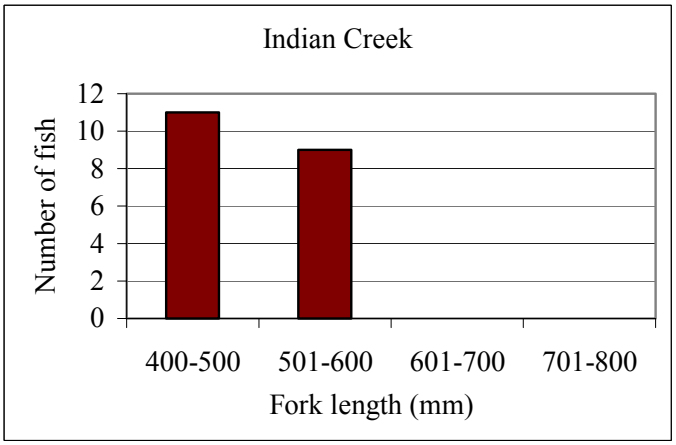
Age class	Fork length (mm)
0	0-50
I	51-100
II	101-150
III	151-200
IV/resident	201+

[a] Adapted from Goetz 1989.

class are problematic to categorize. At this size it is difficult to distinguish between age class IV adfluvial/fluvial juveniles and adult resident fish. For this study, fish larger than 200 millimeters are not distinguished between age class IV juveniles and resident adult fish. Most sampling was not done during spawning, except in Rattlesnake Creek, where large fluvial adults were excluded from sampling. This prevented the snorkelers from distinguishing resident adults based on spawning coloration.

In the three streams where only adult bull trout were sampled, there was also a diversity of sizes represented (Figure 4). Age class/length relationships among adult adfluvial fish is highly variable with available habitat and food sources, but with bull trout iteroparity, size differences within a breeding cohort presumably represent some degree of diversity among year classes. For South Fork Tieton River, where 20 samples are from 1996, and 10 are from 2000, there was no significant difference in average size between years (t = -0.23; P = 0.82).





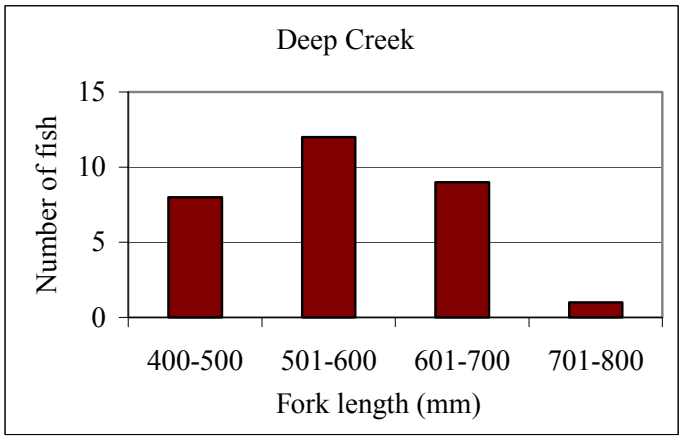


Figure 4 Size class frequencies in the three adfluvial populations where adult bull trout were sampled

Table 3 contains a summary of sampling information, including number of samples collected in each stream, dates of sampling and whether fish were primarily adult or juveniles. Also included are temperatures for each day of snorkel sampling, to show the variation between sites. Extreme temperatures found were a low of 4 °C in Middle Fork Ahtanum in October, to a high of 16 °C at South Fork Ahtanum in August. This temperature is considered the upper limit of bull trout thermal tolerance (Fraley and Shepard 1989), and in this reach of stream, no bull trout were located. Length of stream sampled correlated with number of fish captured is an informal measure of bull trout relative densities in sampled reaches (Figure 5).

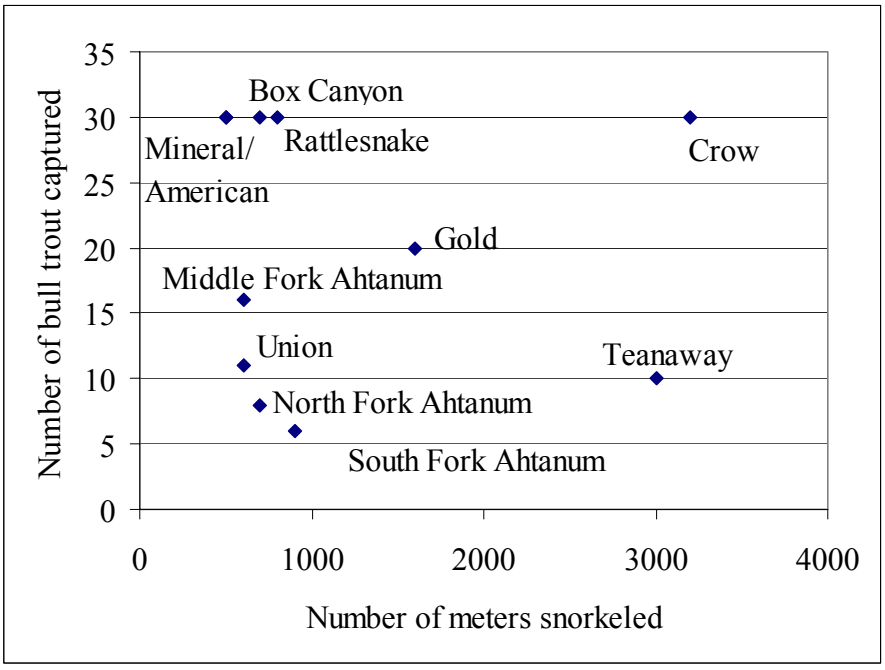


Figure 5 Relative estimates of juvenile bull trout densities in each stream snorkeled

Genetic Results

The six loci that were tested at all populations were found to be generally polymorphic (Table 5). An additional locus, SFO18, was only amplified in one population, Ahtanum Creek. It was found to be monomorphic for the "inland" allele (SFO*150) and was not included in further analysis.

The average number of alleles per locus was low (2.6), with the total number of alleles present per population at all six loci ranging from 9-16 (Table 6). Only one locus, FGT3, had more than two alleles present within a population. Most populations had 5-6 FGT3 alleles present, and four populations: Box Canyon Creek (BX), Mineral Creek (ML), Deep Creek (DP), and North Fork Teanaway (TN) had only two alleles. Allelic richness ranged from 1.5 in North Fork Teanaway to 2.5 in South Fork Tieton (see Table 6). Population size appeared to be a contributing factor to patterns of allelic diversity, as there was a relationship showing a positive trend between average number of redds per year (an indicator of population size) and the total number of alleles observed across all loci (Figure 6; $r = .28$; $F = 3.87$; $P = 0.07$).

Within Population Variation

Expected heterozygosities (H_E) ranged from 0.23 for Deep Creek, to 0.44 for Indian Creek (see Table 6). The average expected heterozygosity was 0.33. There was no significant relationship between population size (based on annual redd count data) and expected population heterozygosities (Figure 7; $r = 0.20$; $F = 2.56$; $P = 0.14$). However, Deep Creek was a significant outlier, and when this population was removed, a

Table 5 Allele frequencies and sample sizes (n) at six polymorphic loci for bull trout in the Yakima River basin[a]

	SFT	IN	AM	UN	RT	CR	BX	ML	DP	GL	AH	TN
Loci/alleles												
FGT-3												
n	**25**	**28**	**29**	**9**	**28**	**26**	**29**	**27**	**21**	**20**	**29**	**10**
157	0.34	0.23	0.05	0.05	0.32	0.14	0	0	0.36	0	0	0
163	0.06	0.03	0.27	0.22	0.05	0.14	0	0	0	0.03	0.65	0
165	0.20	0.35	0.08	0.22	0.08	0.15	0.43	0.54	0	0.48	0	0.20
167	0.04	0	0.08	0	0	0	0	0	0	0.15	0	0.80
173	0.14	0.23	0.29	0.33	0.17	0.12	0	0	0.64	0.20	0.19	0
175	0.22	0.14	0.20	0.16	0.35	0.46	0.57	0.46		0.15	0.16	0
Oneμ7												
n	**26**	**29**	**30**	**11**	**30**	**31**	**29**	**28**	**27**	**19**	**30**	**10**
218	0.92	0.44	1	0.95	0.98	0.98	0.49	0.45	0.28	0.55	0.93	1
244	0.07	0.55	0	0.04	0.01	0.02	0.52	0.55	0.72	0.45	0.07	0
BT73												
n	**21**	**27**	**30**	**10**	**30**	**30**	**29**	**27**	**24**	**18**	**27**	**10**
140	0.20	0.16	0	0	0.02	0.08	0.29	0	0.02	0	0.44	0
144	0.80	0.83	1	1	0.98	0.92	0.71	1	0.98	1	0.56	1
SSA456												
n	**21**	**28**	**30**	**10**	**29**	**30**	**27**	**26**	**26**	**16**	**28**	**10**
157	0.81	0.80	0.87	0.8	0.81	0.83	0.96	1	0.92	1	0.54	0.50
159	0.19	0.20	0.13	0.2	0.19	0.17	0.04	0	0.08	0	0.46	0.50
SSA311												
n	**21**	**28**	**30**	**10**	**30**	**30**	**30**	**26**	**28**	**17**	**27**	**10**
112	0.42	0.39	0.12	0.05	0.42	0.15	0.25	0.52	0.07	0.12	0.67	1
120	0.57	0.61	0.88	0.95	0.58	0.85	0.75	0.48	0.93	0.88	0.33	0
OTS101												
n	**21**	**29**	**30**	**10**	**30**	**29**	**30**	**24**	**25**	**15**	**28**	**10**
100	0.81	0.85	0.57	0.65	0.77	0.66	0.58	0.44	0.92	0.62	0.66	0.45
112	0.19	0.15	0.43	0.35	0.23	0.34	0.42	0.56	0.08	0.38	0.34	0.55

[a] Allele sizes are given as number of base pairs and sample sizes as amplified for each locus. Population identifiers are as in Table 1.

Table 6 Descriptive statistics for bull trout populations in the Yakima River basin[a]

Populations:	SFT	IN	AM	UN	RT	CR	BX	ML	DP	GL	AH	TN
H_E	0.40	0.44	0.29	0.30	0.33	0.32	0.40	0.34	0.23	0.32	0.43	0.23
H_O	0.41	0.33	0.26	0.21	0.36	0.30	0.45	0.38	0.23	0.35	0.36	0.32
Alleles	16	15	14	14	15	15	12	10	12	13	13	9
Richness	2.52	2.41	2.21	2.29	2.19	2.32	1.93	1.67	1.81	2.07	2.12	1.50
F_{IS}	-0.03	0.26	0.12	0.32	-0.40	-0.08	0.05	0.16	-0.15	-0.01	-0.02	-0.02

[a]Included: Expected heterozygosity (H_E), observed heterozygosity (H_O) total number of alleles per population (alleles), allelic richness (richness), and overall F_{IS}. Population identifiers are as in Table 1.

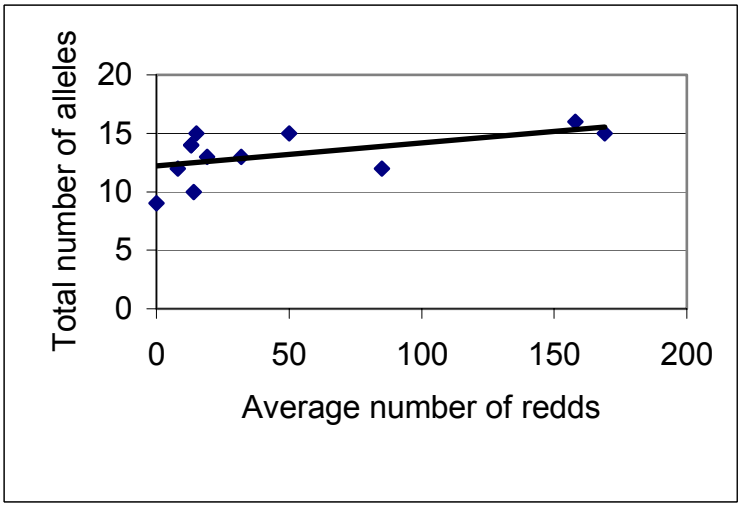
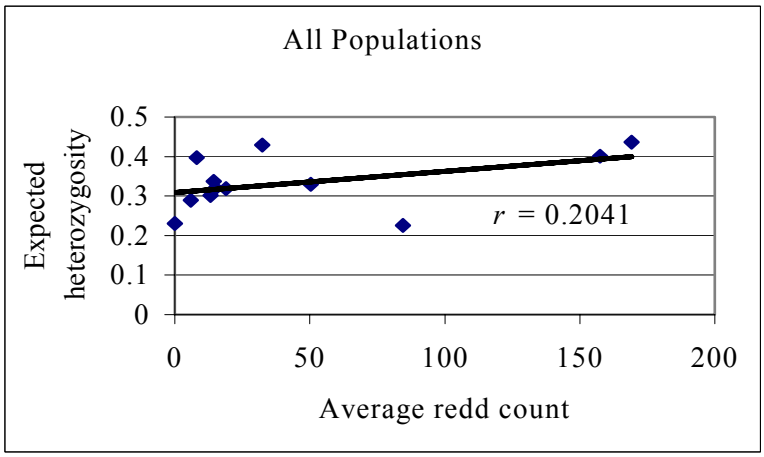


Figure 6 Correlation between the total number of alleles at all six loci per population and average annual redd counts (indicator of population size). There is a positive trend ($r = 0.28$, $F = 3.87$, $P = 0.07$).

A



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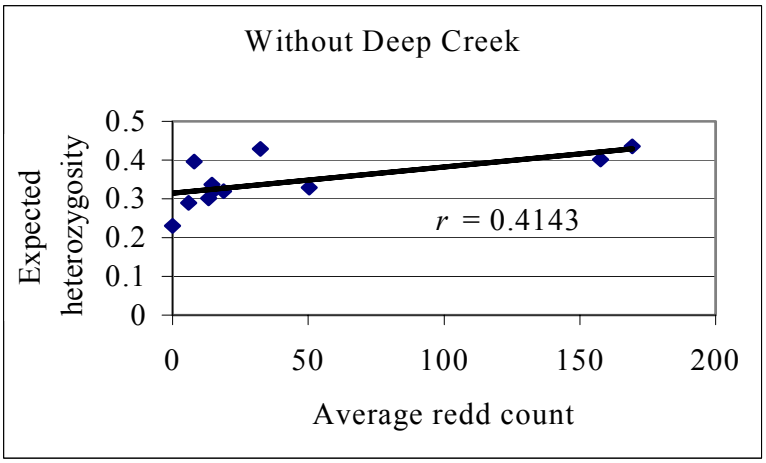

Figure 7 Correlation between expected heterozygosity (genetic diversity) and average annual redd counts for Yakima River basin bull trout populations. A) All of the populations included ($r = 0.20$, $F = 2.56$; $P = 0.14$). B) Without Deep Creek, an outlier population, the relationship is significant ($r = 0.41$, $F = 6.37$; $P = 0.03$).

significant relationship between these variables was found (see Figure 7; $r = 0.41$; $F = 6.37$; $P = 0.03$).

Deviations from Hardy-Weinberg equilibrium were found in 11 of 72 global tests for heterozygosity deficiencies and excess (p-value <0.05; one tailed test). An excess of homozygotes was found in seven populations, none with more than one locus causing the departure. In four populations, excess heterozygosity caused the deviation. Four of the six loci investigated contained at least one deviation from Hardy-Weinberg equilibrium, and eight of the 12 populations. Only North Fork Teanaway River had more than one deviation, and this population had a low sample size with all individuals almost genetically identical. Significant F_{IS} values, as tested over all loci, reflect these within population deviations (see Table 6).

Among Population Variation

The index of genetic diversity measure used to determine the amount of genetic variation among populations (Weir and Cockerman's ΦF_{ST}) was 0.217, reflecting a high degree of genetic differentiation between populations. However, using the log likelihood ratio of exact tests for pair-wise comparisons, there was no significant difference in genotype frequencies between most of the following population pairs: American River, Union Creek, Rattlesnake Creek, Crow Creek, and South Fork Tieton River (Table 7). All of these populations, except for the South Fork Tieton River, are part of the American-Naches fluvial population. All of the other population pairs were uniquely differentiated in all genotype comparisons.

Table 7 Pair-wise comparisons for each population pair of bull trout in the Yakima River basin[a]

	SFT[a]	IN	AM	UN	TN	RT	CR	AH	BX	ML	DP	GL
SFT	*	8	96	96	174	72	85	128	206	213	98	219
IN	0.073	*	104	104	182	80	93	136	214	221	106	227
AM	0.112	0.198	*	0	199	67	32	153	231	238	34	244
UN	**0.084**[b]	0.136	**-0.022**	*	199	67	32	153	231	238	34	244
TN	0.305	0.350	0.429	0.463	*	175	188	175	68	75	218	81
RT	**0.009**	0.128	0.090	**0.081**	0.341	*	56	128	224	261	68	237
CR	**0.059**	0.160	**0.027**	**0.011**	0.418	**0.042**	*	141	237	244	33	250
AH	0.191	0.262	0.248	0.244	0.266	0.238	0.250	*	207	214	154	221
BX	0.149	0.085	0.214	0.181	0.428	0.196	0.145	0.287	*	7	250	39
ML	0.208	0.122	0.269	0.256	0.398	0.227	0.226	0.315	0.068	*	257	46
DP	0.288	0.127	0.335	0.303	0.634	0.321	0.338	0.472	0.284	0.364	*	264
GL	0.158	0.074	0.140	0.097	0.460	0.187	0.138	0.338	0.079	0.111	0.215	*

[a] The upper matrix shows distance in river kilometers between each pair (from the sampling locations). The lower matrix shows genetic distance (Weir and Cockerman's Φ F_{st}). Bolded numbers represent pair-wise comparisons that were not significantly differentiated from one another using log likelihood exact tests for pair-wise comparisons and strict Bonferroni corrections (FSAT; Goudet 1996). Population identifiers are as in Table 1.

A Mantel calculation was made to compare genetic distance (pair-wise F_{ST} comparisons) and geographic distance (river kilometers between each sampling location). A Mantel nonparametric test calculator was used to determine significance in two ways, via the standard normal variate (g) and through randomized replications of the matrix data (Liedloff 1999). A matrix of the fluvial populations (American River, Union Creek, Rattlesnake Creek, and Crow Creek plus South Fork Tieton) shows a significant "isolation-by-distance" relationship (Figure 8). Among the remaining populations this relationship was not significant.

Genetic Relationships

All phylogenetic trees created in PHYLIP show a clustering of the fluvial populations plus South Fork Tieton, and a clustering of Upper Yakima populations. The UPGMA tree (Figure 9) shows branch support for these clusters that are close to 50%, with higher support for branching at some outer nodes. The two spawning populations from Kachess Lake cluster together with 59% support; however, the two populations from Rimrock Lake did not cluster in any of the trees generated.

These same clustering patterns were evident in the principal component analysis (PCA) of population genotypes. PCA results found the first four axes to be significant (according the broken-stick), with the first axis explaining 41% of the variability and the second, 22% (Figure 10). The fluvial populations, plus South Fork Tieton, cluster, as do the Upper Yakima populations of Gold Creek, Mineral Creek and Box Canyon Creek. Deep Creek was a significant outlier, as were Ahtanum Creek and NF Teanaway River.

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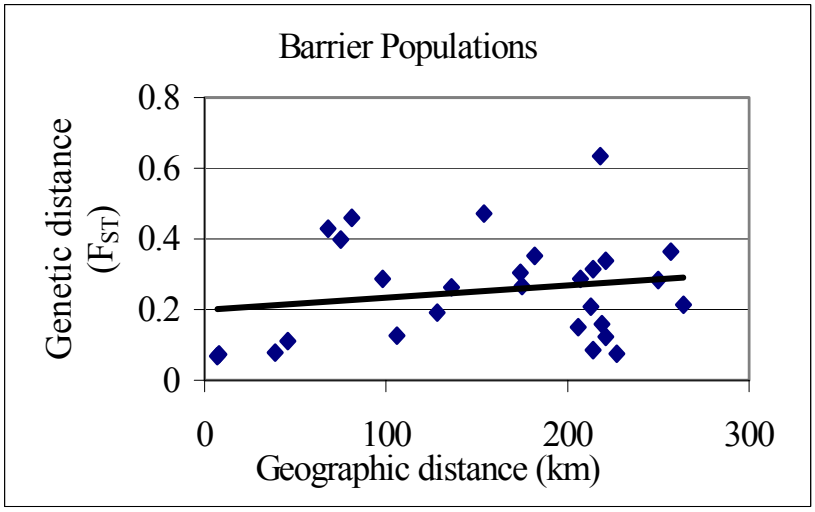

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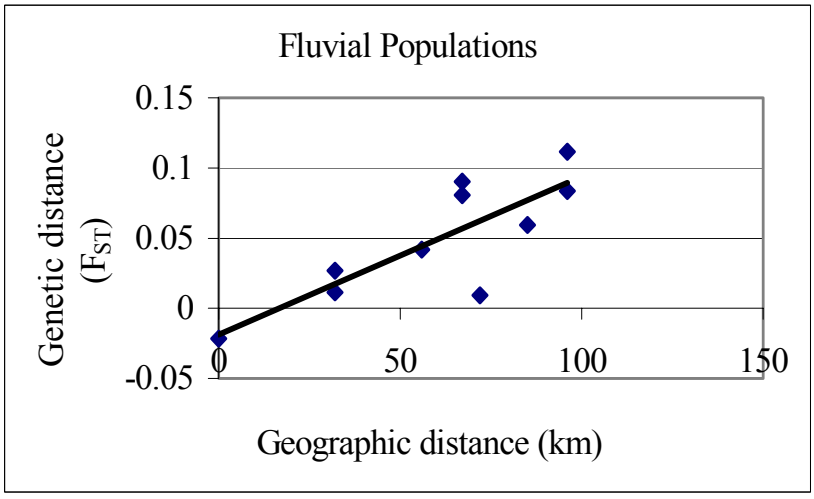

Figure 8 Correlation between genetic distance (pair-wise F_{ST}) and geographic distance (river kilometers between sample sites) for population pairs. A) Among fluvial populations including South Fork Tieton. B) All other (barrier) populations. Mantel correlations show a significant relationship ($r = 0.82$; $g = 2.767$; $P < 0.01$) among the fluvial populations and not among the barrier populations ($r = 0.11$; $g = 0.7388$; $P > 0.05$).

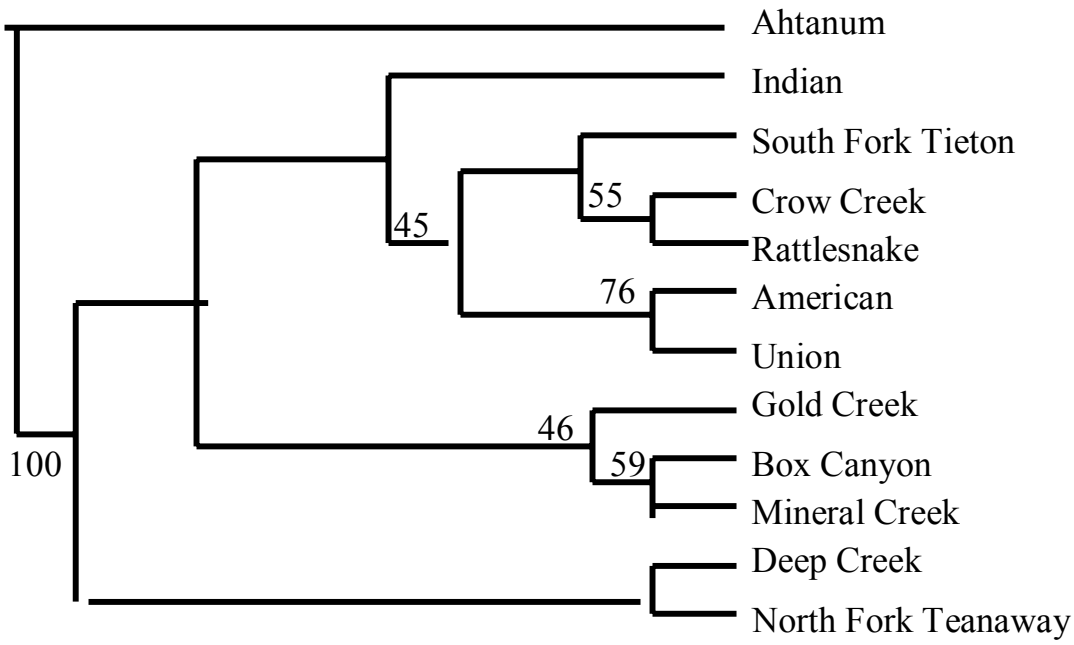

Figure 9 UPGMA consensus tree (PHYLIP; Felsenstein 1993) based on CSE chord distances, visualizing relationships among bull trout populations in the Yakima River basin. Numbers indicate branch support over 40% based on 1000 bootstrap replicates.

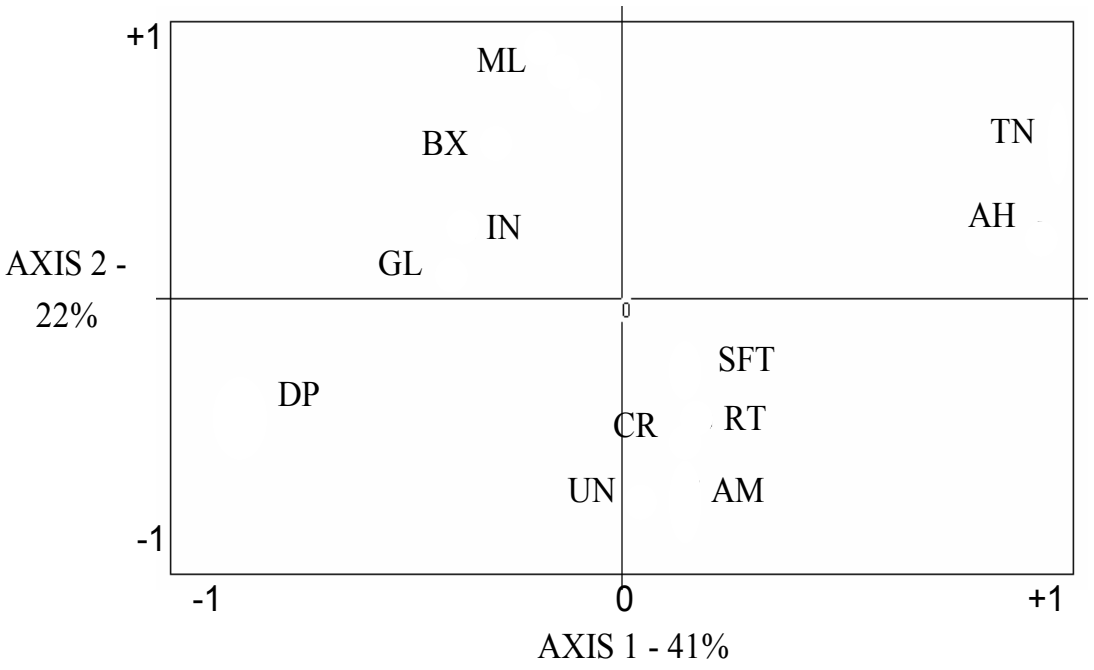

Figure 10 Principal component analysis of Yakima River basin bull trout population genotypes. Shows clustering of the fluvial populations, plus South Fork Tieton, and of the Upper Yakima River populations. Population identifiers are as in Table 1.

CHAPTER IV

DISCUSSION

Field Sampling

To meet the assumption of random sampling as part of a genetic study of a population or group of populations, sampling design is important. For this study, samples were collected from various life stages of bull trout, and across a five-year time period. For adult sampling, the iteroparous nature of spawning behavior in bull trout contributes to a greater confidence that individual fish sampled were not related. Two to four size classes were sampled in Deep Creek, South Fork Tieton and Indian Creek, as well as both male and female individuals (see Figure 4). These size classes likely represent different age classes of fish. In the remaining nine populations where juvenile and/or resident fish were sampled, three to four size/age classes were part of each population sample. This reduces the likelihood of non-random sampling of siblings.

Length of stream sampled and number of bull trout captured is an informal measure of bull trout relative densities in reaches sampled (see Table 2). Other factors, including number of capable snorkelers, visibility, and maneuverability within the stream also contributed to the total number of fish captured and sampled. However, it is interesting to note that the three streams with the highest number of juveniles captured in the shortest distance were Mineral Creek, Box Canyon Creek and American River (see Figure 5). These streams have some of lowest average number of redds per year (see Table 1).

One possible explanation for this in Mineral Creek and Box Canyon Creek is the

limited amount of habitat available for snorkeling, thus it was easier to locate juveniles. Union Creek, however, has a similar amount of habitat and only eleven samples were collected from this stream. This density data supports the possibility that there are unaccounted for resident populations co-existing with migratory bull trout, and that these fish are being overlooked in redd counts and thus adult population estimates. The presence of bull trout with multiple life history traits in one stream system has been previously documented (Rieman and McIntyre 1993; Nelson et al. 2002). It is unknown whether expression of migratory instincts are genetically or environmentally controlled. Further genetic sampling and demographic studies are needed to address this important question.

Genetic Variation Within Populations

The average expected heterozygosity found within these twelve bull trout populations was 0.33. In other salmonid species, genetic diversity within populations is much higher. For example, in a study of redband trout in the McCloud River, Nielsen et al. (1999) found an average expected heterozygosity across microsatellite loci of 0.68. Other studies of brown trout, steelhead trout, Atlantic salmon and chinook salmon have found similar results (Estoup et al. 1993; Fontaine et al. 1997; King et al. 2001; Heath et al. 2002; Kinnison et al. 2002). However, other microsatellite studies of bull trout populations found average expected heterozygosities across loci ranging from 0.24 to 0.39 (Leary et al. 1993; Spruell et al. 1999; Taylor et al. 1999; Kanda and Allendorf 2001; Costello et al. 2003). Thus, the Yakima River basin populations are within the

normal range for population genetic diversity. Though low levels of diversity within populations can reduce evolutionary potential, and thus make populations more vulnerable to disease and stochastic environmental events, bull trout seem to be adapted to surviving at these levels. Kanda and Allendorf (2001) suggest that low variability at a variety of molecular markers in bull trout is a result of small effective population sizes historically. These smaller population sizes may reflect post-glacial dispersal patterns and early bull trout evolutionary history (Taylor et al. 1999; Costello et al. 2003).

However, genetic diversity is expected to be relatively higher among populations with a larger population size. In the Yakima River basin, the populations with the largest average redd counts (South Fork Tieton and Indian Creek) have among the highest heterozygosities, as does Box Canyon Creek, which has low redd counts (see Table 1). This is another possible point of evidence for the presence of spawning resident adults contributing to a population size larger than that accounted for in the annual redd counts.

Another anomalous population is Deep Creek, with an average redd count of 81, and an expected heterozygosity of 0.23 (see Table 1; Table 5). In 1950, rotenone, a poison directed at undesirable fish species, was applied to Bumping Lake (WDFW 1997). This treatment likely impacted the population of adult bull trout residing in the lake, and caused a bottleneck as the population was re-established from the juveniles rearing in Deep Creek at the time of the poisoning, plus any surviving adults. The low levels of genetic diversity found in this population support this presumed genetic bottleneck.

There were deviations from Hardy-Weinberg equilibrium at a several loci. Hardy-Weinberg equilibrium describes a balance in levels of heterozygosity observed at allele

frequencies in discrete populations with random mating (Griffiths et al. 1999). There are several possible explanations for deviations from this pattern. During the amplification of DNA during PCR, there is the possibility for a "null allele" which is an allele that is present but does not amplify, resulting in distorted allele frequencies. If null alleles are not a factor, and there are true heterozygosity deficiencies in a population, these can be caused by non-random mating patterns such as inbreeding. Inbreeding effects are not unexpected in populations with chronically low population counts. Another possible cause is Wahlund's effect. In the case of fish sampled within a rearing stream, Wahlund's effect could be caused by age class substructure, or the presence of resident bull trout that are breeding separately from migratory fish.

Genetic Relationships

Genetic analysis of the data shows a high level of genetic differentiation among bull trout spawning populations in the Yakima River basin. An overall F_{ST} value of 0.217 is greater than that seen in other genetic studies of bull trout population structure within a specific drainage. Spruell et al. (1999) found an F_{ST} value of 0.063 in the Lightning Creek drainage, and Neraas and Spruell (2001) found a value of 0.137 in the larger Lake Pend Oreille system, both in Idaho. Geographic distribution of bull trout differs in these studies from that of the Yakima River basin, but provide a comparison for the degree of structure found. One important differing factor which seems to be contributing to an overall higher F_{ST} value in the Yakima basin is the inclusion of three life history types. F_{ST} is a relative measure of the amount of variance attributable to between population differences, thus by

including isolated resident populations, the overall value will be higher.

There are significant differences in genotype frequencies at most population pairs (see Table 7). However, these differences are not significant among the four fluvial populations sampled, and one adfluvial population, South Fork Tieton. It is significant that South Fork Tieton, an adfluvial population found in Rimrock Lake, shows similarities with non-barrier populations in another stream system (see Figure 1). There are several possible explanations for this result. One possibility is that there is one way gene flow into the fluvial system via bull trout entrainment from the reservoir. Netting studies have shown that adult and sub-adult bull trout are released from the dam during the large, annual drawdown event every September (James 2002). Though a percentage of the fish do not survive the intense water flow, bull trout are found and have been captured in the Tieton River below the dam (P. James, personal communication 2003). But if fish from the reservoir are contributing to the gene pool of fluvial populations, why is South Fork Tieton unique in this grouping? It is reasonable to assume that adfluvial bull trout that spawn in Indian Creek would have an equal chance of entrainment, and ability to contribute to the fluvial gene pool.

Another possible explanation for this pattern is that it reflects historical gene flow. Rimrock Lake is the only reservoir in the Yakima River system that was not constructed on a natural lake. There was therefore no established adfluvial bull trout population prior to 1925. It is likely that South Fork Tieton once had a migratory fluvial population, and possible that Indian Creek was a non-migratory resident population until the reservoir environment became available. The separation of each spawning tributary into a distinct

population, despite a shared lake habitat, is supported by tagging data from Rimrock Lake (James 2002). In six years of marking and recapturing tagged fish post-spawning, there has only been one instance of cross-over between populations. Site fidelity to spawning areas seems to be very high in Rimrock Lake and in bull trout populations in general (Spruell et al. 1999). In a population with a small effective population size, 75 years of isolation could cause significant changes in allele frequency patterns (Rieman and Allendorf 2001), but the South Fork Tieton has a large enough population to buffer these effects. However, if isolation continues, the population will likely become further differentiated.

Another pattern that emerged was the grouping of Upper Yakima bull trout populations. Though the pair-wise comparisons showed significant differences between all pair matches, phylogenetic trees created consistently show a clustering of this grouping, as did principal components analysis (see Figure 9; Figure 10). This result is expected between Mineral Creek and Box Canyon Creek, which are separate spawning tributaries in the same reservoir and have opportunity for gene flow, but there is no longer connectivity possible with Gold Creek, in Keechelus Lake. Similar allele frequencies, particularly at the highly variable FGT3 allele, suggest that these allele patterns were established when gene flow was occurring between populations, and that re-connection could be beneficial as current population sizes are critically low (WDFW 1997).

A part of the Upper Yakima population that is missing from this grouping is the North Fork Teanaway River. This system has suffered from low flows in the downstream

reaches of the river due to irrigation practices since the 1930's (T. Mayo, personal communication 2003). Bull trout in the system are found in the upper reaches, where flows remain more stable. The segregation of North Fork Teanaway River bull trout from the other Upper Yakima populations may reflect that this has always been an isolated, resident population. Another possibility is that the Teanaway population has suffered a large bottleneck. This system has not been well monitored, but it appears that the small population has been adversely affected through various stochastic events, one example being a large flood event of 1995-1996, which reduced bull trout numbers substantially (P. James, personal communication 2003).

Ahtanum drainage appears to support a stable, isolated population of resident bull trout. Genetic diversity was high and yet unique from the other lower Yakima River systems. In this drainage, three forks of the Ahtanum River were sampled and analyzed as one population. The degree of substructure and the amount of movement among these tributaries remains unknown. In addition, if samples from the main-stem Yakima River are collected, there may be evidence of some gene flow between these populations, just as the unsampled Upper Yakima River fluvial fish may be contributing gene flow in the North Fork Teanaway River. Radio telemetry studies of bull trout movements are shedding new light on movements of fluvial fish, and a tagging study that is being initiated in the Yakima River basin will offer new insights into perceptions of life histories and migration (J. De La Vergne, personal communication 2003).

The clustering of the American-Naches fluvial populations does not include Deep Creek, which is geographically the closest adfluvial population. Though the presumed

bottleneck event has likely contributed to the allele frequency differences, this population has unique behavioral differences in spawning timing. In most streams in the Yakima River basin, spawning adults migrate into the tributaries in mid-summer, and spawn during the month of September. In Deep Creek there is a two to three week period in August when fish move into the stream and spawn (James 2002). This evidence points to an isolation of Deep Creek that was established prior to the construction of the irrigation dam. This is an example in the Yakima basin where physical migration barriers may not be the primary reason for observed genetic substructure.

Isolation-by-Distance

An ecological model used to describe populations that are at varying geographic distances is a stepping-stone, or isolation-by-distance model (Wright 1943). This concept applies to the idea that populations closer to one another are more likely to interbreed and to be at equilibrium. Thus, in a scatter plot of population pairs that correlates geographic distance and genetic distance, there is a positive relationship (Hutchinson and Templeton 1999). When this relationship is not apparent, and not significant, the assumptions of gene flow and equilibrium are not met. This can be for various reasons. Schwartz et al. (2002) found no apparent relationship among widely distributed lynx populations, yet a low global F_{ST} (0.033) was found, indicating that lynx were dispersing widely, not interbreeding preferentially with closer neighbors. In the case of Yakima River bull trout populations, with a high global F_{ST} (0.217), the lack of a positive relationship between geographic and genetic distance among barrier populations is more likely a factor of

barriers to migration and small population sizes overemphasizing the effects of drift and restraining gene flow (see Figure 8). When the same correlation is made among the fluvial populations, there is a significant relationship, indicating that populations which are geographically closer do interbreed more often when no barriers to migration are present (see Figure 8).

The issue of migration barriers is very relevant in the Yakima River basin, and in many other river systems where dams and other human-made barriers are limiting migration of bull trout and other fish species. These genetic results indicate that irrigation dams without fish passage are likely preventing gene flow between populations that historically interbred. The South Fork Tieton River appears to have once been connected to the fluvial populations. Yet, it is evident from the minimal gene flow among populations which share the same lake environment, yet have distinct spawning populations, that there are other barriers to gene flow besides physical barriers. For example, in Kachess Lake, where Box Canyon Creek and Mineral Creek both have spawning bull trout populations, there are temporal differences in spawning timing. Adfluvial bull trout in Box Canyon Creek move onto the spawning grounds in July, and spawn in September, at the same time as most of the other Yakima River basin populations (Meyer 2002). Mineral Creek bull trout spawn in October and November, and move into the tributary just prior to spawning (Meyer 2002).

Management Implications

Despite strong spawning site fidelity, temporal differences in spawn timing and other self-isolating behavior, evidence of gene flow among the populations with no barrier to migration implies that there is the possibility to reconnect populations if barriers are removed. The time frame for separation among the populations, in the form of impassable dams, is less than one hundred years. It appears that in the smaller populations, and in the populations that have potentially suffered a genetic bottleneck event, there has been sufficient time for drift to have significantly changed the observed allele patterns at the loci selected for this study. However, microsatellites reflect genetic changes that occur at a much faster rate than quantitative traits, and do not necessarily reflect local adaptations that could be compromised by gene flow (see Moran 2002). One of the risks of introducing gene flow among populations that have been isolated is the potential for outbreeding depression. In areas where environmental conditions are not significantly different, these risks are minimal when compared to the potential benefits of returning connectivity to populations which have had some level of gene flow historically.

Another important application for genetic information can be to determine conservation units on a variety of scales, locally and biogeographically (Moritz 1994; Waples 1995). In the Yakima River basin it would be appropriate to reconsider local WDFW stock delineations based on the results of this study. For example, in Rimrock Lake, the South Fork Tieton River and Indian Creek appear to be functioning as separate spawning populations, and each stream should be monitored and protected independently,

as there is no evidence that re-colonization would take place after a population reducing event. On a larger scale, genetic variation and patterns in the Yakima River basin can be compared to other watersheds and population groups in the Middle Columbia River and throughout the range of bull trout. Though this study focused on within drainage relationships, future work may address questions of genetic variation on a larger scale, as well as temporal changes.

Data generated by genetic studies is especially useful when there is a context of demographic and historical information about the area and populations in question. As genetic studies become a more prevalent facet of managing at-risk species, the most appropriate ways to implement management decisions based on available genetic data are being explored. In Dunham et al. (1999) extinction risk assessments models are examined in light of genetic variation data. Epifanio et al. (2003) present a case study and a model for including genetic data in a recovery plan context. The authors describe a matrix of conservation prioritization based on unique and common traits within populations, and population status, delineated as "vulnerable" or "strong." In their model, the conservation of vulnerable populations, with both unique and common traits, is prioritized.

In the Yakima River basin, application of such a system could aid in allocation of funds for conservation efforts. In the Upper Yakima system, Box Canyon Creek, Mineral Creek, and Gold Creek would qualify as "vulnerable" populations that have allele frequency patterns in common. North Fork Teanaway River is a population that is geographically close, has the potential for connectivity with a fluvial population, and has a unique genetic profile. The unknown factor in the Upper Yakima River is the remnant

fluvial population, which could certainly qualify as “vulnerable,” and though the genetic information is unknown, would be considered “unique” as the last remaining fluvial population in this area. Under the model suggested by Epifanio et al. (2003), this area would be a high priority for focusing recovery efforts, such as restoring connectivity between all populations.

Though the possibility for connectivity does not ensure gene flow in a species such as bull trout with evidence of strong site fidelity, it is a step in the direction of allowing for full expression of all life history traits, and for the potential of larger effective population sizes for chronically low populations. The recovery of bull trout in the Yakima River basin, and throughout their range, will be dependant on a complement of factors, such as protection of habitat, control of non-native species, and preservation of existing populations. Genetic information about relationships between populations can contribute a deeper insight into evolutionary history and migration patterns. The results of this study suggest that though there are barriers to gene flow other than dams, connectivity is and has been important to the genetic structure of bull trout populations. Continued research and conservation efforts are needed to ensure the survival of these resilient and complex fish.

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