BULL TROUT (*SALVELINUS CONFLUENTUS*) SPAWNING MIGRATIONS IN THE DUNCAN RIVER: INSIGHTS FROM TELEMETRY AND DNA.

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Abstract

Radio telemetry and microsatellite DNA analyses were used to describe spawning migrations and the spatial scale of genetic differentiation among populations of bull trout (*Salvelinus confluentus*) in the Duncan River system, southeastern British Columbia. Over two years, 66 radio-tagged bull trout were tracked to destinations in the upper Duncan River. The distribution of bull trout by destination in the upper Duncan River and migration timing among all destinations did not vary between the two study years. One third of bull trout tracked for two spawning migrations switched spawning stream from one year to the next. There was also a trend for increasing size of radio-tagged bull trout with spawning stream size. This suggests that bull trout in the upper Duncan River do not have fidelity at the scale of individual spawning stream. Microsatellite DNA analysis of juvenile bull trout from two spawning streams in the upper Duncan River indicate that there is little between stream variation. Exact tests of population differentiation over allele frequencies of six polymorphic microsatellite loci suggest no significant genetic differentiation between the two spawning streams. An analysis of molecular variance (AMOVA) partitioned a small (< 1%) but significant amount of genetic variance to the between spawning stream variance partition. The estimate of between spawning stream F_{ST} was 0.01. These estimates of low genetic variation between spawning streams, especially when combined with direct evidence from telemetry, suggest that bull trout in the upper Duncan River do not home at the scale of individual spawning streams.

Table of Contents

Table of Tables

Table of Figures

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Chapter 1 - General Introduction

The way species are subdivided into reproductive groups, or populations, is critical for conservation and management (Vogler and DeSalle 1994; Bohonak 1999). Different populations within a species often reflect different current and recent historical evolutionary events. Preserving this within species genetic diversity is thought ensure long-term species viability (Moritz 1994; Avise 1995). To preserve, or manage, this diversity requires an understanding of the geographic scale over which populations occur. Divergence among populations is a function of the strength of local selection and the amount of gene flow among populations.

Important determinants of the amount of gene flow between two populations include dispersal ability and the presence and location of barriers to dispersal (Waples 1998; Bohonak 1999). In the absence of barriers to dispersal, the magnitude of migration and gene flow between otherwise separate populations is partly a function of geographic proximity. Adjacent populations exchange migrants more frequently than populations separated by greater distances. This phenomenon is called "isolation by distance" (Wright 1943; Slatkin 1993).

In organisms that migrate between feeding and breeding locations, the level of population divergence is often a function of degree of fidelity to discrete reproductive sites. If individuals born in different places home (i.e. return) to specific reproductive sites, this could influence interpopulation divergence. Homing decreases gene flow among reproductive sites and, potentially, can re-enforce geographic isolation among populations (Taylor 1991). Without gene flow, populations in different environments will adapt to local conditions, and diverge (Barton and Hewitt 1989). Even in the absence of strong selection, isolated populations will diverge due to genetic drift (Wright 1931); however, the migration of even a small number of reproductive individuals between populations will prevent the fixation of local genotypes (Wright 1931; Hartl and Clark 1989; Mills and Allendorf 1996). From a management or conservation perspective, differentiating among divergent populations is, therefore, a species specific task. The processes leading to interpopulation divergence are well studied in salmonid fishes.

Generally, salmonid fishes tend to home (i.e. return to their natal streams), albeit with varying degrees of fidelity (Scheer 1939; Banks 1969; Nordeng 1977; Quinn 1993; McElhany et al. 2000). In these fish, homing typically leads to geographic isolation and local adaptation, often within individual spawning streams (Scheer 1939; Quinn and Dittman 1990; Taylor 1991). Homing probably is especially important for semelparous Pacific salmon (*Oncorhynchus* spp.): it increases the probability that the one chance for reproduction is successful. Banks (1969) commented that iteroparous Atlantic salmon (*Salmo salar*) may home less accurately than semelparous Pacific salmon, since *Salmo* has scarcely speciated relative to *Oncorhynchus*. Management and conservation of salmonid fishes focuses, or attempts to focus, at the level of genetically distinct units (Allendorf and Phelps 1981; Taylor 1991; Utter and Ryman 1993; Allendorf et al. 1997). Critically important to this management objective is knowledge regarding where genetically distinct groups occur and, in the case of salmonid fishes, this often corresponds to specific spawning streams (Ryman 1983; Taylor 1991).

Bull trout (*Salvelinus confluentus*), is an iteroparous salmonid native to northwestern North America (Cavender 1978; Haas and McPhail 1991). Historically, bull trout ranged over 25° of latitude from the McCloud River in northern California to the Mackenzie River in the North West Territories and between the Pacific Ocean and the eastern slopes of the Rocky Mountains (Haas and McPhail 1991; McPhail and Baxter 1996). Because of population declines in the U.S., bull trout in that country are listed as threatened under the Endangered Species Act (Barry 1999). Usually, these declines are attributed to habitat degradation, habitat fragmentation, and exotic species introductions (Rieman and McIntyre 1993; Watson and Hillman 1997). Bull trout are long lived (potentially > 20 years) that occur as one of four life history types: resident, fluvial, lacustrine, or anadromous (Fraley and Shepard 1989; Goetz 1989; McPhail and Baxter 1996). Bull trout spawn in running freshwater in the fall. Like other *Salvelinus* species, they are adapted to cold water, and their distribution suggests strict temperature requirements (Rieman and McIntyre 1993; Parkinson and Haas 1996). In all but the resident life history form, bull trout migrate between geographically separate spawning and feeding areas. Eggs incubate over winter and hatch early in spring. Juvenile bull

trout remain in the natal stream for as long as three summers before moving to either larger streams, lakes or nearshore marine areas (McPhail and Murray 1979; Goetz 1989; Haas and McPhail 1991; McPhail and Baxter 1996). Age of first maturity may be four years, although more commonly bull trout do not mature until age five to seven (Allen 1980; Fraley and Shepard 1989; Goetz 1989).

Bull trout spawning migrations are not well studied. In a review of migratory timing information, McPhail and Baxter (1996) found that there was no appreciable difference in spawning time across 20^0 of latitude. Generally, bull trout spawning migrations begin in the spring or early summer, with fish arriving at a spawning destination in early to late August. Spawning occurs from early September to mid October, with fish leaving spawning areas shortly after spawning is completed (McPhail and Baxter 1996). Several studies have addressed migratory timing with radio telemetry (McLeod and Clayton 1994; Schill et al. 1994; Swanberg 1997; O'Brien and Chamberlain 2000). These telemetry studies determine timing of migrations more specifically for particular regions, and help to define seasonal habitat use by bull trout for conservation and management strategies. Telemetry also can help identify limits and barriers to dispersal.

Genetic studies suggest that bull trout occur as two major phylogenetic groups: a coastal and interior group (Spruell and Allendorf 1997; Taylor et al. 1999). This broad scale structure is attributed to recent historical isolation within two glacial refugia south of the Cordilleran ice sheet (Taylor et al. 1999). Genetic studies also generally find low genetic diversity in restricted geographic areas, such as within major watersheds of large river systems like the Columbia and Fraser, with relatively high differentiation between such regions (Leary et al. 1993; Spruell and Allendorf 1997; Williams et al. 1997; Costello and Taylor unpubl. MS). Most evidence suggests that bull trout home accurately to natal streams for reproduction (as reviewed by Goetz 1989), although some studies have suggested that homing may not be accurate at the scale of natal stream (McPhail 1979; Oliver 1979; see also references in Goetz 1989).

My thesis focuses on the application of radio telemetry and molecular genetic techniques to an investigation of the relationships between bull trout spawning

migrations, patterns of movement among potential spawning locations, and the spatial scale of population structure. I have organized the thesis into two primary chapters around the telemetry and molecular genetic methods I used. Each primary chapter is written as a stand-alone manuscript; although, because of repeated citations in each chapter, only one list of cited literature is presented. This organization, although convenient for later publication of results, requires repetition of introductory and discussion material in each chapter. The chapters are structured as follows:

Chapter 2. Bull trout (*Salvelinus confluentus*) radio telemetry in the Duncan River: migratory timing and insight into the mechanism of spawning stream selection.

This chapter presents results of a three year (1995-97) radio telemetry study of bull trout migrations in the Duncan River, a tributary to Kootenay Lake in southeastern British Columbia. I describe the migratory timing of bull trout moving from Kootenay Lake to destinations in the upper Duncan River. I compare the number of bull trout migrating to specific destinations over two years, and test whether the size of radio-tagged bull trout is related to the size of the tributaries to which the fish migrated. I estimate of the total number of bull trout in the upper Duncan River in one year on the basis of migrants trapped leaving a spawning stream. I also describe the migration of six bull trout to the upper Duncan River in two consecutive years, and changes in their migratory destinations between years. I use my estimate of total numbers from the trap, and the proportion of fish changing destination to provide a rough estimate of the magnitude of switching among spawning streams.

Chapter 3. An evaluation of genetic differentiation between two bull trout (*Salvelinus confluentus*) spawning streams in the upper Duncan River using microsatellites.

I present microsatellite allele frequency data from juvenile bull trout collected in two spawning tributaries to the upper Duncan River. From these data, I calculate measures of population differentiation and examine population divergence among these

spawning streams. Under the assumptions of the island model of population structure (Wright 1943), I estimate the number of migrants between the two spawning streams. I compare this estimate of migration qualitatively with the telemetry results presented in Chapter 2. I discuss my results in relation to other genetic studies of bull trout at similar geographic scales.

Chapter 4. General Conclusions

In this chapter, I summarize the results presented in Chapters 2 and 3 as they relate to bull trout in the upper Duncan River. I compare the results of the telemetry and genetic methods used. I also discuss the implications of my results in relation to the management and conservation of bull trout in general.

Chapter 2 - Bull trout (*Salvelinus confluentus*) radio telemetry in the Duncan River: migratory timing and insight into the mechanism of spawning stream selection

2.1 Introduction

Bull trout (*Salvelinus confluentus*) is an iteroparous salmonid native to northwestern North America (Cavender 1978; Haas and McPhail 1991). Prior to the taxonomic work of Cavender (1978) and Haas and McPhail (1991), bull trout were considered Dolly Varden (*Salvelinus malma*). Historically, bull trout ranged over 25° of latitude from the McCloud River in northern California to the Mackenzie River in the North West Territories and between the Pacific Ocean and the eastern slopes of the Rocky Mountains (McPhail and Baxter 1996). Because of population declines in the U.S., bull trout are listed as threatened under the Endangered Species Act (Barry 1999). Usually, these declines are attributed to habitat degradation, habitat fragmentation and exotic species introductions (Rieman and McIntyre 1993; Watson and Hillman 1997). In British Columbia (BC), bull trout are 'blue listed', meaning that they are a species of special conservation concern. Bull trout are the only fish species identified by the BC Forest Practices Code as requiring special forest harvesting related management practices (Haas 1998).

Bull trout are long lived (potentially > 20 years; McPhail and Baxter 1996), spawn in running freshwater, and occur as one of four life history types: resident, fluvial, lacustrine or anadromous (Fraley and Shepard 1989; Goetz 1989; McPhail and Baxter 1996). Spawning occurs in the fall. Eggs incubate over winter and hatch in spring. Juvenile bull trout remain in the natal stream for as long as three summers before moving to larger streams, lakes or nearshore marine areas (McPhail and Murray 1979; Goetz 1989; Haas and McPhail 1991; McPhail and Baxter 1996). Age of first maturity may be four years, although more commonly bull trout do not mature until age five to seven (Allen 1980; Fraley and Shepard 1989; Goetz 1989). Spawning frequency is uncertain, although it is unlikely that spawning occurs every year after first maturity (Allen 1980; Goetz 1989; McPhail and Baxter 1996). With the exception of the resident life history, bull trout

migrate between geographically separate spawning and feeding areas. Bull trout migrations are often in excess of 200 km (Clayton and Mcleod 1993; Schill et al. 1994; Swanberg 1997; O'Brien and Chamberlain 2000), and in rare instances, where movement is not blocked, can exceed 500 km (Burrows et al. in press). Until recently, little quantitative migration data were available.

Much of the literature on bull trout migratory behaviour consists of provincial and state fisheries agency reports. Two reviews of this literature (Goetz 1989; McPhail and Baxter 1996) have summarized data from throughout the range of bull trout. In general, it is known that bull trout migrate throughout the summer, arriving at spawning locations from mid August to early October. Spawning activity has been observed as early August (Metolius River tributaries – Ratliff 1987 as cited by Goetz 1989) and as late as October (MacKenzie Creek – McPhail and Murray 1979). In their review, McPhail and Baxter (1996) found no suggestion of differences in the timing of spawning across 20^0 latitude. It appears that the timing of spawning, and presumably the timing of spawning migration, varies little over the range of bull trout.

Swanberg (1996; 1997) conducted a radio-telemetry project in the Blackfoot River in Montana. Bull trout were collected from locations in the Blackfoot River, radio-tagged, and tracked to spawning locations in tributary streams. Spawning migrations began as early as June, with bull trout arriving at spawning locations in early July. Spawning did not take place until late September. Swanberg (1997) documented strong fidelity to feeding locations in the mainstem Blackfoot River. After round trip spawning migrations of up to 150 km, 86 % of bull trout returned to within 20 m of their original point of capture in the mainstem river (Swanberg 1997).

Schill et al. (1994) examined bull trout spawning migrations with radio telemetry in the Rapid River, a tributary of the Salmon River in Idaho. Movement into the Rapid River began as early as April 30, and bull trout generally arrived at spawning locations in early August. Spawning occurred in early September. Schill et al. (1994) documented high post spawning mortality in the Rapid River: only 31% of the tagged bull trout left the Rapid River and returned to feeding areas in the Salmon River.

It is usually assumed that bull trout home to their natal streams (Goetz 1989); however, despite suggestions that this may not be the case (McPhail and Murray 1979),

the mechanism of spawning stream selection by bull trout has not been examined. McPhail and Murray (1979), based on observations from two years of data from a trap on a small bull stream in British Columbia, suggested that as bull trout age and grow, they switch to larger spawning streams. They based this suggestion on the observation of one fish returning to McKenzie Creek in both study years, only to leave the stream without spawning in the second year. The year it left without spawning, this fish was the largest bull trout to have entered the creek. McPhail and Murray (1979) also found that the mean size of bull trout spawning in McKenzie Creek was smaller than the mean size of bull trout captured by anglers from Upper Arrow Lake, into which the creek flows. In addition, they observed that larger streams flowing into Upper Arrow Lake contained larger spawning bull trout.

I used radio telemetry to examine the migratory behaviour of bull trout in the Duncan River, BC. I wanted to determine the extent and timing of spawning migrations of bull trout to destinations in the upper Duncan River. In addition, I wanted to identify bull trout spawning locations. I also tested whether there was a positive relationship between size of destination stream and size of fish as suggested by McPhail and Murray (1979). My study was conducted within a larger project designed to identify bull trout spawning locations in the upper Duncan River and examine the effect of a water storage dam on the Duncan River on bull trout migrations.

The questions I addressed were

- What is the timing of bull trout migrations in the Duncan River?
- What streams in the upper Duncan River are used by bull trout for spawning?
- Is there a relationship between size of bull trout and the size of their chosen spawning stream, as suggested by McPhail and Murray (1979)?

Study Site

The Duncan River, located in southeastern British Columbia, drains from the Selkirk and Purcell mountains into Kootenay Lake and, ultimately, into the Columbia River (Fig. 2-1). On 31 July 1967, Duncan River was impounded by the closure of Duncan Dam, located approximately 10 kilometers upstream of the river's confluence with Kootenay Lake. Duncan Dam is 40 m high and creates a 72 km^2 reservoir. The dam provides water storage (1.73 billion m^3), but has no power generation facilities

(Anonymous 1986). Upstream of the Duncan Reservoir, the upper Duncan River drains an area of nearly 1300 km^2.

The migration route of bull trout from feeding areas in Kootenay Lake to spawning locations in the upper Duncan River requires passage through Duncan Dam. Studies prior to 1967 predicted that the migration route of bull trout would be blocked by impoundment (Peterson and Withler 1965). In 1968, the first senior operator at the dam, 'Dutchy' Wageningen, altered spring and summer discharge at Duncan Dam to allow bull trout access to the upper river. Currently, this 'fish transfer' procedure continues under the supervision of BCHydro, the BC Ministry of Environment, Lands and Parks - Fisheries Section (MELP), and Len Wiens - the present senior operator. Tag recapture information prior to this study suggested that bull trout are able to ascend the dam (Fleck 1977; J. Bell, MELP, pers. comm.), but in 1994, aerial surveys of the upper Duncan River system failed to locate evidence of spawning (C. Spence, MELP, pers. comm.). Tagging studies of bull trout from the Duncan River provide evidence of migrations throughout the larger Kootenay River system, with tag returns ranging from Trout Lake in the north to the confluence of the Kootenay and Moyie rivers in the south (Fleck 1977; Fig. 2-1).

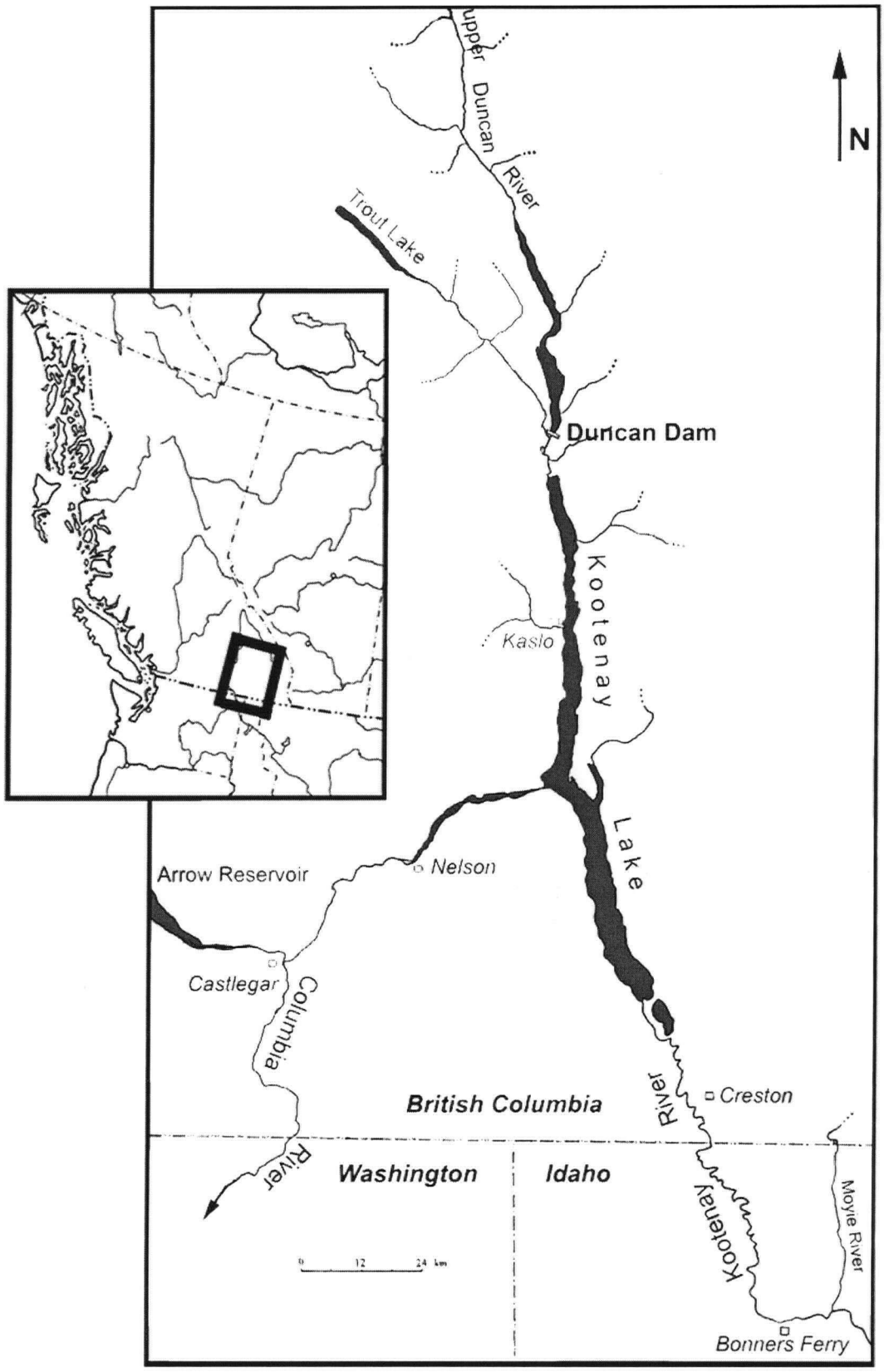

Figure 2-1. Map of the Duncan River system and Kootenay Lake. Inset map locates the study area within British Columbia.

2.2 Methods

Capture and tagging

During this study, I used several different sized radio tags, which varied in transmission duration and frequency (Table 1). All radio tags were digitally encoded. This allowed reception equipment to distinguish individual signals. Lotek Engineering, Newmarket, Ontario manufactured the radio tags and reception equipment.

Table 2-1. Characteristics of radio tags employed in the study.

Channel	Frequency (MHz)	Number deployed	Duration (months)	Diameter (mm)	Length (mm)	Antenna length (mm)	Mass (g)
5	149.400	31	12	11	50	400	8.2
15	148.400	16	6	11	45	370	7.1
20	149.700	15	24	15	50	400	15.5
22	149.740	16	12	11	50	400	8.2
31	150.120	18	12	11	50	400	8.2

I obtained bull trout for radio tagging from four locations in the Duncan River system: the discharge structure at Duncan Dam, the tailrace channel immediately downstream of the dam, the lower Duncan River (between the dam and Kootenay Lake), and the upper Duncan River. Bull trout were captured by angling with spoons at locations other than the discharge structure at Duncan Dam. Those captured at the dam were fish that had actively moved into the flip bucket at the downstream end of the discharge tunnel. Presumably they were attempting to move through the dam. By stopping flow through the tunnel, and lowering water levels in the flip bucket with a pump, I was able to access bull trout gathering there. BCHydro staff at Duncan Dam facilitated my access to bull trout in the flip bucket by manipulating discharge, controlling pumps and assisting with fish capture. All bull trout present in the drained flip bucket were tagged with external individually numbered 'T-anchor' style tags (Floy Tag Inc. Seattle, Washington) and measured (fork length) with a flexible tape. BCHydro, Ministry of Environment and Columbia Basin Compensation Program staff and volunteers assisted with capture and tagging of bull trout in the flip bucket. I selected bull trout from the flip bucket captures for radio tagging.

I did not select bull trout randomly for transmitter application. The weight (in water) of surgically implanted tags should not exceed 2% of the weight (in air) of the fish destined to carry it, and ideally this ratio should not exceed 1% (Winter 1983; Marty and Summerfelt 1986; McLeod and Clayton 1993). By selecting bull trout that exceeded 500 mm fork length (FL), in all cases I kept the weight ratio below 1%.

I attempted to radio tag only male bull trout; however, sex determination before surgery was difficult, because few secondary sexual characteristics were evident. I targeted males to limit effects of radio tagging on reproduction. When possible during surgery, I determined sex by internal examination of the gonads.

I anaesthetized selected fish in a cooler containing 30 L of water with tricane methane sulfonate (MS222) at 100 ppm. Whenever possible, I used the anesthetic bath on two or three fish that did not undergo surgery before anaesthetizing a surgical candidate. This was done to lower the stress of reduced pH brought about by the addition of MS222 (Bidgood 1980). I anaesthetized bull trout to stage 4 for surgery. Stage 4 anesthesia is characterized by loss of both equilibrium and reactivity to external stimuli as well as a slow but regular opercular rate (McKinley et al. 1992).

Before surgery, I measured mass using a spring scale with the fish supported in either a collapsible fish tube or weighing bag of known mass. Subsequent to measuring, a radio tag was inserted into the abdominal cavity (as described by McLeod and Clayton1993). Briefly, I placed the anaesthetized fish on its back in a wet neoprene lined wooden 'V'-trough and covered it, except for the region of incision, with moist towels. In 1995, I made a 2.5 cm long incision into the abdominal cavity immediately anterior to the pelvic girdle and approximately 1 cm off, and parallel to, the mid-ventral line. In 1996, the incision was made directly on the mid-ventral line. This was done to reduce damage to the ventral musculature. I then inserted a stainless steel needle (16 gauge, 5 cm long) through the body wall from posterior to and above the pelvic girdle into the incision. I threaded the whip antenna of the radio tag through the needle, removed the needle, and inserted the tag into the abdominal cavity. I closed the incision with three or four interrupted sutures and applied betadine to the closed incision and antenna exit wound. To recover the fish after surgery, they were held directly in a stream of flowing water. When the fish was capable of maintaining equilibrium (stage 2 anesthesia - McKinley et al.

1992), it was placed in a collapsible fish tube and allowed to recover for 30 - 180 min before release.

Prior to 28 June 1995, all radio-tagged bull trout captured at Duncan Dam (22 fish) were released in the flip bucket at Duncan Dam. I anticipated that the majority of these fish would be tracked as they ascended the discharge tunnel; however, I did not track any of these initial releases in Duncan Reservoir or the upper Duncan River. After 28 June in 1995, I released all bull trout radio tagged at Duncan Dam directly into Duncan Reservoir (25 fish). To ensure a large sample size of migrants to the upper Duncan River in 1996, I released 34 bull trout radio tagged from flip bucket captures into the reservoir. Six bull trout radio tagged at the dam in 1996 were released back into the flip bucket. All bull trout that were radio tagged away from the dam were released at the point of capture.

In 1996, I recaptured six bull trout carrying radio tags from 1995. As these tags should have stopped functioning before the end of the migration period in 1996 I attempted to surgically remove old tags and replace them with new ones. In two of the replacement attempts, I could not remove the old tag and so clipped its antenna close to the body to minimize drag before inserting a new tag. In the other replacements I removed the old tag without incident. I recaptured two radio-tagged bull trout in the flip bucket in 1997, and removed (but did not replace) one of the tags.

In addition to radio tagging in 1996, I operated a fish trap on Houston Creek at the confluence of Houston Creek and the upper Duncan River. The trap was operational for a total of 91 h from 24 September to 3 October 1996. The trap was constructed with 2.5 cm^2 plastic coated wire mesh supported by iron fence posts pounded into the stream bottom. The trap box was a 2.5 * 1.5 * 1.0 m wood framed box enclosed by 1.0 cm plastic mesh, with a funnel entrance on the upstream side. I estimated the number of bull trout present during the spawning period in Houston Creek from the number of radio-tagged and untagged fish captured in the trap and the known number of radio-tagged bull trout present in the creek using the Peterson estimate (as described by Greenwood 1996). In an attempt to roughly quantify the number of bull trout migrating into the upper Duncan River in 1996, I assumed that the proportion of radio-tagged to untagged bull trout was the same in all other location within the upper Duncan River.

I also conducted stream walks (late September) in 1995 and 1996 through the portions of Houston Creek, Westfall River and the mainstem upper Duncan River looking for evidence of spawning. These searches focused on areas where radio-tagged bull trout were located.

Tracking

I used a Lotek SRX-400A version 4.01/W5 receiver to track radio-tagged bull trout by truck, boat and aircraft. While tracking on the ground or by boat, I used either a hand held three element antenna or a short range whip antenna. A Cessna 172 aircraft was used for the majority of aerial tracking, but I tracked from a Eurocopter AS 350B helicopter on two occasions and a Cessna 337 aircraft once. Antenna setup differed by aircraft: the Cessna 172 had a single forward pointing four element antenna attached to the port side wing strut, the AS 350B had a single three element antenna attached to the nose of the aircraft, and the Cessna 337 had a downward-pointing two element antenna attached to each wing strut. In the Cessna 337, a switch-box allowed the receiver to 'listen' to both antennae simultaneously or either individually during tracking to help resolve the direction of receptions. When tracking from aircraft, I recorded location of receptions as both written descriptions and coordinates from a GPS.

Generally, I maintained a bi-weekly schedule of tracking flights during the in-migration and spawning phase in 1995 and 1996 (Fig. 2-2). As the first radio-tagged fish began to leave their spawning sites, flight frequency was increased. In 1997 and 1998, I flew once in the fall to identify number and location of spawning bull trout.

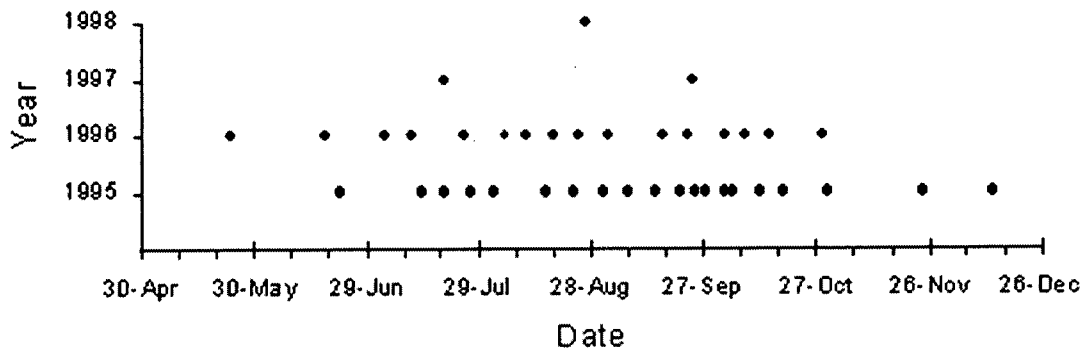

Figure 2-2. Dates of tracking flights in the Duncan River system.

I also used two Lotek SRX-400A version 4.01/W17T receivers as data logging fixed stations during 1996. Fixed station sites consisted of a battery-powered (1 deep-cycle 12V battery) receiver connected to two antennae oriented upstream and downstream to resolve direction of travel. Fixed station sites were located at Duncan Dam and 42.5 km upstream of the dam on the upper Duncan River (Fig. 2.3). I programmed fixed receivers to continuously scan each radio tag frequency in sequence for a five-second interval on each antenna, and record any reception in memory. With this scan cycle, each frequency was scanned for a minimum of 12 min each hour. I downloaded reception information to a portable computer when the receiver battery was replaced (bi-monthly).

Data analysis

I converted raw aerial, ground, and remote reception data to distance upstream (+) or downstream (-) of the Duncan Dam in km. Multiple receptions of an individual bull trout in one day were reduced, by averaging the locations, to a single daily reception. Figure 2-3 illustrates the kilometer designations used throughout the study. To begin analysis of the telemetry data, I characterized the destination of individual radio-tagged bull trout by the tributary or the section of the mainstem upper Duncan River to which they migrated. I then plotted the reception locations for each tagged individual as date vs. distance from Duncan Dam. Only the movements of those bull trout migrating into the upper Duncan River are presented. Unless otherwise noted, all statistics are presented with the 95% confidence intervals.

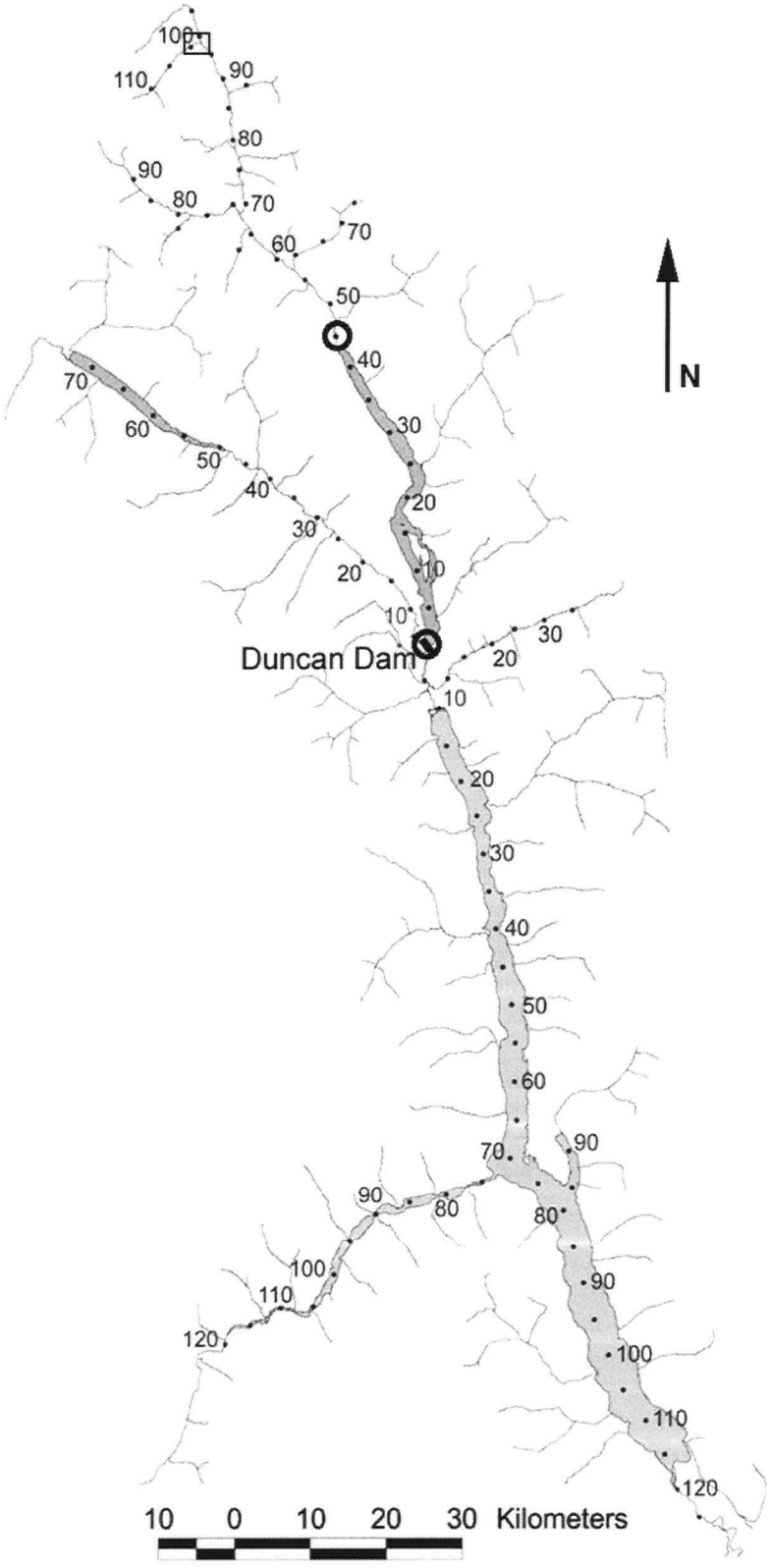

Figure 2-3. Kootenay Lake and the Duncan River with kilometer designations used throughout the study. The location of data logging telemetry fixed stations (circles) and the Houston Creek trap (square) are indicated.

I first compared the distribution of bull trout by destination in the upper Duncan River between 1995 and 1996 using a Fisher exact test. I then divided the movements of bull trout migrating into the upper Duncan River into three phases: the in-migration, spawning site residence, and out-migration. I considered the in-migration phase underway when a radio-tagged individual was released after tagging or continuous upstream movement began, and it continued until upstream movement ended. The spawning site residence phase continued from the end of the in-migration phase until a continuous downstream movement began. I considered the out-migration phase complete when a tag was first detected in the Duncan Reservoir (1995) or when it had passed below the fixed station receiver located near the inlet of the upper Duncan River to the Duncan Reservoir (1996).

To simplify these data, for each destination category I defined a separate arbitrary distance from Duncan Dam to distinguish between the three migration phases. The residency mark was positioned to include all similar migrants to a particular destination and was based on cessation of upstream movement by those migrants. For example, when a radio-tagged bull trout migrating to Houston Creek (a tributary of the upper Duncan River) crossed a mark 100 km upstream of Duncan Dam, I considered the in-migration phase to have ended and the spawning site residency phase to have begun. Similarly, when the 100 km residency mark was crossed in the downstream direction by bull trout leaving Houston Creek, I considered the spawning site residence phase complete and the out-migration phase to have started (Fig. 2-4). Most often, I did not locate radio-tagged bull trout as they crossed the residency mark; I inferred the date from the closest receptions immediately upstream and downstream.

By dividing movement patterns into these three phases (Fig. 2-4), I was able to calculate 9 migration statistics:

- in-migration start date / time (days) / rate (km•day^{-1})
- residency start date / time (days),
- out-migration start date / time (days) / rate (km•day^{-1}) / end date.

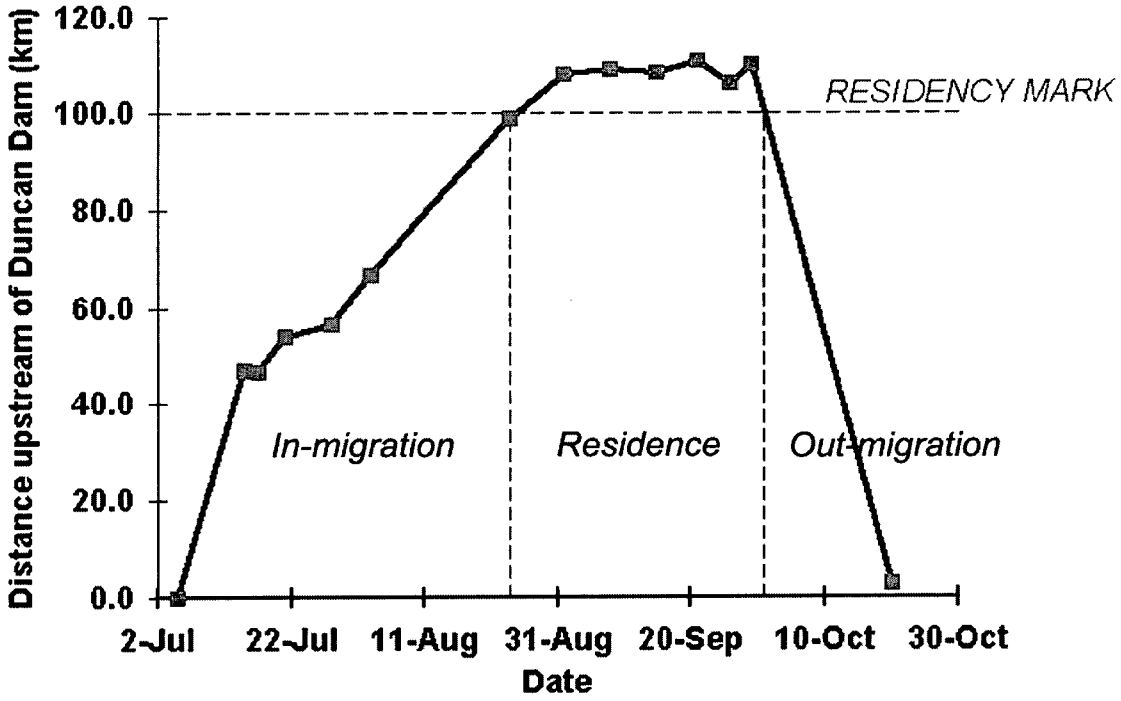

Figure 2-4. Example of a bull trout migration pattern based on radio telemetry location fixes (connected squares) showing the function of the residency mark in defining the boundaries between the in-migration, residence and out-migration phases.

I compared the nine migration statistics, along with FL and mass, over year of migration and destination category using two-factor analysis of variance (ANOVA). This study was not designed to fit the ANOVA model and, as such, I used it only as an exploratory tool. The first factor in the ANOVA analysis was the year of migration. For bull trout with two years of migration data in the upper Duncan River, I considered each migration year as independent in terms of the ANOVA analysis. The second factor in the analysis was destination. Because of small sample sizes to some destinations, I grouped low-use destinations into more broad categories for analysis. Where no differences were found by the two-factor ANOVA analysis, I pooled migration statistics.

I compared, using t-tests, the migration statistics of bull trout migrating through the discharge structure to those that were moved above, to determine if moving bull trout above Duncan Dam changed migration timing.

In order to determine the relative sizes of spawning tributaries in the upper Duncan River system, I estimated the catchment area (km^2) of each targeted tributary from 1:50,000 topographic maps. I regressed instream flow estimates of five tributaries calculated from portable flow-meter transects (Anonymous 1990) against catchment area to evaluate the usefulness of catchment area as a predictor of flows in the upper Duncan River system. The five instream flow measurements were made over a two-day period in October 1996 (stable low flows). I then regressed the mean FL of radio-tagged bull trout against catchment area of their destination tributaries.

2.3 Results

Capture and tagging

Ninety four bull trout were tagged over the duration of the project. Two bull trout died within one hour of the surgical procedure (prior to release). Anaesthesia time averaged 360 ± 28 s (n = 92). Surgery time, measured as the total time from removal of the fish from the anaesthetic to the start of recovery, averaged 628 ± 31 s (n = 95). Post surgery recovery, from removal of the radio-tagged bull trout from the surgical trough until equilibrium and reactivity was regained, averaged 351 ± 35 s (n = 94). The biological data collected from radio-tagged bull trout that migrated to destinations in the upper Duncan River are appended (n = 66).

I found no significant difference in fork length and weight distributions of radio-tagged bull trout across years (t-tests, $P = 0.882$ and 0.468, respectively) and pooled these data. Fork length (Fig. 2-5) and mass of only the radio-tagged bull trout migrating to the upper Duncan River averaged 703 ± 14 mm and 3575 ± 240 g (n = 66). There was no significant difference in mean FL between all bull trout captures from the flip bucket in 1995 and 1996 (two-tailed t-test, P = 0.558) and again I pooled the data. In 1995 and 1996, 533 bull trout (mean FL = 652.8 ± 5.0 mm) were captured and measured in the flip bucket at Duncan Dam. The average FL of radio-tagged bull trout that migrated to the

upper Duncan River was significantly larger than average FL of bull trout captured at Duncan Dam that were not radio-tagged (one-tailed t-test, $P < 0.001$; Figure 2-5).

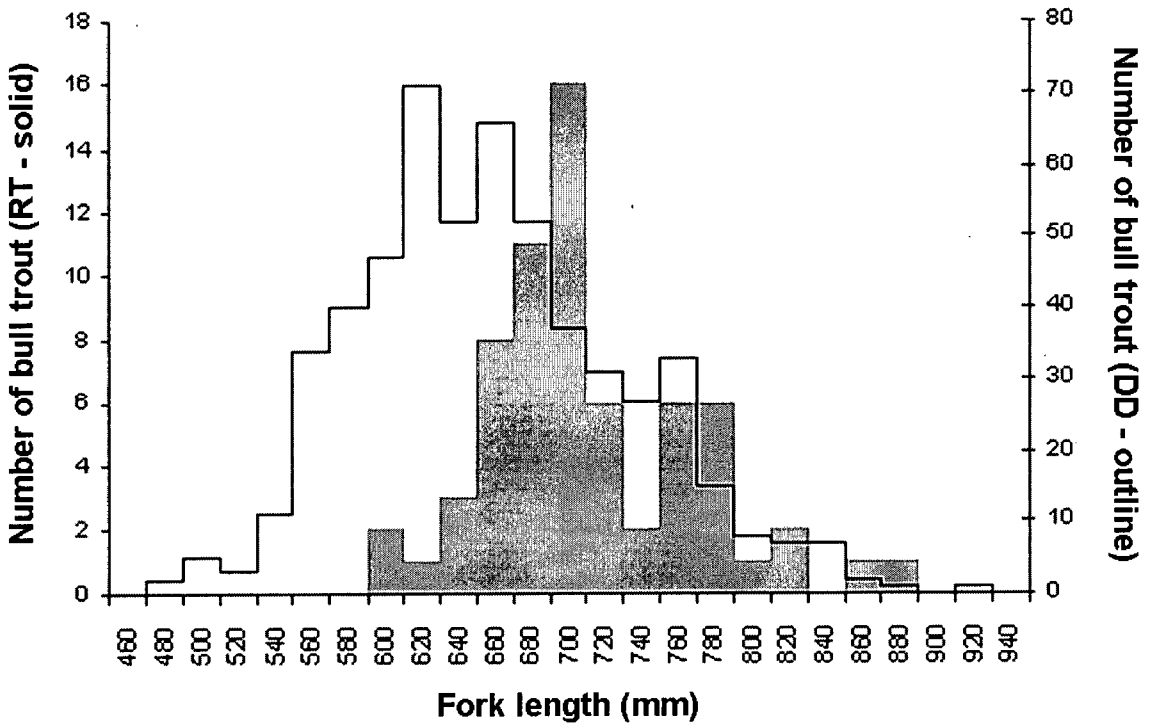

Figure 2-5. The fork length (FL) frequency histograms of all bull trout captured at Duncan Dam in 1995 and 1996 ('DD' - outline and right axis, n = 533) and of all radio-tagged bull trout migrating to destinations in the upper Duncan River ('RT' - gray bars and left axis, n=66).

Destinations in the upper Duncan River

In total, I tracked 66 radio-tagged bull trout to destinations in the upper Duncan River over 1995 and 1996 (Table 2-2). The majority of these bull trout were physically moved from below Duncan Dam and released in the reservoir. On average, each of the fish migrating to the upper Duncan River was located 28.5 ± 7.5 times during the study. Only six radio-tagged bull trout migrated through the discharge structure at Duncan Dam without interference. Figure 2-6 illustrates the upper Duncan River and location of major tributaries. The distribution of bull trout by migratory destination (seven categories; Table 2-2) was not significantly different between 1995 and 1996 (Fisher exact test, P = 0.91). Destinations with small numbers of radio-tagged bull trout migrating to them were grouped, giving a total of four destination categories for ANOVA analysis (Table 2-2).

Table 2-2. Migratory destinations and ANOVA groups of radio-tagged bull trout in the upper Duncan River. Destination 3, mainstem upper Duncan River, is broken into five sub-categories (3.a. – 3.e., *italics*) which are separated by tributary confluences (Fig. 2-6).

Migratory Destination	1995		1996		Analytical
	Migrants	% of Total	Migrants	% of Total	Group
1.) Houston Cr.	8	30.8	13	32.5	1
2.) Westfall R.	6	23.1	10	25	2
3.) mainstem upper Duncan River	9	34.6	8	20	3
3.a.) above Houston Cr.	*2*	*7.7*	*3*	*7.5*	*3*
3.b.) Hatteras Cr. to Houston Cr.	*4*	*15.4*	*2*	*5*	*3*
3.c.) Laidlaw Cr. to Hatteras Cr.	*1*	*3.8*	*1*	*2.5*	*3*
3.d.) Westfall R. to Laidlaw Cr.	*2*	*7.7*	*1*	*2.5*	*3*
3.e.) Giegerich Cr. to Westfall R.	*0*	*0*	*1*	*2.5*	*3*
4.) Giegerich Cr.	2	7.7	5	12.5	4
5.) Stevens Cr.	1	3.8	2	5.0	4
6.) Hatteras Cr.	0	0	1	2.5	4
7.) Marsh Adams Cr.	0	0	1	2.5	4
TOTALS	26	100	40	100	

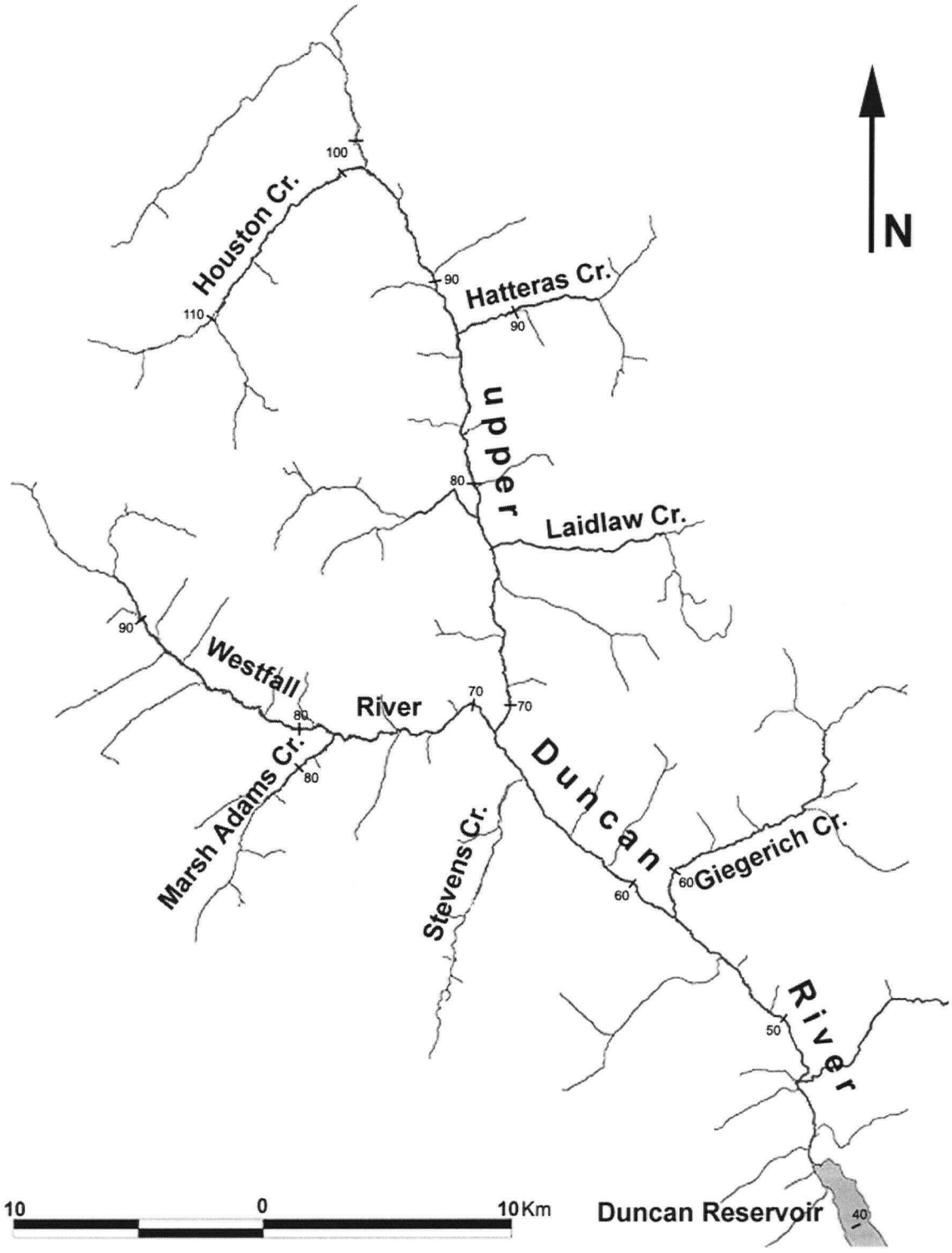

Figure 2-6. Map of the upper Duncan River system with kilometer designations upstream of Duncan Dam.

Migratory timing comparisons

Radio-tagged bull trout migrating to destinations in the upper Duncan River system showed little variation in timing of migration. The two factor ANOVA comparisons of migration rate and timing did not vary by destination, between years, or year by destination interaction. The ANOVA results are summarized in Table 2-3. There was a suggested relationship to destination for FL, mass and out-migration time, but not between years or in terms of the interaction between year and destination. As out-migration time was not significantly correlated (Pearsons correlation coefficient, $r = 0.236$, $P = 0.09$) with out-migration distance I pooled the out-migration time data. I plotted fork length data for each ANOVA group (Fig. 2-7) to show the relationship between length and destination suggested by the two factor ANOVA result. In Figure 2-7, I suggest the mainstem upper Duncan River destinations represent the physically smallest destination category: this is not technically correct, and reflects an assumption that bull trout migrating to these destinations did not spawn. Only the fork length of bull trout migrating to Westfall River and destinations in the mainstem upper Duncan River were significantly different using a Tukey test ($P = 0.01$) in a pooled by destination comparison.

Two of the migration statistics used in this analysis are confounded. Migration rate statistics, in-migration and out-migration rate, are calculated as distance per day. The destination factor of the ANOVA model is also related to distance. As a result, the migration rate calculations are confounded due to the relationship between the distance inherent in both rate and the destination factor of the ANOVA. The ANOVA calculations involving rate are included because they do give insights into influence of the year of migration on migration rates. A table presenting all migration data used in the ANOVA analysis, by radio-tag number and destination category, is appended.

Table 2-3. Two factor ANOVA comparisons of migration data for radio-tagged bull trout by analysis group (see Table 2.2) and year of the study.

Migration Statistic	*P*-value Destination	*P*-value Year	*P* - value Interaction (Dest.•Year)	Pooled Mean ± 95 % C.I. (n)
In-migration Start Date	0.280	0.357	0.833	8 July ± 4.4 days (64)
In-migration Rate **	0.010**	0.332	0.825**	1.80 ± 0.17 km/d (64)
In-migration Time	0.247	0.754	0.981	51.4 ± 4.2 days (64)
Residency Start Date (In-migration End Date)	0.630	0.358	0.482	29 Aug. ± 2.9 days (64)
Residency Time	0.489	0.650	0.533	31.7 ± 3.4 days (53)
Out-migration Start Date (Residency End Date)	0.084	0.154	0.733	28 Sept. ± 2.0 days (53)
Out-migration Rate **	0.350**	0.102	0.711**	9.93 ± 2.78 km/d (52)
Out-migration Time	**0.017***	0.655	0.561	8.99 ± 2.69 days (52)
Out-migration End Date	0.849	0.574	0.619	8 Oct. ± 2.68 days (52)
Fork Length (mm)	**0.043**	0.351	0.360	suggested differences (64)
Mass (g)	**0.019**	0.471	0.257	suggested differences (64)

* out-migration time was not correlated with distance (see text) and pooled

** comparison is confounded by similarity between factor and data variable (see text).

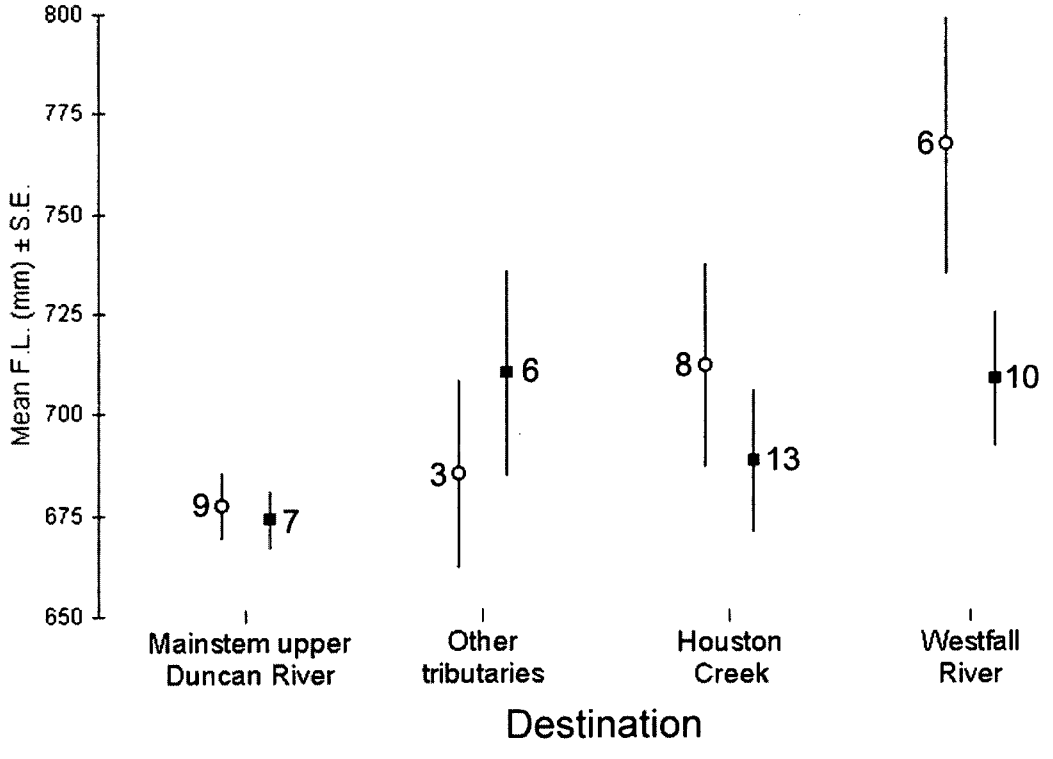

Figure 2-7. Data used in the calculation of bull trout fork length by destination and year two factor ANOVA. Open circles and solid squares are 1995 and 1996 data, respectively. Error bars are ± 1 SE. Destinations (ANOVA groups – Table 2.2) are approximately (see text) ordered by stream size from smallest to largest (left to right). Sample sizes are indicated next to data points.

The migration statistics of radio-tagged bull trout moving through the discharge structure at Duncan Dam were not different from bull trout transported and released upstream (two sample t-tests; minimum P = 0.092 for all migration statistics except in-migration and out-migration rate).

I confirmed spawning activity within the residency area of radio-tagged bull trout in Houston Creek and the Westfall River. Locating bull trout redds was easy in these two tributaries: redds were located at all locations where radio-tagged bull trout were located during the last week of the spawning site residency period. I was unable to locate evidence of spawning in sections of the mainstem upper Duncan River upstream of Hatteras Creek (Fig. 2-6) where radio-tagged bull trout were located, despite thorough searches. I did not search for evidence of spawning in the mainstem upper Duncan River below Hatteras Creek; however, the mainstem river downstream of Hatteras Creek (Fig. 2-6) does not contain habitat usually associated with bull trout spawning (Goetz 1984; McPhail and Baxter 1996). I assume that the bull trout migrating to destinations in the upper Duncan River were not migrating to spawn based on similar bull trout non-spawning migrations observed by Swanberg (1997) and O'Brien and Chamberlain (2000). In total, 49 radio-tagged bull trout migrated to presumed spawning tributaries of the upper Duncan River during the study.

Catchment area was a useful predictor of stream flow in the upper Duncan River system. A linear regression of stream flow estimates on catchment area was significant ($P < 0.001$; Fig. 2-8). Although the sample size is small, the catchment size of different tributaries explained > 99% of the variation in estimated stream flows ($y = 0.029x - 0.636$, $r^2 = 0.997$). Because I was unable to estimate flows from all streams targeted by radio-tagged bull trout, I used catchment area as a surrogate (Table 2.4). I plotted the fork length of all radio-tagged bull trout (1995 and 1996 combined) against catchment area of the destination tributary in Figure 2-9. A linear regression of fork length on catchment area was significant ($P = 0.030$), although little of the variation in length was explained by increasing catchment area ($y = 0.301x + 669.2$ $r^2 = 0.095$; Fig.2-9). The relationship between fork length and catchment area is not robust: when the largest individual (875 mm FL from Westfall River – 230 km^2 catchment; Fig. 2-9) is removed, the regression is not significant ($y = 0.235 + 675.5$, $r2 = 0.065$, $P = 0.077$).

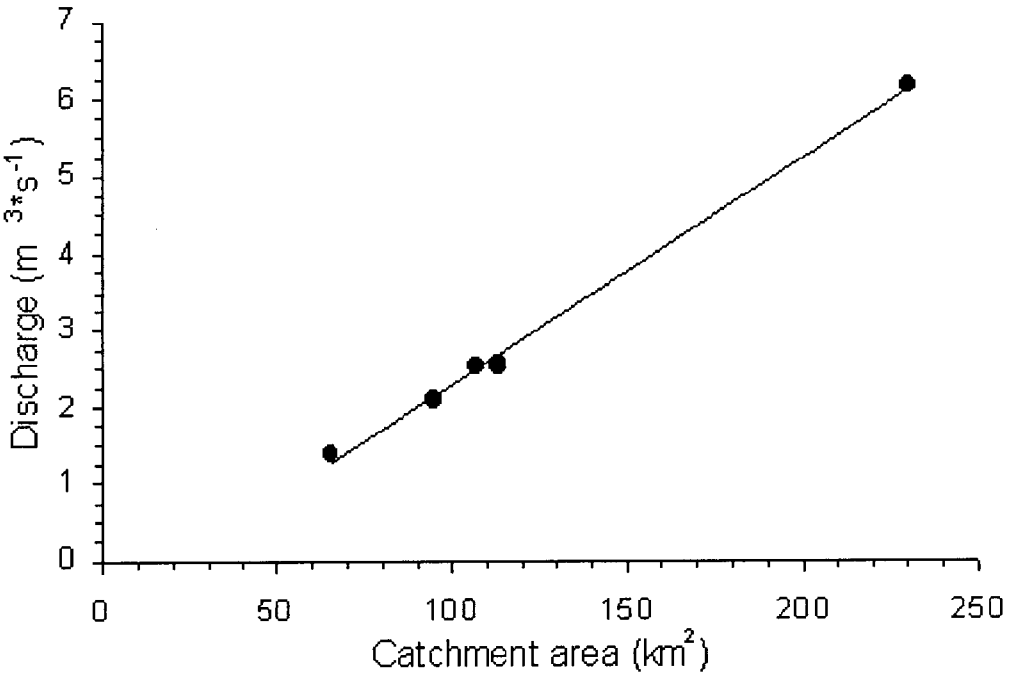

Figure 2-8. The regression of catchment size to instream flow estimate of five tributaries of the upper Duncan River. The regression ($y = 0.029x - 0.636$; solid line) is significant ($P < 0.001$, $r^2 = 0.997$).

Table 2-4. The catchment area of tributaries targeted by radio-tagged bull trout in 1995 and 1996.

Stream	Catchment area (km^2)
Westfall River	230
Giegerich Creek	113
Houston Creek	107
Hatteras Creek	65
Stevens Creek	51
Marsh Adams Creek	46

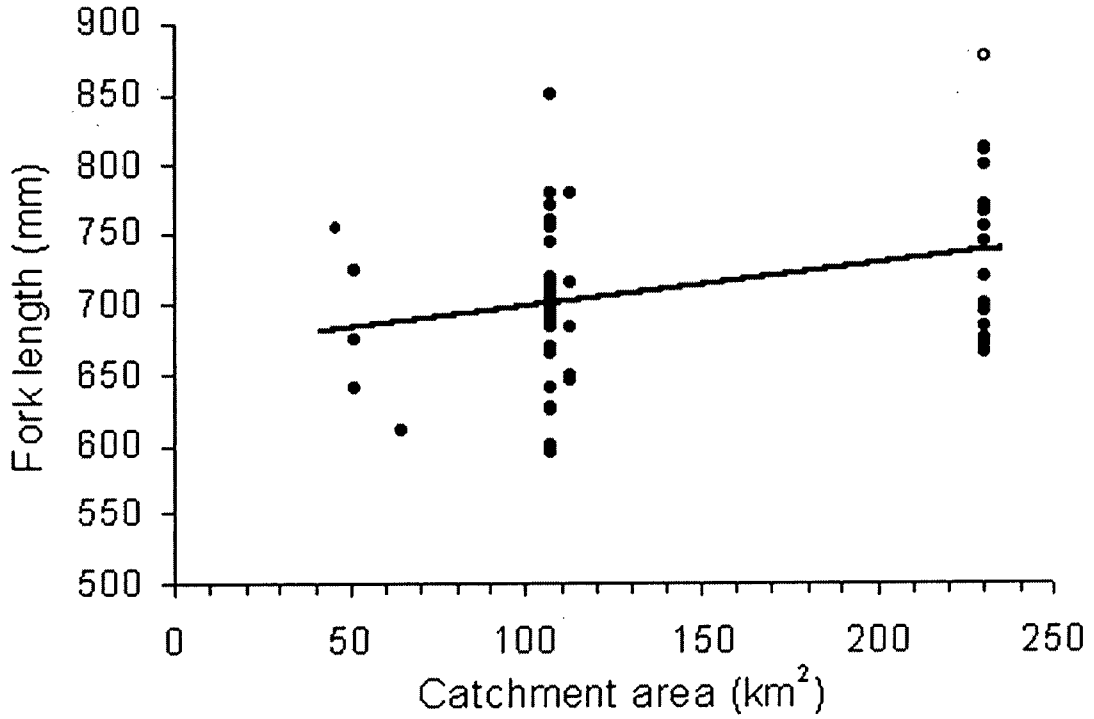

Figure 2-9. Fork length of radio-tagged bull trout (n = 49) by catchment area of destination tributaries in the upper Duncan River. The regression ($y = 0.301x + 669.2$; solid line) is significant ($P = 0.03$, $r^2 = 0.095$). A linear regression excluding the largest fish from this figure (open circle) is not significant ($P = 0.08$, see text).

For analysis of migration statistics, I considered the migration of bull trout to the upper Duncan River over when they returned to Duncan Reservoir. From subsequent locations, *via* both angler recaptures and telemetry fixes, most were tracked back to Kootenay Lake after reaching Duncan Reservoir. Over the winter of 1995/96, 82.6 % (19 of 23) radio-tagged bull trout left the reservoir through the discharge structure. I excluded three bull trout confirmed killed by anglers after the 1995 migration from this calculation. Over the winter of 1996/97, 74.3 % (26 of 35) of radio-tagged bull trout were confirmed to have left the reservoir; excluding three expired transmitters and two presumed mortalities. I rarely located radio-tagged bull trout in lakes, because of radio signal attenuation with depth in water (Winter 1983). I did however track bull trout throughout Kootenay Lake, and also located one radio-tagged bull trout at the confluence of the Moyie and Kootenay rivers in Idaho (Fig. 2-1) the summer after it had been tracked in the upper Duncan River. Radio telemetry confirmed all of the suggested movement patterns of bull trout in the

Kootenay River collected previously using conventional individually numbered Floy-tags (Fleck 1977).

I also determined the date bull trout moved downstream through Duncan Dam. In 1995, I only estimated the date based on first location downstream, but over the winter of 1996/97, I placed a remote data-logging receiver at the base of the dam (Table 2-5) to record actual dates of downstream passage. Over the winter of 1996/97, I tracked 13 bull trout moving downstream through Duncan Dam. On average, bull trout left Duncan Reservoir on 23 January 1997 (± 30 d) after summer and fall migrations to the upper Duncan River in 1996.

Table 2-5. Overwinter out-migration dates of individual radio-tagged bull trout through the discharge structure at Duncan Dam by year of migration to the upper Duncan River.

1995 migration	1996 migration
16-Oct-95*	8-Oct-96*
16-Oct-95*	20-Oct-96*
16-Oct-95*	26-Dec-96
31-Oct-95*	25-Jan-97
25-Nov-95*	1-Feb-97
13-Aug-96*	7-Feb-97
	10-Feb-97
	13-Feb-97
	16-Feb-97
	19-Feb-97
	24-Feb-97
	10-Mar-97
	11-Mar-97

*approximate only - first reception downstream of the dam using manual receiver

Multi-year migrations

Six radio-tagged bull trout were tracked migrating to the upper Duncan River in two consecutive spawning years. Two of the six entered different tributary streams in the second migration year (Table 2-6). If I consider only those bull trout migrating to presumed spawning locations in 1995 (17 fish), two (11.8%) returned in 1996, and both entered a different tributary. In both cases, the bull trout switched to a tributary with a smaller catchment area than their first tributary destination. Because many radio tags expired before the spawning migration in 1997, I cannot estimate the number migrating in both 1996 and 1997.

Table 2-6. Destinations of radio-tagged bull trout migrating to the upper Duncan River in two consecutive years.

Radio tag number	F.L. (mm) at tagging	Sex	Destination		Distance (km) between destinations
			year 1	year 2	
22	675	F	Westfall R.	Stevens Cr.	11.7
30	655	F	Mainstem	Mainstem	11.5
35	755	M	Stevens Cr.	Marsh Adams Cr.	18.7
39	655	M	Mainstem	Mainstem	12.5
40	690	F	Mainstem	Mainstem	3.5
64	685	M	Houston Cr.	Houston Cr.	4.0

Houston Creek fish trap

I captured 47 bull trout emigrating from Houston Creek in a fish trap. Of these out-migrants, three were radio-tagged (6.4 %) and one was marked with a Floy-tag at Duncan Dam. The mean fork length of trapped out-migrants was 564 ± 27 mm (n = 45, Figure 2-10). The mean fork length of fish captured at the trap was significantly smaller than that of fish captured at Duncan Dam that were not radio tagged (pooled 1995 and 1996 dam captures; 1 tailed t-test, $P < 0.001$). Twenty one were female, 23 were male, a single bull trout (FL 411 mm) was of indeterminate sex, and two escaped before examination. All but the smallest bull trout captured at Houston Creek showed clear evidence of spawning. Females were gaunt and all males had recent scarring on the caudal fin and peduncle, suggesting spawning (McPhail and Baxter 1996). All males and females expressed milt or eggs when anaesthetized and handled. The mark recapture estimate of the total number of bull trout in Houston Creek was 167. Assuming the proportion of radio-tagged to untagged fish was the same at all destinations in the upper Duncan River, approximately 500 bull trout migrated into the system in 1996 (Houston Creek was 32.5% of the total number of migrants in 1996 – Table 2-2). Based on the proportion of fish migrating in consecutive years, I estimate that roughly 40 to 50 bull trout switch spawning stream from year to year in the upper Duncan River.

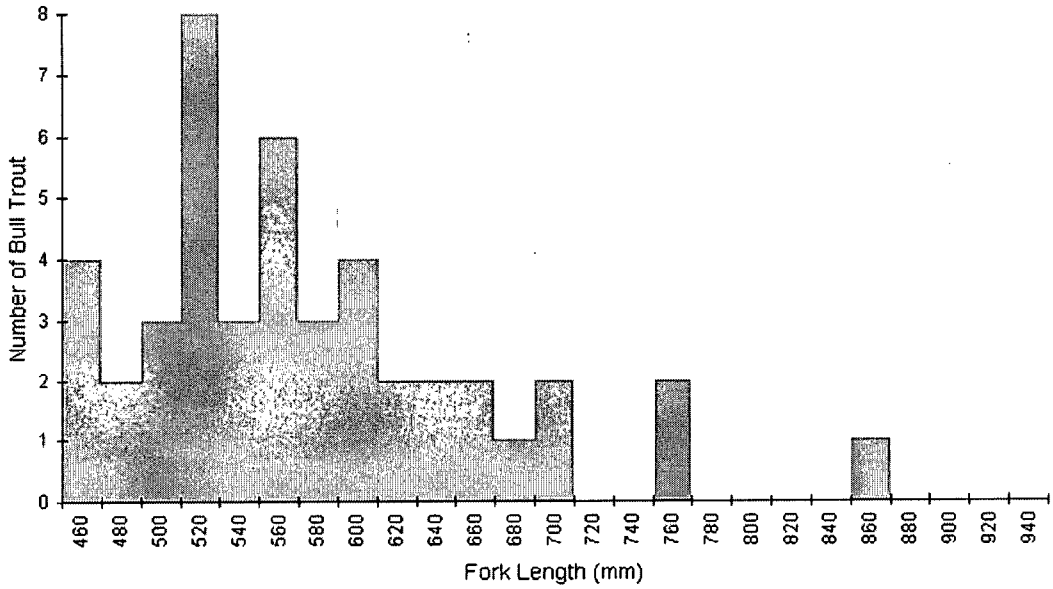

Figure 2-10. The fork length (FL) frequency histogram of bull trout captured at the Houston Creek trap in 1996 (gray bars, n = 47).

2.4 Discussion

The mean fork length of bull trout receiving radio tags (703 ± 14 mm) was significantly larger than the mean size of fish captured at Duncan Dam. This discrepancy reflects my selection of larger bull trout for tagging due to size constraints associated with the implantation of tags.

In this study I have assumed that radio tagging does not change the migratory behaviour of bull trout. Except for a short period immediately following tagging, many studies (Stasko and Pincock 1977; Winter 1983; Marty and Summerfelt 1986; Swanberg 1996; Thorstad et al. 2000) suggest that if the transmitter is small relative to the size of the fish tagged, the effect of transmitter implantation on fish behaviour and movement can safely be ignored. By 'small', I follow the definition of Marty and Summerfelt (1986): the mass (in water) of surgically implanted tags should not exceed 2% of the mass (in air) of the fish destined to carry it and, ideally, this ratio should not exceed 1%. I kept the tag to fish weight ratio below 1% in all cases. In this study, I did not explicitly test the assumption that radio tagging did not effect behaviour, but several of my observations corroborate the findings of others. I found that bull trout carrying transmitters often migrated to the extent of available habitat. I also located untagged bull trout in proximity to radio-tagged fish at all locations I examined, including a tagged bull trout male

spawning with an untagged female in Westfall River. This suggests that their behaviour was similar. Untagged bull trout were also captured during out-migration from Houston Creek at the same time as radio-tagged individuals.

Bull trout migrations in the upper Duncan River

Radio telemetry proved useful in obtaining data on bull trout spawning migrations in the upper Duncan River. The pattern of bull trout migration typically consisted of in-migration to a destination in the upper Duncan River, residence within a short distance of that destination, and a rapid out-migration relative to the time of in-migration and residence. Comparisons of the 1995 and 1996 migration statistics show the two years to be very similar (Table 2-3). Time of in-migration, residence, and out-migration do not differ between destinations in the upper Duncan River or the year of migration (Table 2-3). The average bull trout migrating to a destination in the upper Duncan River began moving upstream of the dam on 8 July. The average bull trout arrived at its destination on 29 August (51 day in-migration) and remained there for 32 days until 28 September. In-migration rate averaged just under 2 $km*day^{-1}$. Moving more than 9 $km*day^{-1}$, the average out-migrant returned to Duncan Reservoir on 8 October. Bull trout leave Duncan Reservoir, and return to Kootenay Lake, throughout the winter (Table 2-5). The timing of spawning migrations in the Duncan River fall within the range of bull trout migratory timing as reviewed by Goetz (1984) and McPhail and Baxter (1996).

The impact of releasing bull trout captured and tagged downstream of Duncan Dam in the reservoir upstream on migratory timing and behaviour is a concern. The migratory timing of bull trout moved over Duncan Dam did not differ from those that moved through it ($P \geq 0.092$), suggesting that the release of bull trout upstream of the dam did not alter migration behaviour. I only tagged fish after they actively moved into the discharge structure at Duncan Dam. Movement into the discharge structure suggests that bull trout were attempting to move through. O'Brien and Chamberlain (2000) compared the timing of bull trout spawning migrations in the Adams River with my results. The dates radio-tagged bull trout arrived and left destination areas in Adams River (no dam) were the same as Duncan River bull trout. This suggests that the impact of the dam on the

timing of spawning migrations is minimal for those fish successfully passing upstream through the dam.

In total, six tributaries and portions of the mainstem upper Duncan River were the destinations of migrating bull trout. The two largest tributaries, Westfall River and Houston Creek, account for over half of the tagged migrants (Table 2-2). The distribution of bull trout among destinations (Table 2-2) did not vary significantly between 1995 and 1996 (Fisher exact test, $P = 0.91$). I evaluated whether this distribution was related to stream size, using catchment area as a surrogate (Fig. 2-8), in terms of the size (Fig. 2-9) of bull trout migrants as suggested by McPhail and Murray (1979). For this test, I used only bull trout migrating to tributary destinations and excluded those migrating to destinations in the mainstem river (see below). There was a slight positive relationship between the size of radio-tagged bull trout and the size of the tributary they migrated too (linear regression, $P = 0.03$), but this relationship was not robust. Perhaps this relationship would be better resolved if smaller bull trout had been radio-tagged.

McPhail and Murray (1979) suggested that bull trout may be physically restricted from smaller spawning streams as they grow. The mean sizes of spawning bull trout McPhail and Murray (1979) captured were 447 and 438 mm standard length and 1260 and 1097 g for males and females, respectively. These were probably first time spawners. Duncan Dam bull trout captures were on average more than 200 mm larger than the bull trout McPhail and Murray (1979) captured in their study. The largest bull trout I captured at Duncan Dam was 920 mm and 9500 g. This amount of variation in size supports McPhail and Murray's (1979) suggestion: as bull trout grow, spawning habitat preferences probably change. If bull trout switch spawning stream based on size, as the regression of bull trout size on tributary catchment area suggests, it explains a reduction in fidelity to natal stream. It suggests that bull trout may not home accurately at the scale of individual spawning streams. It is also possible that the apparent relationship between bull trout size and spawning tributary catchment area could reflect the effect of selection in streams of differing size for appropriately sized bull trout.

I assumed that bull trout migrating to mainstem destinations in the upper Duncan River did not spawn. I based this assumption on both negative spawning survey results and a general lack of suitable spawning habitat at mainstem destinations compared to

literature descriptions (Baxter and McPhail 1996; McPhail and Baxter 1996). Two other radio telemetry studies have suggested that some bull trout that participate in the spawning migration do not spawn. Swanberg (1996; 1997) found that as many as 70 % of bull trout radio-tagged in the Blackfoot River in Idaho did not migrate all the way to spawning areas, and did not spawn. Swanberg (1996; 1997) found that these fish were generally smaller, began migrations later, and left tributaries earlier than spawning fish. Similar results were obtained in the Adams River, BC (O'Brien and Chamberlain 2000). Over two years, approximately 40 % of the radio-tagged bull trout migrating into the Adams River did not leave the mainstem river. In Adams River, bull trout remaining in the mainstem were smaller than those moving into spawning tributaries, and they emigrated from the river prior to the emigration of individuals that spawned in tributaries. Bull trout migrating to mainstem upper Duncan River destinations were generally smaller than other migrants (Fig. 2-7), although all were large enough to be mature based on literature accounts (Goetz 1989; McPhail and Baxter 1996). Unfortunately, I was unable to collect bull trout at mainstem destinations and examine them for their level of maturity.

Swanberg (1996; 1997) suggested that non-spawning migrants were moving to avoid seasonal high temperatures in the mainstem Blackfoot River. Temperatures in the mainstem Blackfoot River can exceed 20 ^{0}C in July. Swanberg (1997) specifically dismissed the hypothesis of foraging for these non-spawning migrants, as prey species were rare in tributaries of the Blackfoot R. Seasonally high temperatures may occasionally exceed 20 ^{0}C in the surface waters of Kootenay lake or the Duncan Reservoir, but such temperatures are easily avoidable with depth. In the upper Duncan River, I did not sample mainstem locations enough to estimate abundances of bull trout prey fish species; although, in all sampling of spawning tributaries (Chapter 3), I encountered only bull trout. Non-spawning migrations in the upper Duncan River do not seem related to temperature or foraging.

Spawning stream switching

Two of six bull trout making repeat migrations switched destination tributary (Table 2-5) in the second migration year. Of the six bull trout tracked in consecutive years, the sex ratio was equal and one male and one female migrated to a different presumed spawning tributary (Table 2-5). Contrary to the hypothesis of McPhail and

Murray (1979), bull trout switching tributaries changed to tributaries with smaller catchment areas.

The observed destination switching suggests that there may be gene flow among spawning streams. I was not able to confirm if stream-switching fish actually spawned in each stream. I was able to directly confirm spawning activity only in Houston Creek and Westfall River because of limited access in other streams. With results like those of Swanberg (1997) and suggested by O'Brien and Chamberlain (2000), where some bull trout migrated without spawning, it is important to determine if the observation of spawning stream switching results in gene flow (see Chapter 3).

Houston Creek fish trap

The bull trout captured during out-migration from Houston Creek were on average much smaller than those captured at Duncan Dam (Fig. 2-10). This suggests that not all bull trout spawning in Houston Creek had migrated from Kootenay Lake. The majority of smaller, and probably younger, bull trout trapped in Houston Creek were bull trout likely migrating from Duncan Reservoir.

I used bull trout captured during out-migration from Houston Creek to estimate the number of bull trout present in Houston Creek for spawning in 1996. Using this estimate (167 fish) I roughly extrapolated the number present in the upper Duncan River in 1996 (~500 bull trout). I assumed the number present in 1995 was similar to 1996, and then estimated numbers of untagged bull trout that change destination tributary each year from the number of tagged bull trout that did so over 1995 and 1996 (approximately 40 to 50 fish). Clearly this extrapolated estimate is imprecise; nevertheless, it is valuable in that it provides me a comparison with estimates of migration from genetic analysis presented in Chapter 3.

Conservation implications

Without confirmation of spawning by bull trout at most locations in the upper Duncan River, the conservation implications of these telemetry results are difficult to interpret. The potentially most important result from a conservation, or management, perspective is the apparent switching between spawning sites by bull trout in consecutive

years. This result suggests that individual spawning streams do not represent genetically divergent populations in the upper Duncan River.

The apparent non-spawning movements of more than 25 % of the radio-tagged bull trout into the upper Duncan River is also important from a conservation perspective. The knowledge that many more bull trout than would be enumerated at spawning sites have migrated nearly the same distance as spawning fish changes suggests habitat protection may be necessary beyond only the area of specific spawning sites. It is important to know that these non-spawning bull trout move at similar times to fish that spawn. Protection measures, such as seasonal angling closures along migration routes, will benefit both spawning and non-spawning migrants.

2.5 Appendix

Table 2-7. The biological data collected from radio-tagged bull trout migrating to destinations in the upper Duncan River. 'Ch.' is tag channel, 'Floy' is the number of the external tag, 'FL' is fork length in mm, and mass is measured in kg.

Fish #	Ch	Tag Code	Date Tagged	Floy	FL	Mass	Sex
4	5	104	13-Jun-95	577W	640	2.80	M
4	20	102	3-Jul-96	577W	715	3.30	M
10	5	111	14-Jun-95	637W	730	4.10	M
10	20	101	14-Jun-96	636Y	770	4.80	M
20	22	123	27-Jun-95	748W	630	2.75	M
20	20	111	14-Aug-96	748W	720	4.20	M
21	22	124	27-Jun-95	749W	710	3.30	M
22	22	125	28-Jun-95	763W	675	2.80	F
23	22	126	28-Jun-95	801W	810	5.45	F
24	22	127	28-Jun-95	802W	655	2.70	M
25	22	128	29-Jun-95	803W	745	4.35	F
26	22	129	29-Jun-95	761W	705	3.35	M
27	22	131	29-Jun-95	804W	670	3.20	F
28	22	133	29-Jun-95	812W	670	3.05	M
29	5	100	5-Jul-95	810W	780	4.80	M
30	22	134	5-Jul-95	829W	655	2.90	F
31	22	135	5-Jul-95	833W	640	2.90	M
32	22	136	5-Jul-95	520W	645	2.80	M
33	22	137	5-Jul-95	775W	685	3.40	M
34	31	138	13-Jul-95	518Y	850	5.90	M
35	31	141	18-Jul-95	520Y	725	3.50	M
35	5	134	14-Aug-96	520Y	755	4.00	M
36	31	142	18-Jul-95	524Y	875	7.60	M
37	31	143	18-Jul-95	525Y	650	2.80	F
38	31	140	19-Jul-95	533W	665	3.10	M
39	31	144	19-Jul-95	890W	655	2.90	M
39	20	112	14-Aug-96	822Y	695	3.40	M
40	31	145	19-Jul-95	554Y	690	3.20	F
41	31	147	19-Jul-95	557Y	665	2.80	F
42	31	148	19-Jul-95	691W	725	4.00	M
43	31	146	20-Jul-95	575Y	715	3.80	M
44	31	149	20-Jul-95	689W	700	3.40	F
45	31	150	20-Jul-95	675W	685	3.10	F
46	31	151	20-Jul-95	619W	680	2.80	F
49	15	2	3-Sep-95	712W	810	5.00	F
51	31	153	6-Jun-96	848Y	780	4.40	M
53	5	121	14-Jun-96	642Y	650	2.80	M
54	5	122	14-Jun-96	643Y	685	3.10	M
55	5	123	14-Jun-96	638Y	695	3.80	M
56	5	124	14-Jun-96	641Y	720	4.00	M
57	31	154	14-Jun-96	637Y	665	3.00	M
58	5	125	18-Jun-96	846Y	685	3.60	M
59	5	126	18-Jun-96	664W	695	3.50	F
60	5	127	18-Jun-96	557W	685	3.00	M
61	5	128	3-Jul-96	815Y	670	2.70	M
62	5	129	3-Jul-96	879W	610	1.80	F
64	20	103	3-Jul-96	812Y	685	3.50	M
65	20	104	3-Jul-96	814Y	765	5.10	M
66	20	105	3-Jul-96	915Y	685	3.20	M
67	15	3	10-Jul-96	817Y	690	3.00	M
68	15	4	10-Jul-96	907Y	595	2.10	M
69	15	5	10-Jul-96	604W	660	2.40	M
71	20	107	10-Jul-96	821Y	755	3.50	M
72	20	108	10-Jul-96	819Y	800	4.60	M
73	20	109	10-Jul-96	816Y	770	4.40	M
74	20	110	10-Jul-96	818Y	755	3.40	M
75	15	6	15-Jul-96	8019Y	745	4.40	M
76	15	7	15-Jul-96	922Y	625	2.80	M
77	15	9	15-Jul-96	8017Y	695	3.50	F
78	15	10	15-Jul-96	828W	700	3.30	M
79	15	11	15-Jul-96	8036Y	675	2.90	M
80	15	12	15-Jul-96	8007Y	600	2.50	M
81	20	114	15-Jul-96	923Y	760	4.90	M
82	20	115	15-Jul-96	611Y	780	5.40	M
83	5	131	7-Aug-96	672W	680	3.50	M
87	5	135	27-Aug-96	8099Y	635	3.60	M

Table 2-8. Summary of migration statistics and destinations of all radio-tagged bull trout migrating to destinations in the upper Duncan River (used in 2 way ANOVA). 'In' is in-migration, 'Res' is residence, 'Out' is out-migration, 'Dest' is destination (see Table 2-2) rate units are km*day^{-1}, and time units are days.

Tag #	Start in	In time	In rate	In/res	Res time	Res/out	out rate	out time	End out	Out time	Dest
26	17-Jun	61	1.64	17-Aug	51	7-Oct	8.08	12	19-Oct	12	1
27	29-Jun	67	1.49	4-Sep	8	12-Sep	6.94	9	21-Sep	9	1
28	29-Jun	52	1.92	20-Aug	34	23-Sep	5.00	20	13-Oct	20	1
29	5-Jul	51	1.96	25-Aug	37	1-Oct	5.11	19	20-Oct	19	1
31	4-Jul	55	1.82	28-Aug	34	1-Oct	6.47	15	16-Oct	15	1
34	13-Jul	55	1.82	6-Sep	28	4-Oct	24.00	2	6-Oct	2	1
38	18-Jul	42	2.38	29-Aug	36	4-Oct	26.25	2	6-Oct	2	1
43	20-Jul	36	2.78	25-Aug	37	1-Oct	11.50	5	6-Oct	5	1
21	14-Jun	61	1.64	14-Aug	44	27-Sep	5.35	10	7-Oct	10	1
55	14-Jun	61	1.64	14-Aug	47	30-Sep	7.64	7	7-Oct	7	1
56	15-Jul	30	3.33	14-Aug	57	10-Oct	53.50	1	11-Oct	1	1
59	15-Jul	38	2.63	22-Aug	36	27-Sep	4.86	11	8-Oct	11	1
62	15-Jul	38	2.50	22-Aug	43	4-Oct	12.13	4	8-Oct	4	1
64	10-Jul	45	2.22	24-Aug	32	25-Sep	3.82	14	9-Oct	14	1
67	10-Jul	47	2.13	26-Aug	32	27-Sep	4.46	12	9-Oct	12	1
68	10-Jul	47	2.13	26-Aug							1
71	10-Jul	55	1.82	3-Sep	23	26-Sep	4.46	12	8-Oct	12	1
73	3-Jul	62	1.61	3-Sep	28	1-Oct	13.38	4	5-Oct	4	1
75	15-Jul	50	2.00	3-Sep							1
76	3-Jul	71	1.41	12-Sep	13	25-Sep	5.94	9	4-Oct	9	1
80	18-Jun	87	1.15	13-Sep							1
81	7-Jun	102	0.98	17-Sep							1
22	28-Jun	67	1.12	3-Sep	24	27-Sep	19.00	2	29-Sep	2	2
23	28-Jun	71	1.06	7-Sep	23	30-Sep	12.50	4	4-Oct	4	2
25	29-Jun	47	1.60	15-Aug	53	7-Oct	11.17	6	13-Oct	6	2
33	5-Jul	30	2.50	4-Aug	54	27-Sep	6.82	11	8-Oct	11	2
36	18-Jul	57	1.21	13-Sep	30	13-Oct	8.83	3	16-Oct	3	2
49	3-Sep	27	0.46	30-Sep	8	8-Oct	14.40	5	13-Oct	5	2
10	14-Jun	70	1.07	23-Aug	36	28-Sep	3.17	9	7-Oct	9	2
20	14-Aug	32	2.34	15-Sep	31	16-Oct	28.50	1	17-Oct	1	2
57	14-Jun	74	1.01	27-Aug							2
61	3-Jul	44	1.70	16-Aug	42	27-Sep	3.17	9	6-Oct	9	2
65	3-Jul	63	1.19	4-Sep	25	29-Sep	3.17	9	8-Oct	9	2
72	10-Jul	46	1.63	25-Aug	30	24-Sep	2.38	12	6-Oct	12	2
74	10-Jul	62	1.11	10-Sep	29	9-Oct	11.25	2	11-Oct	2	2
77	15-Jul	31	2.42	15-Aug							2
78	15-Jul	59	1.27	12-Sep							2
79	15-Jul	36	2.08	20-Aug	36	25-Sep	7.13	4	29-Sep	4	2
24	27-Jun	68	1.25	3-Sep	32	5-Oct	0.74	51	25-Nov	51	3
30	2-Jul	56	1.52	27-Aug	31	27-Sep	4.21	19	16-Oct	19	3
37	18-Jul	44	1.70	31-Aug							3
39	19-Jul	47	2.02	4-Sep	26	30-Sep	18.25	4	4-Oct	4	3
40	19-Jul	52	1.63	9-Sep	21	30-Sep	1.53	19	19-Oct	19	3
41	19-Jul	31	2.42	19-Aug	46	4-Oct	32.50	1	5-Oct	1	3
42	19-Jul	32	2.66	20-Aug	45	4-Oct	3.64	22	26-Oct	22	3
44	20-Jul	33	2.88	22-Aug	32	23-Sep	4.77	11	4-Oct	11	3
46	20-Jul	31	2.58	20-Aug	20	9-Sep	6.25	6	15-Sep	6	3
30	7-Aug	37	2.62	13-Sep							3
39	14-Aug	30	2.83	13-Sep	13	26-Sep	3.50	11	7-Oct	11	3
40	14-Aug	27	3.15	10-Sep	16	26-Sep	1.93	20	16-Oct	20	3
58	18-Jun	58	1.67	15-Aug	42	26-Sep	4.21	12	8-Oct	12	3
60	18-Jun	60	1.00	17-Aug	39	25-Sep	1.93	7	2-Oct	7	3
66	3-Jul	52	1.63	24-Aug							3
69	10-Jul	44	2.20	23-Aug	15	7-Sep	2.20	23	30-Sep	23	3
32	5-Jul	60	1.00	3-Sep	31	4-Oct	17.50	1	5-Oct	1	4
35	18-Jul	62	1.13	18-Sep	11	29-Sep	5.50	5	4-Oct	5	4
45	20-Jul	47	1.28	5-Sep	25	30-Sep	9.50	4	4-Oct	4	4
4	3-Jul	34	1.76	6-Aug	53	28-Sep	1.69	8	6-Oct	8	4
22	12-Jul	35	1.86	16-Aug	40	25-Sep	2.06	9	4-Oct	9	4
35	14-Aug	20	3.90	3-Sep	23	26-Sep	2.63	12	8-Oct	12	4
51	6-Jun	90	0.67	4-Sep	20	24-Sep	0.90	15	9-Oct	15	4
53	14-Jun	86	0.70	8-Sep	13	21-Sep					4
54	14-Jun	70	0.86	23-Aug	47	9-Oct	1.93	7	16-Oct	7	4

Chapter 3 - An evaluation of genetic differentiation between two bull trout (*Salvelinus confluentus*) spawning streams in the upper Duncan River using microsatellites.

3.1 Introduction

Most species are structured into semi-isolated reproductive populations. Such populations tend to diverge from other populations through selection or genetic drift (Slatkin 1987). Preserving this genetic diversity is considered paramount for conservation (Moritz 1994; Avise 1995). Understanding the spatial scale over which such genetic population structure occurs is, therefore, an important conservation priority. The geographic scale over which species are divided into populations is a function gene flow. In general, a small amount of gene flow between otherwise separate populations will counter genetic divergence due to selection or drift (Wright 1931; Hartl and Clark 1989; Mills and Allendorf 1996). The amount of gene flow is related to barriers to dispersal, a species' inherent dispersal ability and reproductive site fidelity (Waples 1998; Bohonak 1999).

The distance animals can move obviously affects population structure. For populations in close proximity, relative to dispersal ability, there should be more gene flow than between distant populations. This is 'isolation by distance' (Wright 1943; Slatkin 1993). Barriers to migration and gene flow enhance isolation among populations, resulting in a finer spatial scale over which species are structured into genetically distinct populations. In marine organisms, where barriers to gene flow are slight (e.g. Palumbi et al. 1997), the amount of genetic differentiation between populations is generally small (Ward et al. 1994; Waples 1998). In contrast, where physical barriers to movement are more common, such as among populations of freshwater fish, genetic differentiation among populations is stronger (Ward et al. 1994).

The degree to which animals show fidelity to natal sites also has important implications for the level of gene flow and resulting genetic population structure. Reproductive site fidelity, or homing, is an adaptive trait, because it ensures that

reproduction occurs where conditions suitable for survival, growth and reproduction occurred in the past (Lindsey et al. 1959; Northcote 1997). Thus, the spatial scale of genetic divergence among populations should be finer in animals that home to their exact birth site, than, for example, animals that home to the general natal area.

The processes that affect population structure, and divergence among populations specifically, have been well studied in salmonid fishes. In salmonid fishes, particularly Pacific salmon, there is evidence that fidelity to natal areas, or homing, results in fine scale population divergence (Scheer 1939; Banks 1969; McIsaac and Quinn 1988; Quinn and Dittman 1990; Taylor 1991). Distinct populations are detectable, through morphological, behavioural or genetic differences, in salmonids from streams in close geographic proximity (Quinn et al. 1987; Taylor 1991; Angers et al. 1995), within the same stream (Taft and Shapovalov 1938; Tallman and Healey 1994; Costello and Taylor unpubl. MS), or even within the same stream reach (Hansen et al. 1997; Carlsson et al. 1999). Homing may be especially important for semelparous Pacific salmon, in that it increases the chance that their only reproduction is successful. Clearly homing is not perfect in Pacific salmon, since much of their current range was recolonised following deglaciation (Quinn and Dittman 1990). Rates of straying among natal streams can be high quite high (>50%) in some species of Pacific salmon (Shaklee and Varnavskaya 1994; McElhany et al. 2000), especially in fish of hatchery origin (Quinn 1993). As suggested previously, less accurate homing will tend to increase the geographic area of populations.

The bull trout is an iteroparous salmonid native to north western North America (Haas and McPhail 1991). Because of population declines in the southern portion of its range (Barry 1999), bull trout are the subject of conservation concern. These declines are usually attributed to habitat loss, habitat fragmentation and exotic species introductions (Goetz 1984; Rieman and McIntyre 1993; Watson and Hillman 1997). To help define the spatial scale at which conservation efforts should be focused (Vogler and DeSalle 1994), it is important to define the geography of distinct bull trout populations. Unfortunately, the strength of natal stream fidelity is not known in bull trout (McPhail and Murray 1979; Goetz 1984; McPhail and Baxter 1996).

The spatial scale of genetic population structure in bull trout has been studied at three different levels: a species wide level (Spruell and Allendorf 1997; Taylor et al. 1999), large regional levels (Leary et al. 1993; Spruell and Allendorf 1997; Williams et al. 1997; Taylor et al. 1999; Latham and Taylor in press; Costello and Taylor unpubl. MS), and smaller local or small watershed levels (Spruell et al. 1999; Costello and Tayor unpubl. MS). Over the range of the species, both mitochondrial DNA (mtDNA) and microsatellite studies (Spruell and Allendorf 1997; Taylor et al. 1999) suggest that bull trout are broadly divided into a coastal and an interior group. Subdivision on this broad geographic scale is attributed to historical isolation in two glacial refugia south of the Cordilleran ice sheet (Taylor et al. 1999). At a regional level, below that of large drainages like the Columbia or Fraser rivers, bull trout are structured into clusters of similar groups within regions that are differentiated from other such groups (Leary et al. 1993; Spruell and Allendorf 1997; Williams et al. 1997; Taylor et al. 1999; Latham and Taylor in press; Costello and Taylor unpubl. MS). At these two relatively broad scales, bull trout generally display low levels of genetic variability. This low variability is attributed to historical demographic processes such as founder effects resulting from postglacial dispersal (Leary et al. 1993) and more recent bottlenecks (Spruell and Allendorf 1997; Taylor et al. 1999; Costello and Taylor unpubl. MS).

Two earlier studies characterized genetic population structure at the level of individual watersheds. Spruell et al. (1999) examined Lightning Creek, a tributary to the Clark Fork River in Idaho, that drains an area of 220 km^2. Costello and Taylor (unpubl. MS) examined population structure within and between Peace and Kootenay river tributaries in British Columbia and Red Deer and Oldman rivers in Alberta. The spatial scales that Costello and Taylor (unpubl. MS) examined within river systems ranged from roughly 300 to 3000 km^2. In all but the case of one Kootenay River tributary (Wigwam River) both studies found significant subpopulation variation within watersheds (Spruell et al. 1999; Costello and Taylor unpubl. MS).

In Chapter 2, I presented telemetry evidence that over a two year period some bull trout in the upper Duncan River switched spawning streams. This suggests that bull trout may not home to specific streams in the upper Duncan River system. The upper

Duncan River drains an area of nearly 1300 km^2. As discussed in Chapter 2, the observation that some bull trout change streams does not necessarily mean successful reproduction at both locations. I did not confirm spawning at all locations. Also, because the natal stream(s) of the radio-tagged bull trout are unknown, I cannot use the data in Chapter 2 to make direct inferences about stream fidelity, other than noting that fish switched migration destinations over two years. If, over their lives, bull trout switch spawning streams this should tend to increase the geographic area over which bull trout remain a single, genetically homogeneous population.

I used microsatellites to compare two spawning tributaries within the upper Duncan River. The purpose was to test the prediction (based on the telemetry data) that bull trout in the upper Duncan system are a single genetic population. Microsatellites are selectively neutral nuclear loci that show high levels of allelic variation and, as such, they are useful for differentiating closely related species, populations, or even individuals (Jarne and Lagoda 1996). I collected microsatellite allele frequency data from Houston Creek and the Westfall River. These streams were chosen because of their relatively high use by radio-tagged bull trout (Chapter 2) and the observation that bull trout spawned in both streams. The mouths of Houston Creek and Westfall River are separated by approximately 30 km, and their primary spawning areas (identified from telemetry) are separated by approximately 60 km. I used the allele frequency data to estimate the level of genetic differentiation between Houston Creek and Westfall River to address the following questions:

- Do bull trout in different spawning streams in the upper Duncan River belong to genetically distinct populations?
- How does spawning stream switching (as estimated from telemetry) compare to estimates of gene flow derived from microsatellite data?

Based on the suggestion of high gene flow from my telemetry results (Chapter 2), and despite the results of genetic studies at similar spatial scales, I predict that bull trout in Houston Creek and Westfall River represent a single, genetically homogeneous population.

3.2 Methods

Tissue collection

I captured juvenile bull trout by backpack electrofishing at sites in Houston Creek and the Westfall River. The relatively wide spacing of sample locations in Westfall R. (Fig. 3-1) compared to those in Houston Cr. (Fig. 3-2) is a function of access in the two systems. A road extends along the length of Westfall R. and allows relatively easy access. We accessed all but one site in Houston Cr. by foot from the confluence of the creek and the upper Duncan River mainstem. One site in Houston Cr. was accessed by helicopter. At each site, a GPS coordinate was recorded, and a single electrofishing pass made through suitable juvenile habitat. I focused on side channels, woody debris and channel margins (Baxter and McPhail 1996). Electrofishing continued at each site until the target numbers of individuals were captured (see below). The type of tissue collected varied as a function of fish size. Tissue samples of young-of-the-year bull trout consisted of the entire animal. For juveniles > 55 mm, the adipose fin was removed. In all cases, the tissue was placed in an individually numbered snap top centrifuge tube containing 95% ethyl alcohol and refrigerated until processed in the laboratory. No mature bull trout were encountered during this sampling. I separated tissue samples from each stream into three age classes based on fork length (FL) frequency, and in this way grouped samples for further analysis. In Westfall R., bull trout < 54 mm were considered 0+, 1+ from 54 to 93 mm, and 2+ from 105 to 155 mm (Fig. 3-3). In Houston Cr., I considered fish < 60 mm 0+, from 60 to 100 mm 1+, and those > 110 mm 2+ (Fig. 3-4). By setting age breaks at these lengths, I avoided including samples that were difficult to clearly place in a specific age category. I attempted to reach a sample size of 20 individuals from each age class in each stream, but 2+ bull trout were rare in my samples from Houston Cr. Most of the tissue sampling occurred in 1997; however, I used four 1+ samples collected from Houston Cr. in 1996 to bolster the sample size in the 2+ age class from 1997.

Allendorf and Phelps (1981) cautioned specifically against attempting to estimate population structure from juveniles, because allele frequencies estimated from juveniles may not reflect the allele frequencies of adults. In a study evaluating the

problem of relatedness when sampling juveniles for molecular analysis, Hansen et al. (1997) found that young-of-the-year brown trout (*Salmo trutta*) captured within short stream sections came from a small number of full-sibling families. This biased estimates of allele frequencies. To reduce the likelihood of sampling families, I used no more than three young-of-the-year bull trout from each sampling site, and maintained a minimum of 250 m between all sample sites. Although Hansen et al. (1997) found that the problem of sampling families was not apparent in age classes older than young-of-the-year, I also used no more than three fish of older age classes from each sample site.

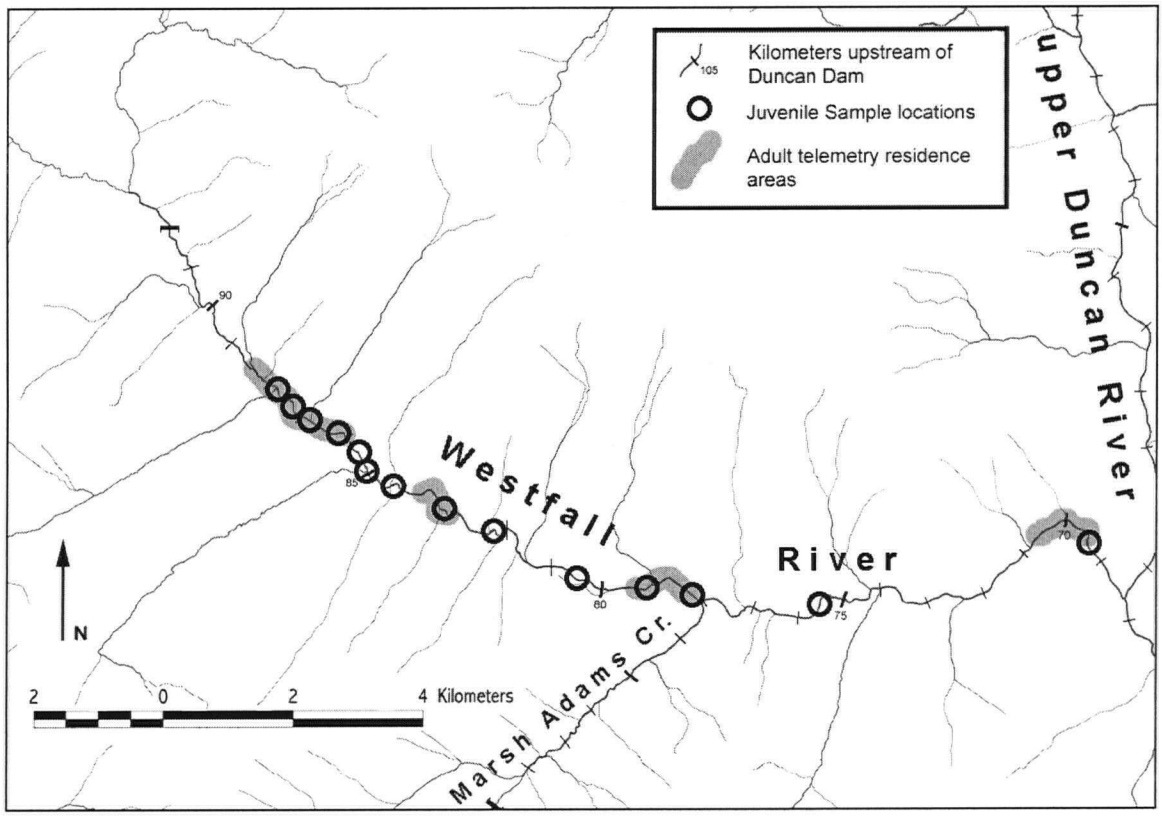

Figure 3-1. Juvenile bull trout tissue sampling locations (n = 14) in Westfall River.

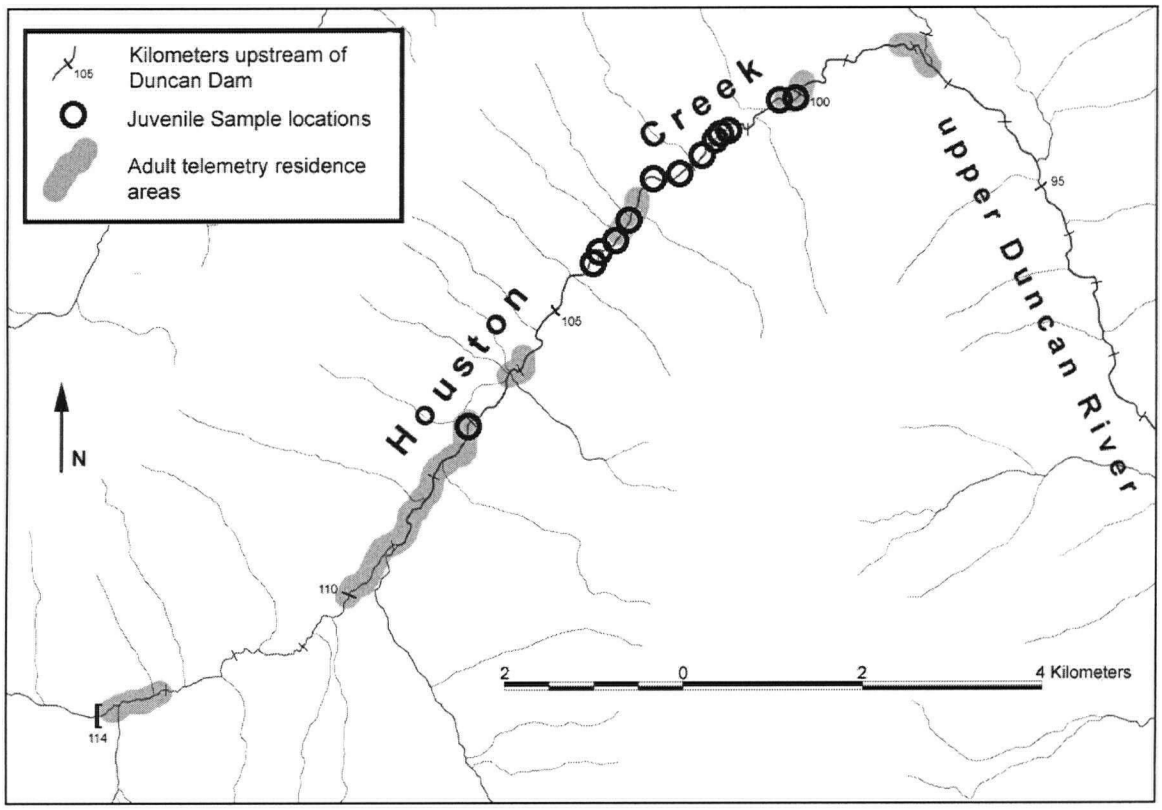

Figure 3-2. Juvenile bull trout tissue sampling locations (n = 13) in Houston Creek.

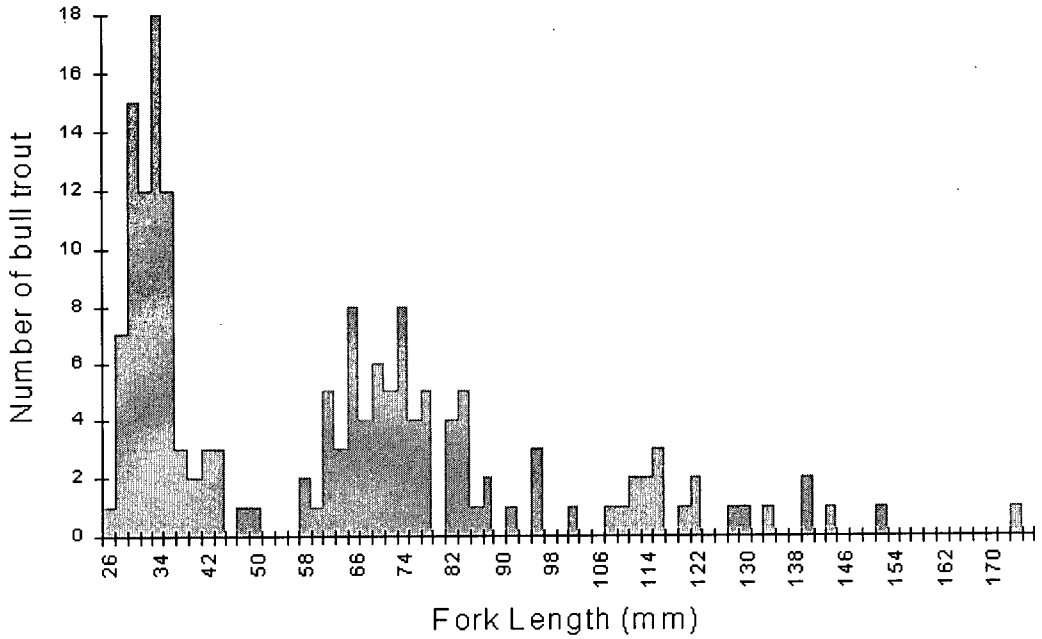

Figure 3-3. Fork length frequency of juvenile bull trout captured in the Westfall River, 15 July – 12 August 1997 (n = 167).

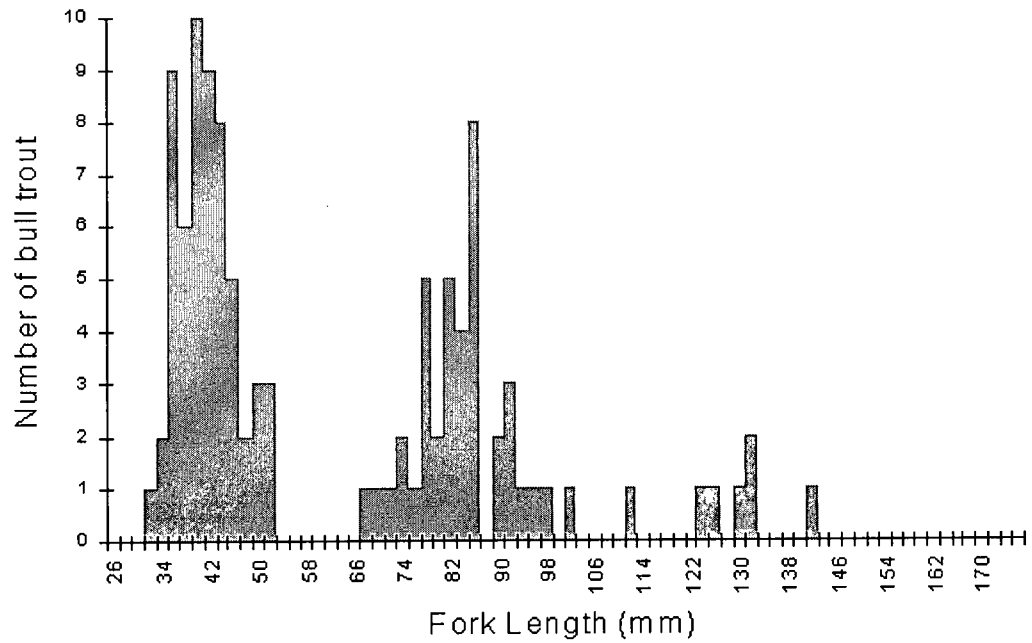

Figure 3-4. Fork length frequency of juvenile bull trout captured in the Houston Creek, 20 – 22 August 1997 (n = 104).

Microsatellite amplification

Total DNA was extracted as described by Taggart et al. 1992 and Taylor et al. 1996. Briefly, I suspended tissue in 2 mM EDTA and digested with Pronase (24 h at 37 °C with rotation) and RNAase (1 h at 37 °C). The DNA was extracted using standard phenol:chloroform protocols followed by ethanol precipitation. I resuspended the extracted DNA in TE buffer and quantified the concentration by spectrophotometry. I stored extracted DNA samples at -20 °C.

From extracted DNA samples, nine microsatellite loci were amplified separately with the polymerase chain reaction (PCR). The microsatellite loci I used came from two sources: seven were isolated from other species (*Ogo* 2 – Olsen et al. 1998; *Omy* 77 – Morris et al. 1996; *Ots* 101 – Nelson and Beacham 1999; *Ssa* 197 – O'Reilly et al. 1996; *Ssa* 311 and *Ssa* 456 – Slettan et al. 1995; *Sfo* 18 - Angers et al. 1995) and two were isolated from a bull trout genomic library (*Sco* 19 and 23; E.B. Taylor, unpubl. data). All of the loci isolated from other species are polymorphic in populations of bull trout in British Columbia and Montana (Spruell et al. 1999; Costello and Taylor unpubl. MS).

One primer from each primer pair was P^{32} end-labeled, using a Polynucleotide Kinase kit (New England Biolabs). Locus specific PCR protocols are appended. All PCR reactions were run on a MJ Research PTC-100 thermocycler. All samples were amplified in 10 μL reaction volumes containing: 100 ng template DNA and 0.5 U *Taq* DNA polymerase (Gibco/BRL) in 10 μl of 20mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5mM $MgCl_2$, 800 μM dNTPs, 0.5 pmol of $[\gamma^{32}P]$-dATP labeled forward primer, 2.5 pmol unlabelled forward primer, and 6 pmol of reverse primer in dH_2O. Four of the loci (*Ogo* 2, *Ots* 101, *Sco* 19, *Ssa* 311) also included 10% dimethyl sulfoxide (DMSO) in the reaction volume (Winship 1989). Amplification products were size fractionated on 6% denaturing polyacrylamide gels and autoradiographed. Alleles sizes were determined using a size standard (M13 sequence) run in two or more lanes of each gel.

Data analysis

Allele frequencies, heterozygosity (both observed and expected), and tests for departures from Hardy-Weinburg Equilibrium (HWE) were calculated using TFPGA 1.3

(Miller 1997) for all loci scored by age class and stream. I excluded monomorphic loci from subsequent analyses. I used GENEPOP 3.2 (Raymond and Rousset 1995) to test for genotypic linkage disequilibrium among loci and to calculate across loci pairwise exact tests of genetic differentiation based on allele frequencies among age classes within streams. In all simultaneous statistical tests, I used the sequential Bonferroni correction (Rice 1989) to adjust alpha values.

To estimate the amount of genetic differentiation between Houston Cr. and Westfall R., I used TFPGA and RST CALC 2.2 (Goodman 1997) to calculate locus specific and across loci estimates of F_{ST} and R_{ST}, respectively, for samples pooled by stream. Allele data were entered as the number of repeat units (with the smallest allele size being one repeat unit) for calculation of R_{ST} estimates using RST CALC. I also calculated locus specific and across loci exact tests of genetic differentiation based on allele frequencies for samples pooled by stream (TFPGA).

As an alternate test of differentiation between Houston Cr. and Westfall R., I calculated hierarchical structuring of genetic variance using analysis of molecular variance (AMOVA; Excoffier et al. 1992) with ARLEQUIN 2.0 (Schneider et al. 2000). I used only a F_{ST} based genetic variance matrix for AMOVA. In addition, I calculated AMOVA for both age structured samples by stream, and for samples pooled by stream.

I calculated the number of migrants from the pooled by stream across loci estimate of F_{ST} ($F_{ST} \approx 1/(4N_em + 1)$ - Wright 1943; Whitlock and McCauley 1999) to compare qualitatively with the magnitude of movement (migration) estimated from telemetry (Chapter 2). I used bootstrapped 95% confidence intervals of the F_{ST} estimate to calculate approximate confidence intervals of the migration estimate.

3.3 Results

Microsatellite loci

Of the nine loci I amplified, four were polymorphic (95% criteria; Hartl and Clark 1989), two had rare second alleles (polymorphic at the 99% criteria), and three were monomorphic (Table 3-1). I will use the 99% criteria as my definition of polymorphic, meaning that the most common allele is at a frequency ≤ 0.99 (Hartl and Clark 1989). A composite autoradiograph of the six polymorphic loci is presented in Figure 3-5. For the polymorphic loci, the mean number of alleles is 3.7, but over all nine scored loci, the mean is 1.6. Westfall River samples had two private alleles (*Ogo* 2 – 148bp and *Ssa* 456 – 159bp) that occurred at frequencies of 0.008 and 0.017, respectively. Although I found all loci pairs in linkage equilibrium across stream level sample groups ($P \geq 0.164$), within age class samples $P = 0.027$ for the *Ssa* 197 – *Sco* 19 pair comparison in the Houston Creek 0+ sample group. After sequential Bonferroni correction (Rice 1989; 15 simultaneous tests, $\alpha = 0.003$), this P value is not significant. Sample sizes and number of alleles by specific stream, age class group, and locus are presented in Table 3-2. Observed and expected heterozygosities are presented in Table 3-2. Estimates of P values for tests of HWE indicated no significant departures from equilibrium allele frequencies across all age sample groupings ($P \geq 0.056$; Table 3-2). The mean expected heterozygosity over all nine scored loci by age class group is 0.220 (min. 0.00 – max. 0.770). Over the six polymorphic loci only, the mean expected heterozygosity is 0.330 (min. 0.00 - max. 0.770; Table 3-2). Allele frequency plots for the six polymorphic loci by age class group and stream are appended.

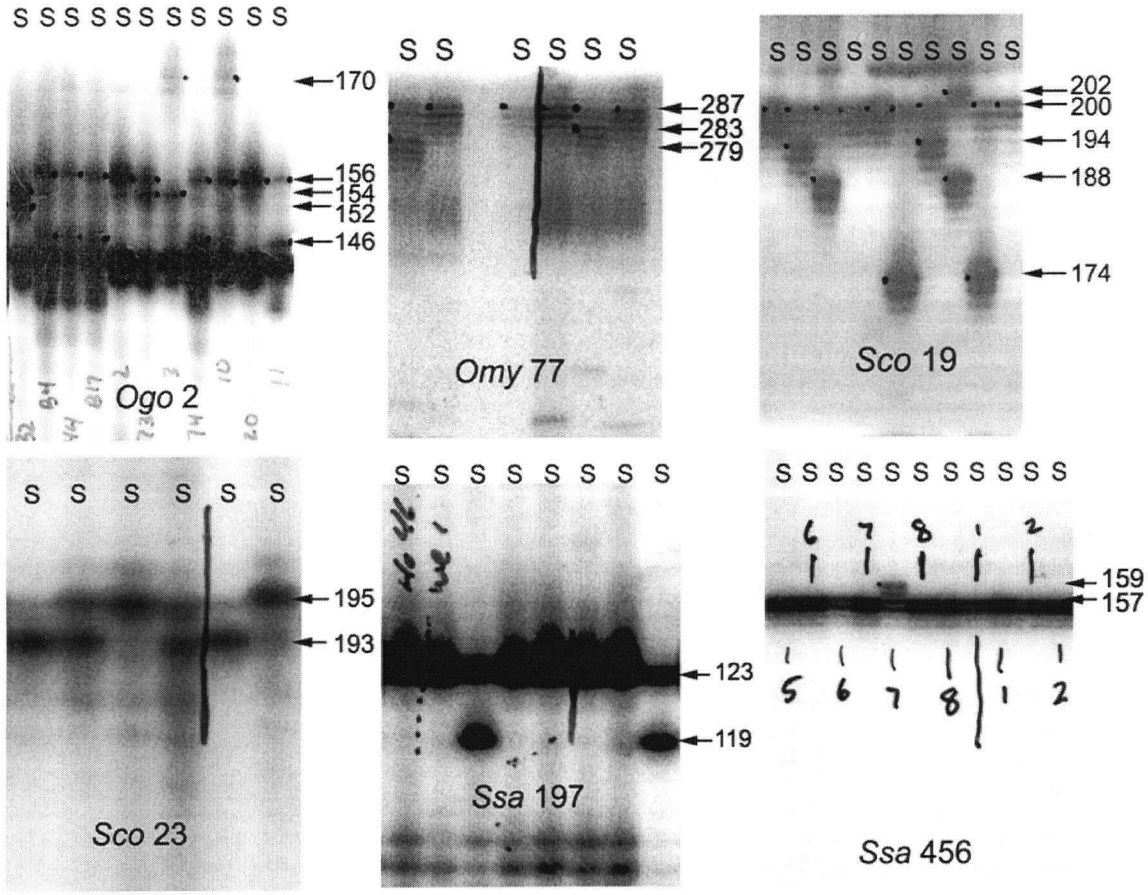

Figure 3-5. A composite autoradiograph of the six polymorphic microsatellite loci scored in bull trout from Houston Creek and the Westfall River. Sample lanes are indicated with an 'S'. Allele sizes (bp) are indicated (arrows).

Table 3-1. Allele size (bp) and frequency (freq.) over all bull trout samples for nine microsatellite loci.

Locus		Allele						
		1	2	3	4	5	6	7
Ogo 2	bp	146	148	152	154	156	170	
	freq.	0.315	0.005	0.095	0.257	0.293	0.036	
Omy 77	bp	279	283	287				
	freq.	0.086	0.054	0.860				
Ots 101	bp	100						
	freq.	1.000						
Sco 19	bp	174	188	194	200	202	204	206
	freq.	0.068	0.064	0.109	0.682	0.050	0.018	0.009
Sco 23	bp	193	195					
	freq.	0.559	0.441					
Sfo 18	bp	150						
	freq.	1.000						
Ssa 197	bp	119	123					
	freq.	0.050	0.950					
Ssa 311	bp	120						
	freq.	1.0000						
Ssa 456	bp	157	159					
	freq.	0.991	0.009					

Table 3-2. Sample size (n), number of alleles amplified (A), and observed (H (obs)) and expected (H (exp)) heterozygosity values by stream, age class group, and locus, as well as estimated probability values (*P*) of HWE tests.

Locus		Westfall			Houston		
		0+	1+	2+	0+	1+	2+
Ogo 2	n	20	20	20	20	20	11
	A	5	4	6	5	4	4
	H (obs)	0.900	0.800	0.750	0.700	0.700	0.636
	H (exp)	0.734	0.696	0.770	0.744	0.671	0.665
	P	0.913	0.730	0.721	0.271	0.423	0.105
Omy 77	n	20	20	20	20	20	11
	A	3	3	3	3	3	2
	H (obs)	0.200	0.350	0.150	0.250	0.350	0.273
	H (exp)	0.184	0.301	0.141	0.304	0.304	0.236
	P	1.000	1.000	1.000	0.056	1.000	1.000
Ots 101	n	20	20	20	20	20	10
	A	1	1	1	1	1	1
	H (obs)	-	-	-	-	-	-
	H (exp)	-	-	-	-	-	-
	P	-	-	-	-	-	-
Sco 19	n	20	20	20	20	20	10
	A	6	6	6	5	4	2
	H (obs)	0.550	0.550	0.500	0.600	0.350	0.200
	H (exp)	0.596	0.600	0.486	0.616	0.306	0.185
	P	0.266	0.206	0.733	0.096	1.000	1.000
Sco 23	n	20	20	20	20	20	11
	A	2	2	2	2	2	2
	H (obs)	0.450	0.600	0.450	0.500	0.600	0.546
	H (exp)	0.469	0.495	0.399	0.420	0.455	0.496
	P	1.000	0.659	1.000	0.618	0.326	1.000
Sfo 18	n	20	20	20	20	20	10
	A	1	1	1	1	1	1
	H (obs)	-	-	-	-	-	-
	H (exp)	-	-	-	-	-	-
	P	-	-	-	-	-	-
Ssa 197	n	20	20	20	20	20	10
	A	2	1	2	2	2	1
	H (obs)	0.200	-	0.150	0.150	0.050	-
	H (exp)	0.180	-	0.139	0.139	0.049	-
	P	1.000	-	1.000	1.000	1.000	-
Ssa 311	n	20	20	20	20	20	11
	A	1	1	1	1	1	1
	H (obs)	-	-	-	-	-	-
	H (exp)	-	-	-	-	-	-
	P	-	-	-	-	-	-
Ssa 456	n	20	20	20	20	20	11
	A	1	2	2	1	1	1
	H (obs)	-	0.050	0.050	-	-	-
	H (exp)	-	0.049	0.049	-	-	-
	P	-	1.000	1.000	-	-	-

Genetic population structure

I found little evidence of genetic divergence between Houston Creek and Westfall River bull trout with the microsatellite loci I amplified. After sequential Bonferroni correction, no pairwise age class comparison had significantly differing allele frequencies ($P \geq 0.011$, 15 simultaneous tests, $\alpha = 0.003$ - Table 3-3), so I pooled samples by stream for further analysis. Locus-specific estimates of F_{ST} and R_{ST} (Table 3-4) again suggest little genetic differentiation between streams. The across loci estimates of F_{ST} and R_{ST} are 0.010 and 0.006 respectively. The bootstrapped 95% confidence intervals of both across loci F_{ST} and R_{ST} estimates include zero (Table 3-4). The *Sco* 23 specific estimates are higher than the other loci (Table 3-4) but generally there is concordance among the polymorphic loci I scored. The number of effective migrants (N_em) calculated from the across loci estimate of F_{ST} is 24.8. The approximate 95% confidence intervals are $7.2 < N_em < \infty$.

The between stream exact test of population differentiation across all loci is not significant ($P = 0.284$; Table 3-5). The probability value estimated for the *Sco* 23 specific exact test, $P = 0.011$, is not significant after Bonferroni correction (6 simultaneous tests, $\alpha = 0.008$; Table 3-5).

Table 3-3. Estimated probability values of exact tests of population differentiation from allele frequencies across loci between all age class groups (0+ - 2+) from Houston Creek (H) and Westfall River (W).

Age group	H0+	H1+	H2+	W0+	W1+
H1+	0.011				
H2+	0.143	0.311			
W0+	0.910	0.147	0.403		
W1+	0.475	0.387	0.716	0.566	
W2+	0.050	0.015	0.238	0.391	0.171

Table 3-4. Estimates of F_{ST} and R_{ST} within and across loci for pooled age class samples by stream. Confidence intervals (C.I.) for across loci estimates are included.

Locus	Fst	Rst
Ogo 2	-0.006	-0.009
Omy 77	-0.001	-0.002
Sco 19	0.007	-0.002
Sco 23	0.045	0.044
Ssa 197	-0.005	-0.005
Ssa 456	0.007	0.008
Across loci estimate	0.010	0.006
Across loci 95% C.I.	0.034>Fst>-0.005	0.168>Rst>-0.001

Table 3-5. Estimated probability values of exact tests of population differentiation, based on allele frequency differences (Infinite Alleles Model), between Houston Creek and Westfall River samples (pooled across age classes) within loci, and across all loci (bold). 'SE' is the standard error of the estimate.

Locus	P value	SE
Ogo 2	0.694	0.016
Omy 77	0.547	0.009
Sco 19	0.480	0.015
Sco 23	0.011	0.002
Ssa 197	0.762	0.002
Ssa 456	0.503	0.003
Across loci estimate	**0.284**	

Partitioning of genetic variation (AMOVA) for both pooled and separate age classes within streams are presented in Table 3-6. I present the AMOVA for separate age classes, despite the lack of pairwise differences between them, to compare the proportion of genetic variance partitioned among age classes within streams and between streams. Pooling age classes resulted in a 0.7% increase in genetic variance partitioned to the between stream partition (0.2 to 0.9%, Table 3-6 and Fig. 3-6). The between stream variance partition of the AMOVA is significant when age classes are pooled by stream (P = 0.036) but not when age classes are separate (P = 0.416; Table 3-6). All other variance partitions in the AMOVA are significant (Table 3-6).

Table 3-6. Analysis of Molecular Variance (AMOVA) partitioning for both pooled and separate age classes by stream. Estimated probability values associated with variance partitions are included. 'SE' is the standard error of the estimate.

AMOVA Partitions	
pooled over age classes by stream	
Within streams	99.1%
Between streams	0.9%
P value estimate (SE)	0.036 (0.006)
separate age classes	
Within age class	97.6%
P value estimate (SE)	0.001 (0.001)
Among age classes within streams	2.2%
P value estimate (SE)	0.002 (0.001)
Between streams	0.2%
P value estimate (SE)	0.416 (0.014)

3.4 Discussion

I was unable to collect many 2+ bull trout from Houston Creek. The apparent lack of 2+ bull trout in Houston Cr. probably reflects the smaller portion of the stream sampled (Fig. 3-1 vs. Fig. 3-2) and lower total catch, because the relative frequency of different age classes between Houston Cr. and Westfall R. appears similar (Figs. 3-3 and 3-4). The apparent size differences between the same age classes from the two streams (Figs. 3-3 and 3-4) probably represent differences in sampling date (> 30 d in most cases) rather than stream specific growth rates.

More than half of the microsatellite loci amplified (Table 3-1) were monomorphic by the 95% criterion. I used these loci specifically because they were polymorphic in other populations of bull trout (Spruell et al. 1999; Costello and Taylor unpublished manuscript). There were no significant departures from HWE. Heterozygosities are low when averaged over loci (mean across loci = 0.220; Table 3-2), but this appears to be normal in bull trout (Spruell and Allendorf 1997), and as mentioned previously, the general finding of low genetic variability in bull trout has been attributed to historical demographic processes (Leary et al. 1993; Spruell and Allendorf 1997; Taylor et al. 1999; Costello and Taylor unpubl. MS).

Although not significant, the estimated P values of pairwise exact tests between Houston Cr. 0+ and Houston Cr. 1+ (H0+ *vs.* H1+), H0+ and Westfall R. 2+ (H0+ *vs.*

W2+) and H1+ *vs.* W2+ samples (Table 3-3) suggest some allele frequency differences. In the case of the H0+ *vs.* H1+ comparison, the loci driving the differences were *Sco* 19 and *Ogo* 2. These two loci have the most allelic diversity in my samples (*Sco* 19 – seven alleles, *Ogo* 2 – six alleles; Table 3-1), and the difference suggested by the low estimated P value (Table 3-3) probably result from my within age group sample sizes. My sample sizes are certainly of issue at the individual age class level, particularly in the 2+ age class from Houston Cr. (Table 3-2). For the other two comparisons with low estimated P values (H0+ *vs.* W2+ and H1+ *vs.* W2+; Table 3-3), the locus driving the result was *Sco* 23 (two alleles; Table 3-1), suggesting that my sample sizes were not as important. After Bonferroni correction (Rice 1989), none of the pairwise age class exact tests were significantly different (Table 3-3).

In calculated estimates of genetic differentiation, F_{ST} is consistently higher than R_{ST} (Table 3-4). The microsatellite allele sizes identified in Houston Cr. and Westfall R. bull trout are consistent with those identified by other bull trout studies with the same loci (Spruell et al. 1999; Taylor and Costello 1999; Latham and Taylor in press; Costello and Taylor unpublished manuscript). Because the same sized alleles exist at the same loci elsewhere in the range of bull trout, this suggests that they did not arise *via* mutation in Houston Cr. and Westfall R. If microsatellite alleles did not arise by mutation, and instead arrived when the area was recolonized, the estimate of R_{ST} is not as good an indicator of genetic differentiation as F_{ST}. Taylor and McPhail 2000 argue that demographic processes (random genetic drift, founder effects and bottlenecks) likely outweigh the effect of mutation on genetic differentiation in threespine stickleback (*Gasterosteus aculeatus*) postglacially recolonizing BC lakes from the sea. With evidence for the importance of the same demographic processes in bull trout (Leary et al. 1993; Spruell and Allendorf 1997; Taylor et al. 1999; Costello and Taylor unpubl. MS), mutation likely plays a minor role in the microsatellite differentiation I found.

There is little genetic differentiation between juvenile bull trout sampled from Houston Cr. and Westfall R. The *Sco* 23 locus (Table 3-4) appears to drive the only differences I found between the two streams. Besides *Sco* 23, there is good concordance across the loci I scored: they all indicate no differentiation between the streams (Table 3-

4). These results are reflected in the non-significant exact test of differentiation based on allele frequencies across loci (P = 0.284; Table 3-5).

The amount of genetic differentiation I found (Table 3-4) is very small compared to estimates from other fish species (Ward et al. 1994; Waples 1998), particularly other freshwater fish. My estimate of F_{ST} (0.01) between Houston Cr. and Westfall R. is six times less than the mean for anadromous fishes (mean marine F_{ST} estimate = 0.06), and more than an order of magnitude lower than the mean estimate for freshwater fishes (mean freshwater F_{ST} estimate = 0.222; as reviewed by Ward et al. 1994). Because of fewer strong barriers to dispersal, marine organisms generally have lower levels of genetic differentiation across their distributions (e.g. Palumbi et al. 1997; reviewed by Waples 1998). The low mean estimate of F_{ST} from the studies reviewed by Ward et al. (1994) may be because many were surveys of allozyme differentiation, which usually have lower resolution of genetic variation than microsatellites (Ferguson et al. 1995; Jarne and Lagoda 1996). Most of the loci I used had low allelic diversity (only two loci had > 4 alleles; Table 3-1) compared to many microsatellite systems (Hedrick 1999; Nelson and Beacham 1999); however, I still found diversity, just very little differentiation between Houston Cr. and Westfall R.

Another way to evaluate my result is to compare it with the amount of differentiation expected between isolated populations diverging *via* genetic drift since founding from a common source. Imagine that Houston Creek and Westfall River represent two such populations. Assuming discreet generations of five years, no gene flow between populations of constant effective population size (N_e = 300), and that colonization occurred 10,000 years ago, $F_{ST} \cong 0.964$ based on genetic drift alone (Nei and Chakravarti 1977 as cited in Waples 1998).

I also used AMOVA (Table 3-6) to quantify genetic divergence between Houston Cr. and Westfall R bull trout. Because of the previous discussion of the applicability of R_{ST} based measures, I only calculated the AMOVA with F_{ST} based distances (Excoffier et al. 1992). With pooled age classes by stream, a significant portion (0.9%, P = 0.036; Table 3-6) of genetic variance is partitioned to differences between streams. Although sample sizes are small, I presented the AMOVA analysis with

separate age classes (Table 3-6), specifically to compare the amount of genetic variation attributed to the between stream partition relative to the among age classes within streams partition (Fig. 3-6). Just over 2 % (P = 0.002) of the total variation is attributed to the among age class partition in the analysis (Table 3-6). The pairwise among age classes (Table 3-1) and between stream exact test (Table 3-5) are both contradicted by these AMOVA results. This may reflect a difference in power between the two methods. Despite this apparent discrepancy, the amount of genetic differentiation between Houston Cr. and Westfall R. is still small. Less than one percent of genetic variance is explained by between stream differences. This ANOVA result, along with F_{ST} estimates and exact tests of population differentiation, suggest that the spatial scale of population divergence in upper Duncan River bull trout is larger than the spawning stream.

As discussed in the methods section, a problem with this general result is the use of allele frequency data collected from juveniles. I replicated my samples over age classes (time), took precautions against sampling families within a year, and used multiple loci in an attempt to alleviate (as suggested by Hansen et al. 1997) the biases introduced by the Allendorf-Phelps effect (Allendorf and Phelps 1981; Waples 1998). As detailed by Waples (1998), the net bias introduced by the sampling of juveniles instead of the adult population would be to inflate estimates of population subdivision. My data, with low differentiation between age classes and in pooled between stream comparisons, suggest that the Allendorf-Phelps effect has not been important in my analysis.

Sample sizes are also a concern in the interpretation of the general lack of between stream differences. The θ estimator of F_{ST} I used (Weir and Cockerham 1984) adjusts population specific estimates by sample size; however, in one age class group (Houston Cr. 2+) my mean sample size over loci was 10.6 individuals (Table 3-2). When two of four polymorphic loci used have more than five alleles, this sample size is not sufficient. My pooled age class between stream analyses are based on mean sample sizes over six loci of 50.6 individuals from Houston Cr. and 60 individuals from Westfall R.

Utter et al. 1992, describe a similar result when comparing chinook salmon (*Oncorhynchus tshawytscha*) populations in the Snake and Klamath rivers. Originally, attempts to differentiate these two groups of chinook were not successful using allozyme

markers (Utter et al. 1992). Only later, when more variable allozyme loci were used, did the two populations become distinguishable (Utter et al. 1992). With additional loci, perhaps more differentiation between Houston Cr. and Westfall R. bull trout would be apparent; although, in terms of the pooled age class test, my sample size ($n \geq 50$ per locus and stream) and number of loci (six polymorphic by the 99% criteria) are greater than many similar studies (e.g. Spruell et al. 1999; Latham and Taylor in press; Costello and Taylor unpubl. MS).

Finally, a potential problem with attempting to define population structure (or lack thereof) using highly variable markers like microsatellites was raised by Hedrick (1999). In this study, with little evidence of population differences, this lack of differentiation may only reflect these specific microsatellite markers. The problem is that other loci, potentially loci defining adaptively significant traits, may give rise to biologically meaningful differences (Hedrick 1999). I tried to minimize this possibility by amplifying nine loci that were known to be polymorphic in bull trout. Averaging over more independent loci would certainly increase the accuracy of estimates of genetic differentiation (Waples and Teel 1990). Although I did not measure many adaptive traits, it is clear that migratory timing between the two streams does not differ (Chapter 2). The relative proportions of different juvenile age classes present during sampling for microsatellite analysis (Figs. 3-3 and 3-4) suggest that juvenile rearing times are similar. General life history, with migrations to and from Kootenay Lake, appears the same in bull trout from both streams. There was a significant, but not robust, trend for larger radio-tagged bull trout in larger tributary streams, but this may also reflect a lack of precise homing (Chapter 2).

Number of migrants

As described by Whitlock and McCauley (1999), estimation of the number of migrants (N_em) from F_{ST} is not accurate. The assumptions of the "Fantasy Island" model (Wright's island model of population subdivision – Wright 1943; Whitlock and McCauley 1999), which should be met for calculation of number of migrants from F_{ST}, have been violated in my calculations. Specifically, Wright's island model assumes: that the number of populations is infinite; population sizes are equal and do not fluctuate;

breeding is random; generations are discreet; each population contributes the same number of migrants, and the probability of migration to one population is equal to all others; the migration rate is small; and that there is no selection or mutation. Telemetry data showing variable number of tagged bull trout spawning in various spawning stream 'populations' suggest that population size is different among streams. Telemetry also suggests that the migration rate may be quite large. I also only sampled two of an unknown number of possible populations that have overlapping generations. Despite these breaches of the assumptions of the model, I decided that the estimation of N_em was justified to allow at least a qualitative comparison of movement rates between spawning streams in the upper Duncan R. based on genetic and telemetry data.

The problem of estimation of N_em from F_{ST} is particularly acute at small values of F_{ST}, as found in this study. When I used the bootstrap estimates of the 95% confidence interval to calculate approximate confidence intervals for my estimate of N_em, the range (from 7.2 to an infinite number of individuals) highlights this problem. N_em estimates are very sensitive to small changes in F_{ST} when $F_{ST} < 0.01$ (Waples 1989; Whitlock and McCauley 1999).

On a purely qualitative level, my estimates of 'migration' between spawning streams based on telemetry and genetic data are similar. As reported in Chapter 2, an approximate estimate of the total number of bull trout changing spawning destination in the upper Duncan R. over two consecutive years is 40 to 50 individuals. The F_{ST} based estimate of 24.8 effective migrant individuals per generation equals approximately 5 effective migrants per year, between Houston Cr. and Westfall R. specifically, with a five year generation time. This qualitative agreement suggests two important things: first, that my violation of assumptions of the calculation of number of migrants from F_{ST} was not completely unrealistic; and second, that the telemetry spawning stream switching result was not spurious, and likely reflects a high level of current and historical gene flow between, at least, Houston Cr. and Westfall R.

The impact of gene flow is dependent on population size (Hartl and Clark 1989). To best evaluate the effects of gene flow on population structure, I need an estimate of migration rate (m). The effective population size, N_e, is the idealized number of breeding

individuals giving rise to observed levels of random genetic drift (Hartl and Clark 1989). In fishes, the proportion of census size that N_e reflects is generally less than 20% (Frankham 1995; Miller and Kapuscinski 1997). If I assume that the number of spawning individuals in both Houston Cr. and Westfall R. average approximately 100 to 150 individuals (from Houston Cr. trap data - Chapter 2), N_e may be as small as 20 to 30 individuals in each stream. With $N_e = 25$, $m \cong 1$, suggesting that nearly all bull trout move between Houston Cr. and Westfall R. for spawning. With N_e equal to estimates of spawning census size (N_e =150), migration rate is approximately 15 to 25 % per generation. In the most conservative case, using the lower 95% C.I. of the N_em estimate (7.8 individuals) and census size as N_e ($\cong$150), migration rate is still > 5 %. A migration rate of 5% would tend to genetically homogenize otherwise separate populations (Hartl and Clark 1989; Mills and Allendorf 1996).

Comparison to previous bull trout studies

My results differ from those of other fine-scale bull trout genetic studies (Spruell et al. 1999; Costello and Taylor unpublished manuscript). Spruell et al. (1999) estimated F_{ST} at 0.063 between samples of bull trout from five tributary populations within the Lightning Cr. drainage, and found significant differentiation between all population pairs. Lightning Cr. is tributary to the Clark Fork River, Idaho, and drains approximately 220 km^2 into Lake Pend Oreille (Spruell et al. 1999), thus, it is less than 20 % the size of upper Duncan River. Costello and Taylor (unpubl. MS) estimated F_{ST} values ranging from 0.01 to 0.31, 0.01 to 0.68, and 0.08 to 0.93 across bull trout populations within watersheds tributary to the Peace, Kootenay, and Red Deer and Oldman rivers, respectively. Sample sites in Costello and Taylor's study (unpubl. MS) were separated by as much as 200 km in each system. Generally, where Costello and Taylor (unpubl. MS) estimated small, but significant, pairwise F_{ST} values, sample sites were within the same watershed or even the same stream. Only in one small watershed did Costello and Taylor (unpublished MS) find no significant genetic differentiation among samples (see below). One difference between my study and the other studies is that Houston Cr. and Westfall R. seem to support only migratory bull trout (Chapter 2). Costello and Taylor (unpublished manuscript) found that if they removed samples collected from above

barriers to migration (populations geographically isolated from migrant populations) from their analysis, it reduced within watershed estimates of F_{ST} by approximately one half. Spruell et al. (1999) give no specifics detailing which, if any, of the populations they sampled were isolated. Perhaps sampling isolated populations inflated their estimates of genetic differentiation.

Latham and Taylor (in press), examined population structuring among bull trout spawning streams tributary to Arrow Lake in the Columbia River, adjacent to Duncan River. Maximum distance between sampled streams in Arrow Lake and the Columbia was approximately 200 km (Latham and Taylor in press). They found evidence of genetic divergence among these streams, specifically a north – south shift in both microsatellite allele frequencies and mtDNA haplotype patterns. Latham and Taylor (in press) estimated a F_{ST} of 0.11 for microsatellites across their sampling area, excluding above barrier populations. They interpreted their results as strong evidence for either accurate homing or low reproductive success in straying fish. Latham and Taylor's (in press) general result, like that of Spruell et al. 1999 and most areas examined by Costello and Taylor (unpubl. MS) stand in sharp contrast to my results.

My findings are consistent with genetic results from one other watershed. Costello and Taylor (unpubl. MS) found insignificant pairwise F_{ST} among three sample sites within the Wigwam River, a tributary of the Elk River that drains nearly 800 km^2. The Elk River flows into Kokanoosa Reservoir, on the Kootenay River upstream of Kootenay Lake. Like the upper Duncan River, a lacustrine population of bull trout (from Kokanoosa Reservoir) spawns in Wigwam River; although, prior to the construction of Libby Dam (1972), this would have been a fluvial population.

There are several possible reasons why my findings from the upper Duncan River differ from most other bull trout studies. One possible reason, as described previously, is that my sites were not isolated from each other. Telemetry results (Chapter 2), and the apparent absence of large mature fish prior to arrival of migrating adults, suggest that Houston Cr. and Westfall R. do not contain resident adult bull trout. Another possibility is that the spatial scale of my comparison is too small; however, one purpose of my study was to determine if more than one population spawned in the upper Duncan

River. Although the streams I sampled for this comparison are geographically close together, distances are apparently more than enough for significant population divergence in bull trout (Spruell et al. 1999; Costello and Taylor unpubl. MS), and certainly other salmonids (Quinn et al. 1987; Taylor 1991; Tallman and Healey 1994; Angers et al. 1995; Carlsson et al. 1999).

Perhaps Duncan Dam has had an impact on genetic population structure within the upper Duncan R., by restricting access from Kootenay Lake. In 1997, the dam had been present for 30 years. At most, this equates to six bull trout generations and the impact of gene flow and other demographic processes on allele frequency differences among populations are usually integrated over longer time scales than this (Whitlock 1992). If bull trout from separate spawning streams in the upper Duncan River represented genetically distinct populations prior to 1967 and the effect of the dam was to make all of these populations breed randomly with one another, six generations would be enough to homogenize any genetic differentiation that had existed. This scenario seems unlikely, given evidence for movement through the dam in both directions by radio-tagged bull trout (Chapter 2).

Conservation implications

The genetic comparison between Houston Cr. and Westfall R. bull trout suggests that the switching behaviour suggested in Chapter 2 produces gene flow among upper Duncan tributaries. Although the microsatellite comparison includes only Houston Creek and Westfall River, the telemetry results indicate that this result applies across the streams entered by radio-tagged bull trout in the upper Duncan River. Thus, the population boundary of bull trout in the upper Duncan River apparently includes all spawning tributaries. So, at what spatial scale are bull trout migrating to the upper Duncan River divided into genetically distinct populations? Without comparisons outside the upper Duncan River, it is only possible to speculate. Based on other studies of genetic differentiation, the next available watershed unit above individual spawning streams is the whole upper Duncan River. Perhaps, a comparison of migrant bull trout from Kootenay Lake entering the Duncan and Lardeau rivers might reveal genetic divergence between these major Kootenay Lake inlet rivers. My results suggest that from

the perspective of conservation and management, the bull trout that migrate from Kootenay Lake to the upper Duncan River should be considered part of the same, essentially panmictic, population.

3.5 Appendix

Table 3-7. Loci specific PRC protocols. Reaction step abbreviations are: D – denature, A – anneal, E – extend. Time notation is m:ss. The reactions of starred loci included DMSO (see text).

		PCR Profile											
		Step 1				Step 2				Step 3			
Locus		D	A	E	cycles	D	A	E	cycles	D	A	E	cycles
Ogo 2 *	temp.	95			1	94	56	72	5	93	55	72	25
	time	3:00				1:00	1:00	1:00		1:00	1:00	1:00	
Omy 77	temp.	95	56	72	1	94	56	72	5	92	54	72	25
	time	3:00	2:00	1:00		1:00	1:00	1:00		1:00	0:30	0:30	
Ots 101 *	temp.	95			1	94	56	72	5	93	55	72	25
	time	3:00				1:00	1:00	1:00		1:00	1:00	1:00	
Sco 19 *	temp.	95	52	72	1	94	52	72	5	92	50	72	25
	time	3:00	2:00	3:00		1:00	1:00	1:00		1:00	1:00	1:00	
Sco 23	temp.	95	61	72	1	94	61	72	5	92	60	72	25
	time	2:00	1:00	1:00		1:00	1:00	1:00		0:45	0:45	0:30	
Sfo 18	temp.	94	65	72	15	94	65	72	20				
	time	1:00	0:35	0:10		0:35	0:35	0:10					
Ssa 197	temp.	95	58	72	1	94	58	72	5	94	57	72	25
	time	2:00	1:00	0:30		1:00	1:00	0:30		0:45	0:45	0:30	
Ssa 311 *	temp.	95			1	94	56	72	5	93	55	72	25
	time	3:00				1:00	1:00	1:00		1:00	1:00	1:00	
Ssa 456	temp.	95			1	94	56	72	5	93	55	72	25
	time	3:00				1:00	1:00	1:00		1:00	1:00	1:00	

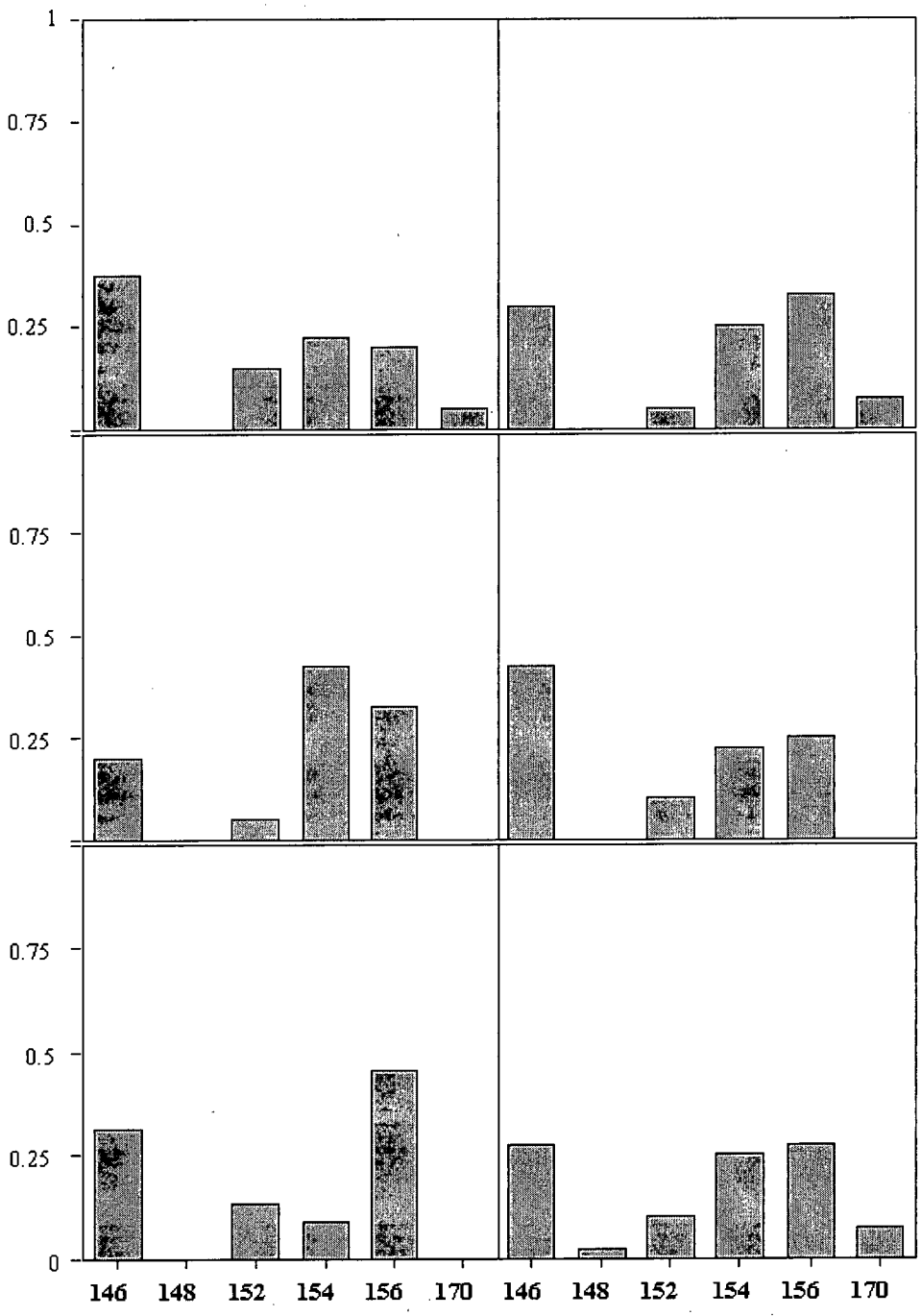

Figure 3-6. The allele frequencies of microsatellite locus *Ogo* 2 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

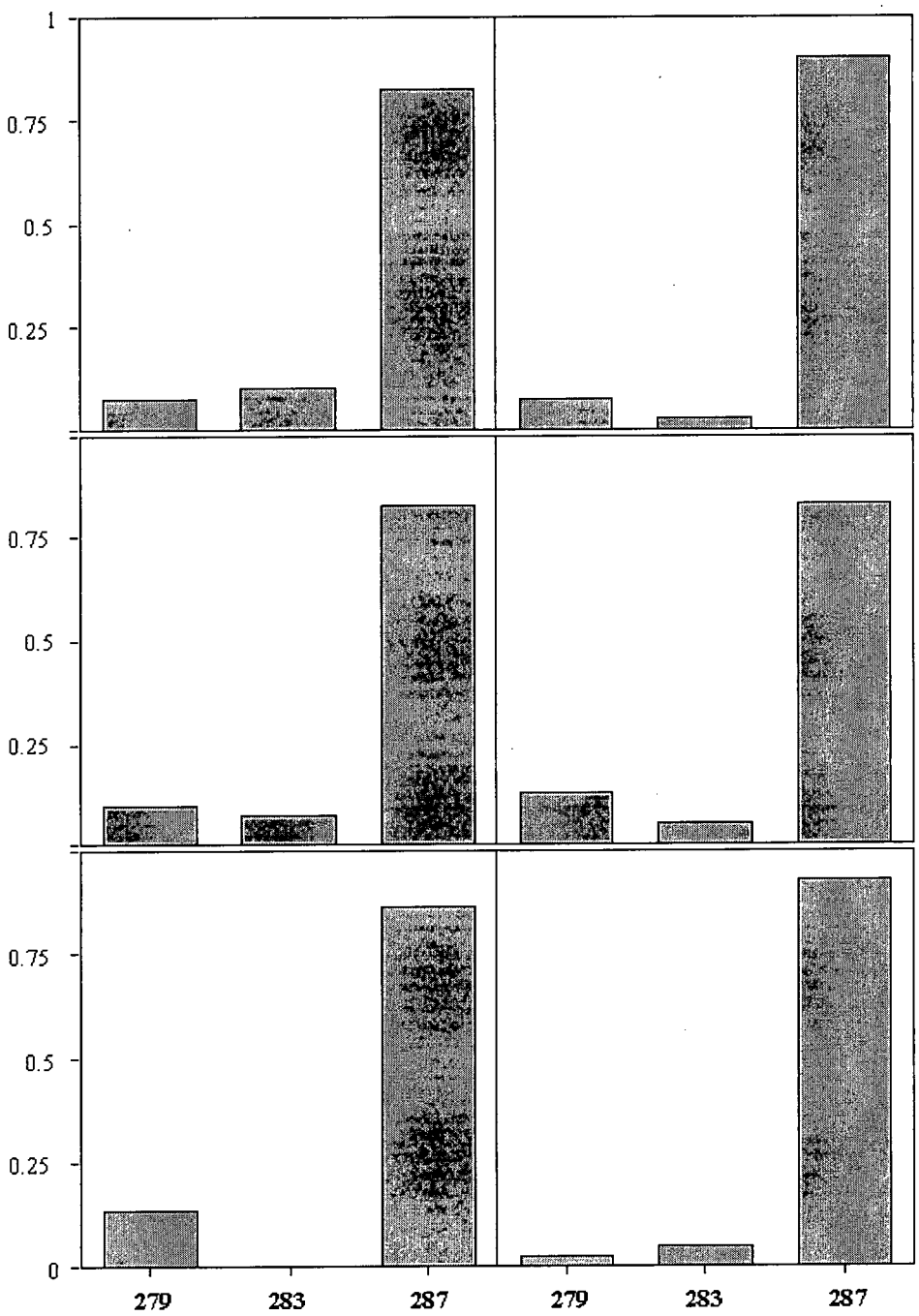

Figure 3-7. The allele frequencies of microsatellite locus *Omy* 77 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

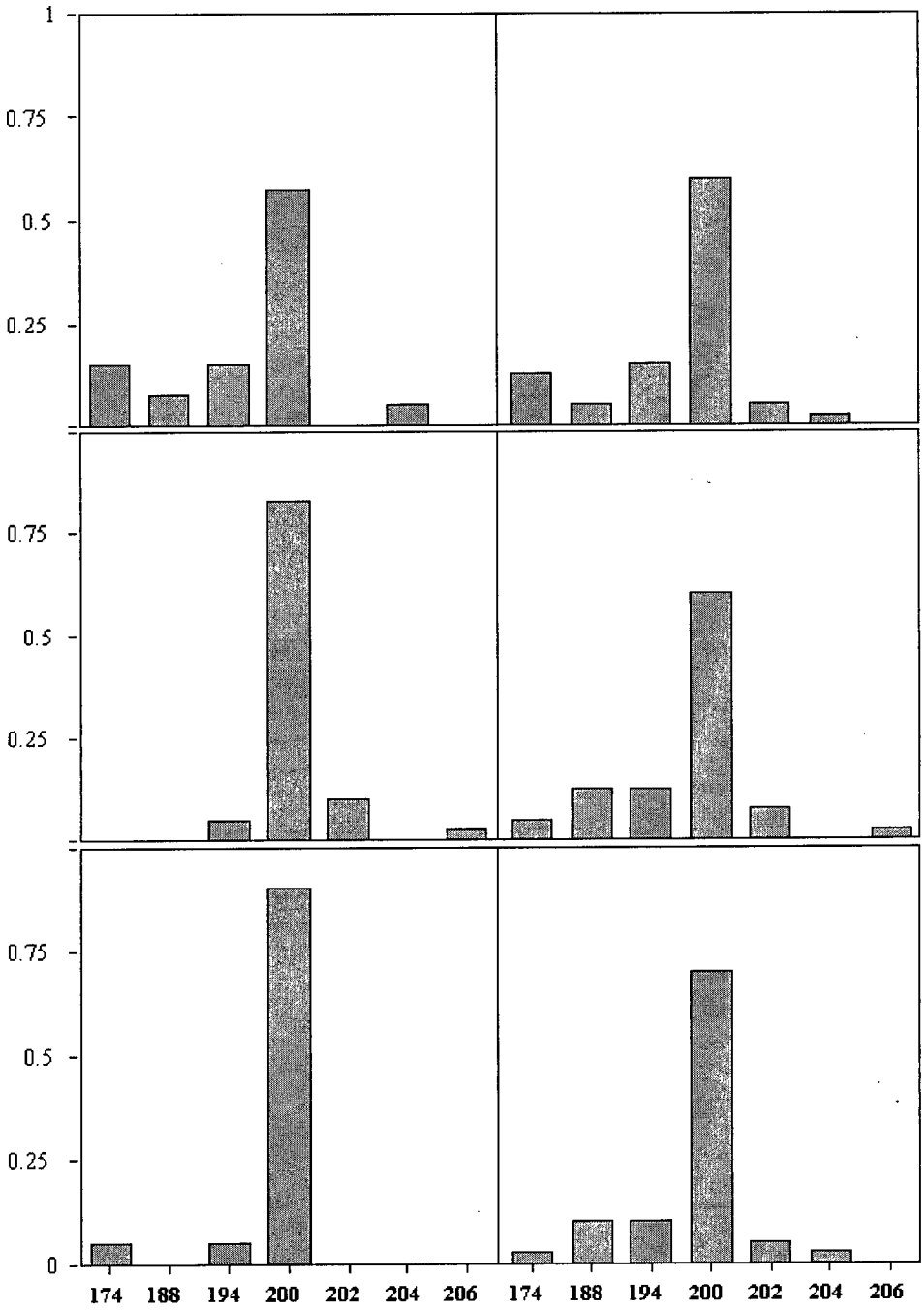

Figure 3-8. The allele frequencies of microsatellite locus *Sco* 19 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

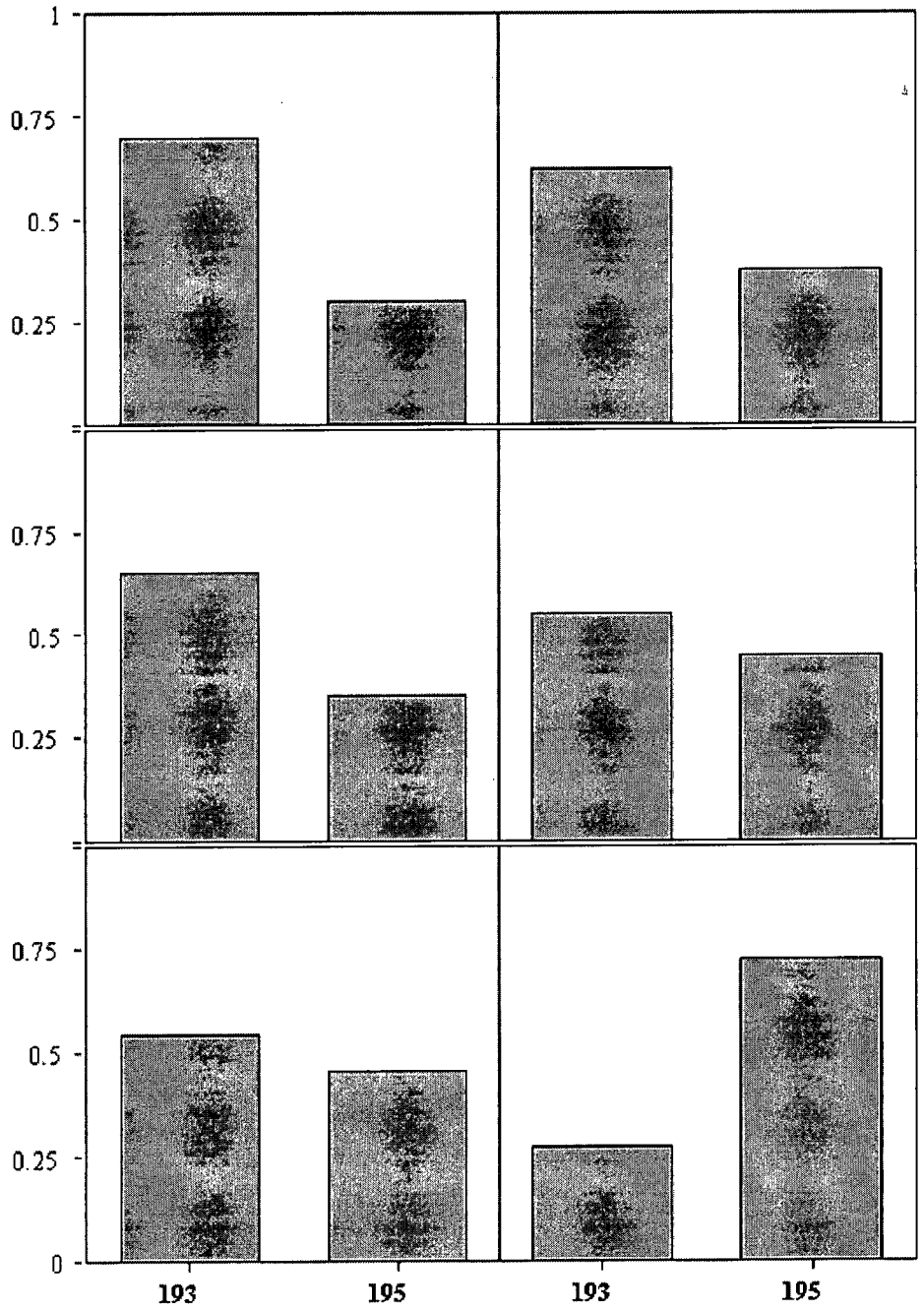

Figure 3-9. The allele frequencies of microsatellite locus *Sco* 23 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

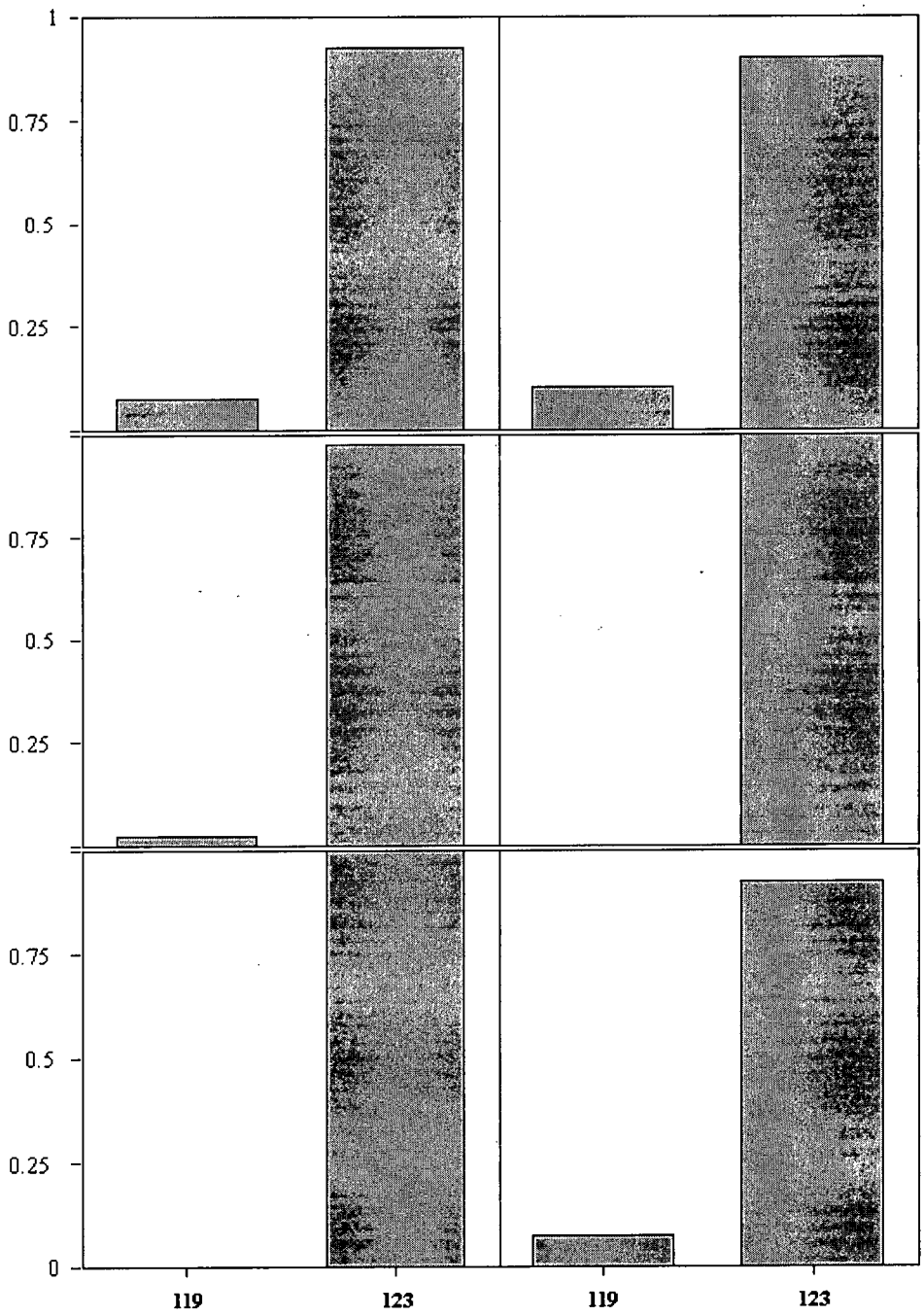

Figure 3-10. The allele frequencies of microsatellite locus *Ssa* 197 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

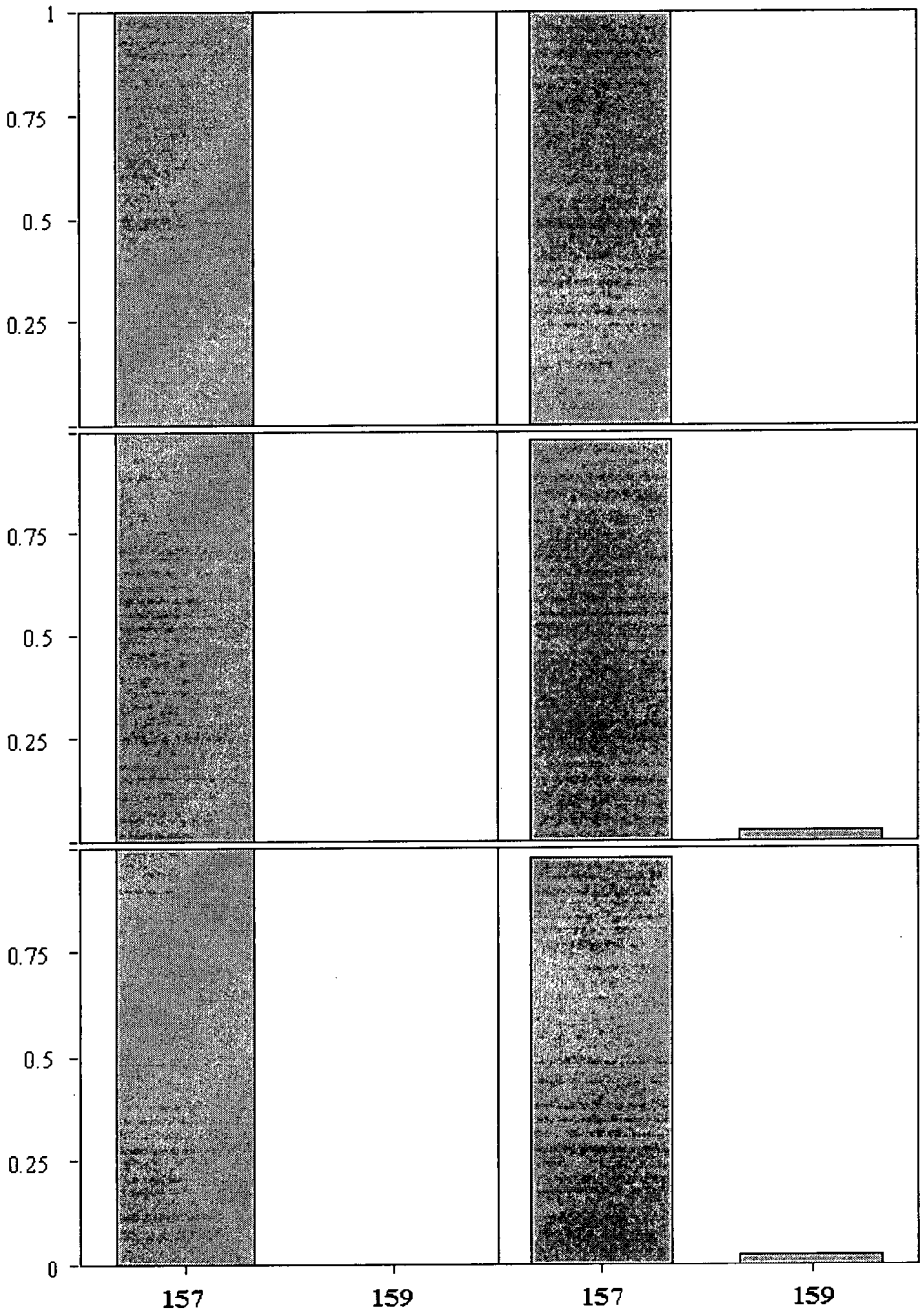

Figure 3-11. The allele frequencies of microsatellite locus *Ssa* 456 from sampled bull trout by age class (0+, 1+, and 2+ top to bottom) and stream (Houston Creek – right, Westfall River – left).

Chapter 4 – General conclusions

Migratory timing and spawning locations in the upper Duncan River

I wanted to determine the timing of bull trout migrations in the Duncan River. In addition, I wanted to identify which streams bull trout use for spawning in the upper Duncan River. Using radio telemetry, I recorded the migration of bull trout from Kootenay Lake to spawning locations in the upper Duncan River, where I identified six putative bull trout spawning streams. Over two years, the proportion of bull trout migrating to these streams and destinations in the mainstem upper Duncan River did not change. The timing of migrations did not vary over the two years of detailed telemetry. These predictable migrations allow conservation and management measures, such as angling restrictions, land use decisions, or flow releases from dams, to focus on specific locations and times that will best benefit the migrating fish.

Apparently, bull trout migrating to destinations in the mainstem upper Duncan River do not spawn. Swanberg (1997) suggested that similar findings for bull trout in the Blackfoot River were related to avoidance of high summer water temperatures. The high summer temperatures in Kootenay Lake and Duncan River probably are not warm enough to explain these non-spawning migrations. The non-spawning movements were not related to feeding, as prey fish were scarce in the upper Duncan River during the summer and fall sampling periods. This result suggests that adult bull trout use more river habitat in the summer than suggested from spawning area use alone. Perhaps to best protect bull trout, habitat protection measures should focus on the upper reaches of spawning systems, not just specific spawning locations.

Spawning stream selection

I examined the hypothesis that bull trout select spawning stream based on size, as suggested by McPhail and Murray (1979). The size of radio-tagged bull trout, both in length and mass, differed by destination in the upper Duncan River. There was a significant, but not robust, relationship between fish length and catchment area of the tributary they entered. This result suggests support for the suggestion that bull trout

switch to larger spawning streams as they age and grow (McPhail and Murray 1979). The result may also mean that there is a general lack of accurate homing at the level of spawning stream within the upper Duncan River. If bull trout spawning habitat requirements change as they grow, then perhaps the lacustrine life history of Kootenay Lake bull trout, and the large sizes they attain, result in reduced homing accuracy.

Of six bull trout tracked migrating into the upper Duncan River in consecutive years, four changed their destination in the upper Duncan River by 10 km from the previous year. Two of the six migrated to putative spawning tributaries in both tracking years, and both switched to a stream with a smaller catchment area during the second year. This result is contradictory to McPhail and Murray's (1979) hypothesis. The stream switching behaviour of these fish suggests that bull trout do not home to specific spawning streams in the upper Duncan River. Without confirmation of spawning at both locations, switching tributaries does not necessarily reflect gene flow between spawning locations.

I used fish trap captures and known numbers of radio-tagged fish to extrapolate a rough number of spawning-stream-switchers. This extrapolation is very crude, but represents my attempt to qualitatively compare the results of telemetry with estimates of the number of migrants inferred from microsatellite allele frequency data.

Population structure and gene flow

I evaluated the spatial scale of population structure in the upper Duncan River. I asked if bull trout in different spawning streams act as genetically distinct populations. I was also interested in qualitative comparisons between the amount of movement between spawning streams and estimates of gene flow from molecular data. I collected microsatellite data from juvenile bull trout sampled in Houston Creek and Westfall River (two confirmed spawning tributaries in the upper Duncan River). A Fisher exact test of differentiation between the two tributaries using six polymorphic loci was not significant. An AMOVA, however, suggests a small (< 1%) but significant component of genetic variation is attributable to the between stream variance component. This suggests that bull trout in the upper Duncan River, as suggested by telemetry results, but contrary to

most other bull trout genetic studies done at similar spatial scales, are not divided into separate populations in different spawning tributaries.

I calculated the number of effective migrants from estimates of genetic differentiation between spawning streams. This estimate is similar to the extrapolation of the number of spawning-stream-switchers using telemetry. A conservative estimate of migration rate is > 5 %.

The difference between this result and other results from studies of bull trout and other fish species is of concern. The spatial scale I examined is small; however, significant differentiation has been found at smaller geographic scales (Spruell et al. 1999; Costello and Taylor unpubl. MS). Another possibility is that some of the differentiation found in other studies was related to sampling resident bull trout populations. In some cases there is no clear indication of the status of populations sampled (Spruell et al. 1999), while in others (Latham and Taylor in press; Costello and Taylor unpubl. MS), above barrier populations were removed and analysed separately. My results are reasonable considering that pooled sample size is ≥ 50 for six polymorphic microsatellite loci in each spawning stream. It is possible that uncovered differentiation, and perhaps adaptive differences, exist between the two streams I compared (Utter et al. 1992; Hedrick 1999).

Results similar to this study were found in the Wigwam River (Costello and Taylor, unpubl. MS). Wigwam River bull trout have a similar life history to the bull trout migrating into the upper Duncan River (Oliver 1979), and sample sites are from areas below barriers to migration (Costello and Taylor, unpubl. MS). Perhaps the similar life history, and growth to large size, plays an important role in the apparent lack of population divergence within the Wigwam and upper Duncan rivers.

Comparison between methods

Direct evidence of bull trout switching spawning stream from telemetry was reflected in estimates of genetic differentiation between Houston Creek and Westfall River. This result is not surprising. Often, a criticism of direct monitoring of dispersal or movement for making inferences about gene flow is that rare but biologically important movements are easily missed (Whitlock and McCauley 1999). In this study, by

restricting focus to the upper Duncan River, I was able to document a relatively common movement that indicated potential for high levels of gene flow. The microsatellite comparison presented in chapter 3 was certainly justified, as the telemetry data only suggested gene flow. On a qualitative level, the estimate of spawning-stream-switchers from telemetry was similar to the equivalent estimate, the number of effective migrants, from microsatellite allele frequencies. This suggests that all of the bull trout that switch spawning tributaries in the upper Duncan River successfully spawn in both locations. Also, this result suggests that the simple relationship between F_{ST} and N_em I used was robust to violations of the assumptions of the Wright's (1943) island model of population structure.

Conservation and management implications

The movement data I have collected allows conservation and management activities in the Duncan River to focus specifically on migrating fish. Habitat protection, angling regulations, and dam operation can be seasonally modified to better protect and assist bull trout movement and reproduction. As suggested above, the migration of non-spawning bull trout to areas downstream of spawning locations also provides information relevant to conservation and management. It seems that more habitat than would be predicted based on location of spawning sites is important for migrating bull trout. Perhaps habitat protection efforts should include more the upper reaches of bull trout spawning systems, not just spawning locations.

The bull trout is an iteroparous salmonid, and as such accurate homing may provide less selective advantage for spawning than in semelparous species like Pacific salmon (Banks 1969). A missed opportunity for reproduction in bull trout may not greatly reduce fitness. In addition, bull trout appear specialized for cold water habitats (Rieman and McIntyre 1993; Parkinson and Haas 1996). Perhaps the ephemeral nature of cold water habitats (often associated with mountainous terrain and glaciation) contributes to a lower selective advantage for homing to the natal stream. Indeed, some radio-tagged bull trout in the upper Duncan River migrated to the upper extent of available habitat. This suggests the species may have exploratory behaviour that is adaptive in ephemeral habitats.

Comparison of the result from Houston Creek and Westfall River to other bull trout genetic studies suggest that barriers to migration play an important role in genetic divergence in bull trout. For conservation purposes, when surveying bull trout genetic variation, care must be taken to identify populations isolated by migration barriers. It is important to note that barriers to migration are some times obvious, such as waterfalls or mountain ranges; however, they are not always readily apparent (Waples 1998; Bohonak 1999). My results suggest that where bull trout have open access to habitat, the spatial scale of populations may be larger than the typical unit of conservation and management - individual spawning streams - in salmonids (Taylor 1991).

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