

MOVEMENT PATTERNS, TIMING OF MIGRATION AND GENETIC POPULATION  
STRUCTURE OF BULL TROUT (*Salvelinus confluentus*) IN THE MORICE RIVER  
WATERSHED, BRITISH COLUMBIA

by

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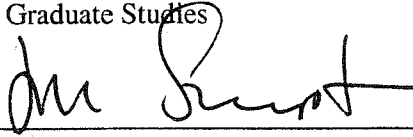
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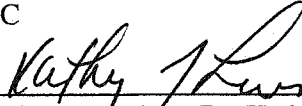
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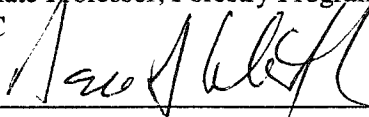
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## Abstract

Movement patterns, timing of migration and genetic population structure of bull trout (*Salvelinus confluentus*) in the Morice River watershed, British Columbia.

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Bull trout (*Salvelinus confluentus*), native to western Canada and the United States, have become a species of special concern (blue listed) in British Columbia due to declining population sizes. Limited biological information exists for bull trout in the Morice River watershed, the area of focus for this research. My research had three goals: a) to determine if bull trout in the Morice River watershed demonstrate multiple life history forms; b) to determine if their movement was influenced by temperature and discharge; and c) to determine genetic structure within the population. Using radio telemetry, I found that one life history form of bull trout exists in the watershed; however, five geographically separated tributary areas were important for spawning. Average daily movement increased with temperature as migration to spawning areas began, and directional movement to spawning habitat spanned June to September. Date of entry into tributary habitat was also negatively correlated with water depth. A genetic analysis using eight polymorphic microsatellite loci revealed three weak clades. The three clades did not correspond directly with spatial clusters defined by the radio telemetry data; hence the Morice River watershed should be viewed as a panmictic breeding unit, and managed at the watershed level.

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## **Chapter 1 : Introduction**

Bull trout (*Salvelinus confluentus*) are char, members of the family Salmonidae. Their historic range extended from 41° to 60° N latitude, and they are native to British Columbia, Alberta, Yukon, Washington, Idaho, Montana, Oregon, Nevada and California (Cavender 1978). Their taxonomy has historically been confused with Dolly Varden (*Salvelinus malma*) until they were presented as a distinct species in 1978 (Cavender 1978). A linear discriminant function based on morphology was developed to distinguish bull trout from Dolly Varden and has provided a reliable means of identifying the two species (Haas and McPhail 1991). Since then, there has been a heightened awareness of bull trout, and researchers began to discover that populations were in decline.

Studies of declining populations have revealed that bull trout are specific in their habitat requirements and seem to be sensitive to habitat disturbance (Rieman and McIntyre 1993). Anthropogenic change such as road building, logging, mining, grazing and the building of dams is common in many of the southern watersheds that bull trout use (Fralely and Shepard 1989; Swanberg 1997a, 1997b). As a result, migratory corridors have been restricted, hydrology has changed due to landscape alterations, and some waters are now unsuitable for bull trout (Leary et al. 1993). Bull trout are now considered a species of concern ("blue listed") in British Columbia under the Conservation Data Centre of the Ministry of Water, Land and Air Protection, and as a threatened species under the Endangered Species Act in the United States (Office of the Federal Register 59[June 10, 1994]:30254). Blue listed species are considered vulnerable taxa in British Columbia because of characteristics that make them particularly sensitive to human activities or natural events. They are at risk, but not extirpated, endangered or threatened ([srmwww.gov.bc.ca/atrisk/red-blue.htm](http://srmwww.gov.bc.ca/atrisk/red-blue.htm)).

Bull trout are known to be highly selective in their choice of spawning sites (Baxter and McPhail 1996) and often prefer to use areas of groundwater infiltration (Fraley and Shepard 1989; Baxter and McPhail 1996, 1999). Populations of bull trout have been observed to use the same spawning sites each year, despite the abundance of what appears to be suitable spawning habitat throughout the system (Baxter and McPhail 1999). As well, geographical separation of spawning habitat used by bull trout has been shown to be related to genetic differentiation (Spruell et al. 1999; Kanda and Allendorf 2001). Bull trout that segregate by spawning area may be genetically distinct, and these reproductively isolated groups are often referred to as stocks (Larkin 1972; Behnke 1993). A stock is a population of organisms which, sharing a common environment and participating in a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed (Larkin 1972). Loss of genetic diversity due to multiple reproductively isolated populations being treated as one breeding unit by managers may occur in the case of bull trout (Spruell et al. 1999). As a result, conservation efforts often focus on preserving stock structure in an effort to conserve genetic diversity.

Genetic diversity in a population may also be represented by fish that exhibit multiple life history strategies (Rieman and McIntyre 1993). Multiple life history forms or strategies can exist within a geographic region and have been suggested for bull trout (Goetz 1989; Rieman and McIntyre 1993). Life history strategies can lead to the differentiation of isolated breeding units when migratory fish home to natal streams to spawn (Rieman and McIntyre 1993). In the broadest terms, life history forms of bull trout are often divided into two categories; migratory and resident (Fraley and Shepard 1989; Rieman and McIntyre 1993, 1996; Saffel and Scarnecchia 1995; McPhail and Baxter 1996; Jakober et al. 1998). These

life history forms, however, are often distinguished by age and size at maturity. Resident forms are generally dwarfed, inhabit small headwater streams and do not make large migrations to spawn (Rieman and McIntyre 1993, 1996; McPhail and Baxter 1996; Jakober et al. 1998). Migratory forms spend the majority of their life in mainstem rivers or lakes and migrate to smaller tributaries to spawn (Fraley and Shepard 1989; Rieman and McIntyre 1993, 1996; McPhail and Baxter 1996). Migratory bull trout have often been subdivided into forms based on the habitat they utilize; fluvial if they remain in rivers and spawn in tributaries (Goetz 1989; Rieman and McIntyre 1993, 1996; Baxter and McPhail 1996; McPhail and Baxter 1996; Swanberg 1997a, 1997b) and adfluvial or lacustrine if they inhabit lakes and spawn in rivers or tributaries (Goetz 1989; Rieman and McIntyre 1993, 1996; Baxter and McPhail 1996; McPhail and Baxter 1996; Beauchamp and Van Tassell 2001). Therefore, different life history forms should demonstrate behavioural differences in habitat selection and patterns of movement.

The goal of my thesis was to determine if bull trout within a single large watershed showed multiple life history forms, if their movement was influenced by environmental variables of temperature and discharge and if they showed genetic population structure. The Morice River watershed has several features that make it a good location to find multiple life history forms. First, the watershed has a large lake at its headwaters with rearing and overwintering potential. Second, the Morice and Nanika rivers are large rivers with suitable rearing, spawning and overwintering habitat. Third, the watershed also has many tributary systems that have suitable spawning habitat (Bustard 1997, 1999; Triton Environmental Consultants Ltd 2000).

I used radio telemetry to investigate movement patterns, habitat use and timing of migration of bull trout in the Morice River watershed during 2000 and 2001. Ninety-three radio transmitters were surgically implanted into bull trout and movements were tracked. Based on the radio telemetry data, I determined if different life history forms could be identified in the watershed (Chapter 2). Using temporal patterns of movement to and from tributary habitat, I then determined if migration timing was influenced by physical factors such as water temperature or discharge (Chapter 3). Lastly, I used microsatellite DNA analysis to assess population structure of the radio tagged individuals, and determined if genetic structure corresponded to movement and spawning locations identified from radio telemetry (Chapter 4).

Integration of the three chapters presented in this thesis provides new information on variation in movement that exists within a single watershed and demonstrates the potential for environmental and genetic factors to influence bull trout. Knowledge gained from this research also has potential to be applied to conservation and management of bull trout in other watersheds within northwestern B.C..

**Chapter 2 : Spatial and quantitative differences in movement patterns of bull trout (*Salvelinus confluentus*) in the Morice River watershed, northwestern British Columbia.**

## **Abstract**

Movement patterns of bull trout (*Salvelinus confluentus*) were examined using radio telemetry in the Morice River watershed, northwestern British Columbia, between April 2000 and November 2001. Ninety-three bull trout were implanted with radio transmitters and tracked using aerial flights and fixed stations. For each radio tagged fish, average spatial location and mean distance traveled per day within the watershed were calculated. Hierarchical cluster analyses were performed for spatial data, movement data, and a combination of both. Analysis of spatial data identified five groups within the watershed; four were separated geographically by tributary use and one used the Morice River mainstem exclusively. Analysis of average distance moved per day yielded five groups of bull trout; those with Small ( $0.06 \pm 0.01$  km/day), Moderate ( $0.41 \pm 0.03$  km/day), Intermediate ( $0.84 \pm 0.03$  km/day), Large ( $1.26 \pm 0.03$  km/day) and Extensive ( $2.2 \pm 0.14$  km/day) movements. Bull trout that demonstrated Intermediate, Large and Extensive movements made long migrations to tributaries, presumably to specific spawning areas. The combined analysis of spatial and movement data resembled the spatial analysis; however, fish within spatial clusters showed a wide range of average movement per day. Variation in spatial and movement data appears to be linked to availability of suitable habitat.



## ***Introduction***

Temporal and spatial diversity in movements of bull trout (*Salvelinus confluentus*) are thought to stabilize populations in highly variable environments (Rieman and McIntyre 1993, 1996). Diversity in movements of fish, therefore, can reduce rates of local extinction, permit recolonization if local extinctions should occur, and influence population genetics and community composition (Jackson et al. 2001). Bull trout that use different areas of a watershed may be part of a metapopulation and this structure is thought to provide a mechanism for spreading risk and supporting weaker populations (Rieman and McIntyre 1993; Rieman and Myers 1997).

Several authors have suggested that bull trout have multiple life history forms or strategies. Migratory and resident bull trout (Fraley and Shepard 1989; Rieman and McIntyre 1993, 1996; Saffel and Scarnecchia 1995; McPhail and Baxter 1996; Jakober et al. 1998) differ in age and size at maturity. Identification of these different forms has characteristically been determined by habitat used and patterns of movement. Fluvial and adfluvial migratory forms spend the majority of their life in mainstem rivers or lakes and migrate to smaller tributaries to spawn, in contrast to resident forms that do not make large migrations to spawn (Fraley and Shepard 1989; Goetz 1989; Rieman and McIntyre 1993, 1996; Baxter and McPhail 1996; McPhail and Baxter 1996; Swanberg 1997a, 1997b; Beauchamp and Van Tassell 2001). These definitions lack a quantitative measure of rate and extent of movement.

Identification of life history strategies is important for conservation and management because these strategies represent diversity in the way fish have adapted to local habitat conditions (Walters and Korman 1999). In this study, my objective was to determine if bull

trout within a single large watershed show differential use of habitat, patterns of movement and extent of movement. The Morice River watershed has several features that make it a good location to find all three life history forms. First, the watershed has a lake at its headwaters with rearing and overwintering potential. Second, the Morice and Nanika rivers are large rivers with suitable rearing, spawning and overwintering habitat. Finally, the watershed also has many tributary systems that have suitable spawning habitat. For this reason, I radio tagged bull trout in the Morice River watershed in northwestern British Columbia and tracked their movements for two years. My goal was to determine if different life history forms could be identified within a single watershed based on radio telemetry data. Spatial patterns were identified by mapping movements within the mainstem and tributaries for individual bull trout and then examining groups of individuals that used habitat areas in geographical proximity to one another.

## **Methods**

### ***Study Location***

The Morice River watershed is located in northwestern British Columbia near the town of Houston (54°24'00", 126°40'00") (Figure 2-1). The watershed is 4 438 km<sup>2</sup> in size, and is the largest tributary of the Bulkley River which drains into the Skeena River. This watershed has a lake at the headwaters and also has two large tributary systems where bull trout have been identified (Bustard 1997, 1999; Triton Environmental Consultants 2000): the Nanika River watershed (895.3 km<sup>2</sup>), and the Thautil River/Gosnell Creek (534.9 km<sup>2</sup>) tributary system.

In addition to bull trout, the Morice River watershed supports other salmonids such as chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), sockeye salmon (*O. nerka*), pink salmon (*O. gorbuscha*), steelhead and rainbow trout (*O. mykiss*), cutthroat trout (*O. clarki*), lake trout (*Salvelinus namaycush*), Dolly Varden (*S. malma*), and Rocky Mountain whitefish (*Prosopium williamsoni*).

The Morice River was divided into three sections based on general habitat features; lower (56.4 km), middle (19.7 km) and upper (15.8 km). The lower section has long riffles and glides as well as pools and large woody debris, in contrast to the middle section that fluctuates in depth and is braided with large debris jams. The upper section differs from the other two because it consists of deep glides, some pools and has good spawning gravel. Morice Lake is glacial fed and is located at the headwaters of the Morice River. It has an area of 97.1 km<sup>2</sup> and is approximately 41.2 km long. The Nanika River drains into Morice Lake, is approximately 24.1 km long and has an impassable barrier (Kidprice Falls) at its headwaters near the outlet of Kidprice Lake. Bull trout must pass through the northeast bay of Morice Lake to access the Nanika River. Gosnell Creek is 58.1 km long, and the lower

20.5 km has been labeled as lower Gosnell Creek. Thautil River is 39.4 km long, and the lower 23 km serves as a migratory corridor for fish to access Denys Creek. Gosnell Creek and Thautil River have similar channel width and discharge however differ in habitat features.

#### ***Transmitter Implantation and Fish Tracking***

Seventy radio transmitters were implanted into bull trout that weighed more than 500 grams between April 12 and August 16, 2000. In the autumn of 2000, 23 transmitters were recovered and were implanted into new bull trout in the spring of 2001 between April 30 and June 14. Angling with roe and lures was the primary method of capture. Bull trout were angled from the upper Nanika River (n=12), Morice Lake (n=1), Thautil/Gosnell system (n=9), lower Morice River (n=26), middle Morice River (n=3) and upper Morice River (n=42). Upon capture, bull trout were held in black zippered polyethylene tubes with circular mesh ends until surgery was performed.

Fish were anaesthetized in a 20 L bath containing 45 ppm clove oil (10 ml of 1 part clove oil:10 parts ethanol) (Anderson et al. 1997; Prince and Powell 2000). While in the anaesthetic bath, fork length, standard length, branchiostegal ray number, anal fin ray number, girth, and upper jaw length were determined. These measurements were used to confirm speciation according to the linear discriminant function developed by Haas and McPhail (1991). Fork length was measured as the distance from the most anterior part of the head to the tip of the middle ray of the caudal fin, whereas standard length was measured as the distance from the most anterior part of the head to the posterior margin of the last whole vertebral centrum (Scott and Crossman 1973). All radio tagged bull trout were greater than 400 mm in fork length, and their girth was measured immediately anterior to the dorsal fin. When the fish

reached stage 4 anaesthesia (loss of equilibrium and slowed opercular movement; Bell 1987), it was weighed using a spring scale and then transferred to a v-shaped surgery trough. Prior to surgery, the leading right pectoral fin ray of each bull trout was separated from the pectoral fin using a scalpel and clipped as close as possible to its insertion with the body. Fin rays were dried and sent to North/South Consultants Ltd. in Winnipeg, Manitoba for aging. Opercular movement was monitored during surgery and the gills were irrigated with freshwater throughout the procedure. If the fish began to recover from Stage 4 during the surgery by showing increased movement, approximately 150 ml of the anaesthetic bath was added to the irrigation water.

Bull trout were implanted with Coded Microprocessor Transmitters (Lotek MCFT series, 59 mm length, 43 cm whip antenna) that allowed identification of individual fish while tracking. Transmitters emitted digitally encoded signals every six seconds at 149.360 MHz and 149.400 MHz for 540 days, weighed 10 g (in air) and were less than 2% of the animal's body weight (in air) as suggested by Winter (1996). Surgical procedures followed were a modified version of those outlined by Winter (1996) and Ward and Miller (1988). The incision site was swabbed with Ovadine, and transmitters were inserted into the intraperitoneal cavity through an incision, less than 2.5 cm long, located lateral to the ventral midline of the fish, anterior to the left pelvic fin. A hollow needle was then inserted into the incision toward the caudal peduncle where it was pushed through the body wall to exit anterior to the anal fin. The transmitter's whip antenna was threaded inside the needle and out through the exit hole before the needle was removed. Transmitters were pulled toward the antenna exit to allow the maximum length of antenna to protrude exterior to the fish. The incision was closed with 2-4 skin staples (3M Precise Disposable Skin Staplers-PGX 35W).

Surgery took less than ten minutes and the combination of surgery and measurements did not exceed 18 minutes. Prior to release, bull trout were transferred to the recovery tube and held until they maintained equilibrium and resisted handling.

Fish movements were monitored by three receivers mounted at fixed stations as well as aerial tracking by helicopter. Forty telemetry flights were taken between May 14, 2000 and November 5, 2001 (see Bahr 2002). During flights, a 3-element Yagi antenna received transmitter signals, a Trimble TDC2 GPS unit logged positions every second and Lotek SRX-400 receivers recorded frequencies, codes and transmitter power signals. Flights were flown at speeds of 100 to 160 km/h, approximately 50 m above the ground, and completed in approximately three hours. Movements were monitored more intensively during spawning season (every four to seven days in late August through September) in both years, and were reduced to once per month during winter 2000-2001.

During flights, two receivers were used to scan transmitter frequencies. Scan delays were set for 6 seconds and receivers were offset from each other to ensure continual monitoring of both transmitter frequencies. Receiver and GPS times were synchronized prior to each flight and fish locations were assigned at the time of the strongest signal. Radio signal detection depended on factors such as weather conditions, topography, flight speed and elevation, fish depth, and interference from other structures such as power lines, radio towers and power boats (Winter 1996). Generally, radio signals could be detected for approximately 10 seconds when moving toward or away from a fish; however, flight speed was decreased in areas of the watershed where aggregations of bull trout were identified. Considering these factors, tracking efficiency was estimated by a similar method to Burrows et al. (2001) who used the strongest signal detection (1 second away from the fish at 100 km/h) and the weakest

(10 seconds away from the fish at 160 km/h) to estimate the accuracy of fish locations. When including GPS error ( $\pm 20$  m), accuracy ranged from  $\pm 50 - 475$  m for locations of bull trout in the Morice River watershed, therefore a margin of error of  $\pm 475$  m was used for interpreting movement data. Fish positions were not differentially corrected because I was interested in large scale fish movement.

Fixed stations consisted of a Lotek SRX 400 receiver connected to two 4-element Yagi antennas mounted at each site; one pointing upstream and one downstream. One fixed station was located near the confluence of the Morice and Bulkley rivers to record fish passage at the lower end of the Morice River when fish left or entered the study area. Another station was set approximately 300 m downstream of Morice Lake and primarily functioned to track fish that traveled upstream into the lake. The third fixed station was located in lower Gosnell Creek and recorded fish passage during the spawning migration. Fixed stations were maintained from May through October 2000 and 2001 and were removed during the winter months when station accessibility was difficult.

Spawning of bull trout was assessed in Morice and Nanika rivers during nine downstream snorkel float surveys, four of the Nanika River (September 16, 23, 2000; September 20, 26, 2001) and five of the upper Morice River (September 15, 2000, September 9, 14, 26, October 10, 2001). Snorkel surveys were conducted using three observers spread across the wetted width of the river, floating parallel downstream. If necessary, sections of rivers were floated repeatedly to confirm observations. During 2001, spawning was visually confirmed in tributaries by viewing pairs of bull trout on redds. If a pair of adult bull trout were not observed on a redd, the redd was only designated as a bull trout redd if it had similar dimensions and was located in similar habitat to those on which adult pairs were

observed. Spawning was assumed for bull trout that migrated to the same spawning areas in 2000.

### *Statistical Analysis*

To determine spatial patterns of fish distribution within the watershed, a single, Euclidean, hierarchical cluster analysis (Everitt 1993) was conducted using Systat ® 7.0. Cluster analysis was chosen because it is a set of methods for constructing a “sensible and informative” classification of an initially unclassified set of data, using the variable values observed on each individual (Everitt 1998). This analysis was chosen over an ordinal approach because it identified groups of fish and their spatial structure, rather than projecting variables onto axes to display their relationships in multidimensional space as occurs using principal component analysis (Clifford and Stephenson 1975). The hierarchical cluster analysis identifies groups of similar samples and arranges them into a dendrogram (a rooted tree in which the nodes link together the clusters being classified) (Clifford and Stephenson 1975) whereas nonhierarchical methods identify groups of similar samples but do not characterize relationships among clusters (Sneath and Sokal 1973; Gauch 1982). This is an agglomerative approach that starts with a set of separate samples and groups these into successively fewer sets until all samples are combined in a single group (Sneath and Sokal 1973; Duran and Odell 1974). I chose this method in contrast to a divisive approach that subdivides all samples of one set into increasingly finer partitions (Sneath and Sokal 1973) because I wanted to examine groups of bull trout that exhibited similar patterns of spatial separation and movement. The single linkage is a method of joining the most similar pair of entities into the same cluster and building the hierarchical tree using this principle. This method is also known as the “nearest neighbour” approach (Sneath and Sokal 1973; Clifford



and Stephenson 1975; Duran and Odell 1974). Cluster distances were computed with the normalized Euclidean distance (root mean squared distances) that is appropriate for quantitative variables (Systat ® 7.0).

Two variables were considered for each bull trout; geographical location within the watershed and distance moved per day between tracking flights. Observations for this analysis were restricted to one of the migratory seasons of 2000 or 2001; June 4-September 23, 2000 or June 3-October 3, 2001 was considered for each fish. Migratory seasons differed in length each year; dates chosen encompassed the duration when bull trout first entered tributary habitat until all fish had migrated out, as determined by tracking flights. In the event that a fish migrated to spawn one year and not the other, the year including the migration was chosen for the analysis.

Average spatial (geographical) location of each bull trout within the watershed was assigned to a river kilometre on the Morice River. For this procedure, the Morice River, a corridor through Morice Lake, and the Nanika River were divided into one kilometre segments beginning at the confluence of the Morice and Bulkley rivers (kilometre 0) and terminating at Kidprice Falls, located at the upstream end of the Nanika River (kilometre 116). The location of each bull trout identified by tracking flights was assigned the closest kilometre number on the river network, a number between zero and 116. Bull trout using tributary habitat were assigned the kilometre number nearest the confluence of the tributary stream with the mainstem river for the duration they remained in the tributary. Gosnell Creek and Thautil River meet approximately 200 metres upstream of the Morice River at km 73.5, therefore fish using these tributaries were assigned kilometre numbers upstream and downstream of the confluence (Gosnell Creek = 79; Thautil River = 68) to differentiate these

two watersheds. Kilometre numbers were assigned to each bull trout on a daily basis during the migratory season. The kilometre number assigned from one contact was assigned each day following that contact until a new location was determined by telemetry flights. These daily kilometre numbers were averaged for input into the analysis. Average values were arranged from smallest to largest, and each value was divided into the highest average kilometre number. This yielded a value for each bull trout relating its average location to the location of the bull trout with the highest average kilometre number. These standardized values were then entered into the cluster analysis.

Average distance moved per day was calculated for each bull trout by dividing the distance moved between tracking flights by the number of days between flights. A minimum of five and up to 16 distance measurements for each individual was calculated during the specified time period. The grand mean of these distances represented the average distance moved per day for the bull trout. It was also standardized using the same method as spatial location and used in the cluster analysis.

Of the 93 tagged bull trout, 67 were included in the cluster analyses; three fish were never located after tagging, four were presumed dead and the remaining 19 were excluded as they were located on less than five telemetry flights during the sample period. The decision of where to cut the dendrogram to form groups is subjective in that no criteria are used to determine when significant changes occur in the distance between clusters (Wilkins et al. 1990). I chose to form clusters that were the most biologically relevant to the bull trout in the Morice system and represented the patterns clearly seen from the individual maps of fish movement.

Mean fork length, age, weight and condition factor were calculated for individuals in groups resulting from the cluster analyses. The homogeneity of variances was tested among the groups using the Levene statistic. Analysis of variance (ANOVA) was used to determine significant differences among groups when their variances were homogeneous. A Krustal-Wallis one-way analysis of variance by ranks test was used to determine significant differences among groups when their variances were not homogeneous. A multiple comparisons test determined which groups were different when the Krustal-Wallis test was used (Siegal and Castellan 1988) and a Tukey test (Zar 1984) determined which groups were different when the ANOVA was used. Condition factor was calculated as  $\text{weight (g)} / (\text{fork length (cm)})^3 \times 100$  (Anderson and Neumann 1996).

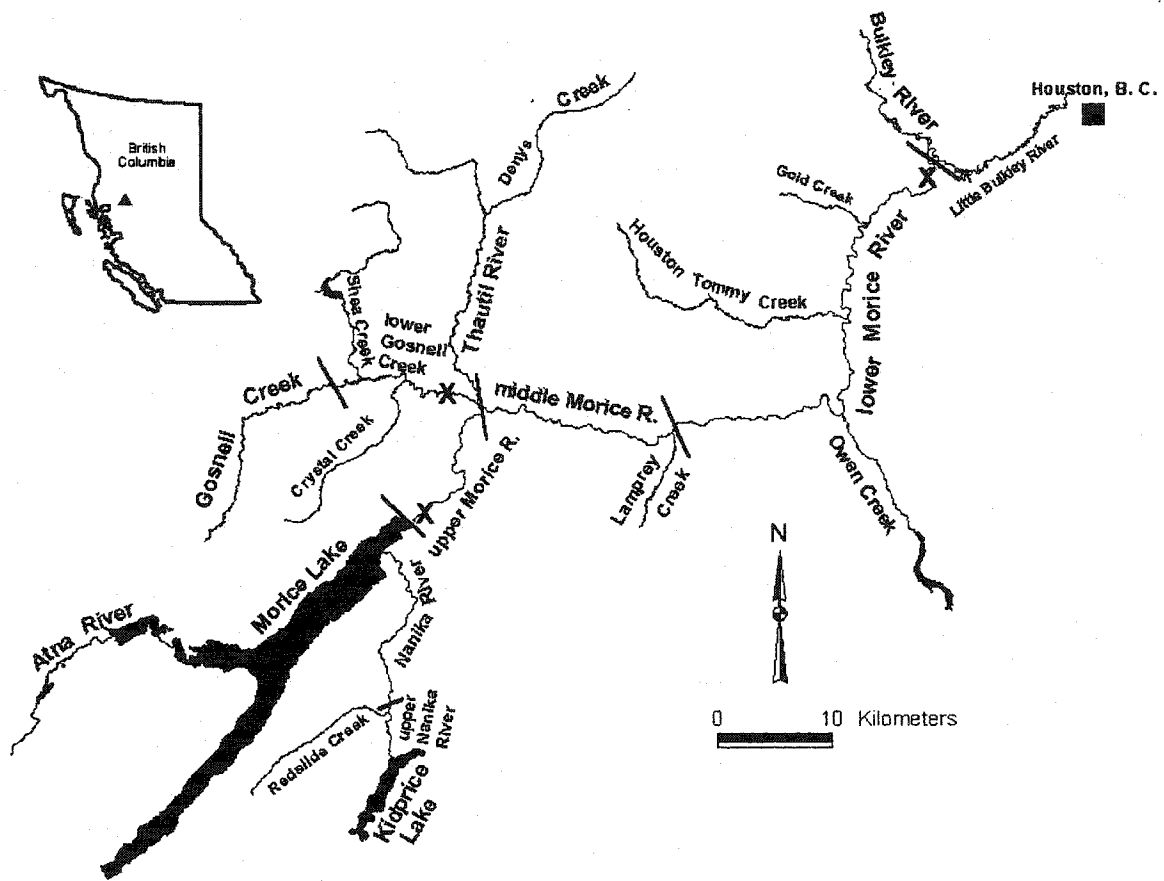


Figure 2-1. Overview map of the Morice River watershed. Short lines denote lower, middle and upper regions of Morice River, Nanika River and Gossnell Creek. Fixed stations are marked by an X.

## **Results**

Of the 67 fish tagged and used in this analysis, 23 were tracked over two years. Of these 23, three fish migrated to spawning areas in 2000, seven fish migrated to spawning tributaries in 2001, three migrated to spawn both years, and ten fish spent both years in mainstem rivers. Spatial analysis of the radio telemetry data divided bull trout into five groups (Figure 2-2); a Nanika/Redslide (NR) group (n=23), an upper Morice (UM) group (n=5), a Thautil/Gosnell (TG) group (n=25), a middle Morice (MM) group (n=8) and a lower Morice (LM) group (n=6). The names describing these groups indicate the geographic areas in the watershed where the fish were found, as outlined on the location map (Figure 2-1). The Nanika/Redslide group and the Thautil/Gosnell group are clustered on either side of the upper Morice group. Some individuals found to spawn in these two areas were tagged and used habitat in the upper Morice River prior to and after spawning. Fish that clustered in the Thautil/Gosnell group spawned in mainstem Gosnell Creek (82 redds), Crystal Creek (8 redds) and Denys Creek (17 redds). Not all of the radio tagged bull trout migrated to tributary habitat and those that remained in mainstem rivers were assumed not to have spawned. Bull trout in the upper Morice group tended to reside in a short 6.5 km section of the river just downstream of Morice Lake. Similarly, the Nanika/Redslide group contained nine individuals that inhabited a small 4.7 km section of the upper Nanika River. The middle Morice group included bull trout that moved and spawned in all parts of the watershed excluding the Nanika River watershed. The lower Morice group included bull trout that used only the lower Morice River, and spawned in Houston Tommy Creek, Gold Creek, or Denys Creek (Thautil/Gosnell). Redds were visually identified in Gold Creek (n=2). Mean fork length, weight, condition factor and age of these groups are shown in Table 2-1. Analysis of

variance indicated that groups were not significantly different in weight ( $p=0.24$ ;  $F=1.41$ ;  $df=4,60$ ) or age ( $p=0.32$ ;  $F=1.12$ ;  $df=4,61$ ), but were significantly different based on fork length ( $p<0.001$ ;  $F=3.74$ ;  $df=4,62$ ) and condition factor ( $p<0.001$ ;  $F=3.76$ ;  $df=4,60$ ).

Significant differences in both fork length and condition factor resulted because the calculation of condition factor requires the use of fork length. A Tukey test (Zar 1984) indicated that the Nanika/Redslide group was significantly larger than all other groups except the lower Morice group. As well, the lower Morice group had a significantly larger condition factor than the Nanika/Redslide group.

The hierarchical cluster analysis of average distance moved per day divided bull trout into five broad groups (Figure 2-3); Bull trout in the Small movement group (S) ( $n=12$ ) moved an average of  $0.06 \pm 0.01$  km/day (distance  $\pm$  SE) and showed restricted movements within specific reaches of the mainstem, such as the upper Morice River, and the Nanika River. Fish in the Moderate movement group (M) ( $n=28$ ) moved an average distance of  $0.41 \pm 0.03$  km/day. Although this group of fish moved greater distances than those in the Small movement group, they remained in the mainstem of the Nanika and Morice rivers, or migrated to nearby tributaries, such as Redslide Creek or the lower reaches of Gosnell Creek. Bull trout in the Intermediate (I) ( $n=6$ ) and Large (L) ( $n=13$ ) movement groups included fish that moved mainly among sections of the Morice River and the Gosnell and Thautil watersheds; the Intermediate group moved an average of  $0.84 \pm 0.03$  km/day and the Large group moved  $1.26 \pm 0.03$  km/day. Bull trout in the Extensive movement group (E) ( $n=8$ ) moved an average of  $2.2 \pm 0.14$  km/day. Fish in this group made extensive migrations throughout the watershed. For example, one fish moved between the Nanika, Morice and Thautil rivers to spawn in Denys Creek; another moved between Thautil River to Gosnell

Creek, back up Thautil River to spawn in Denys Creek, and then downstream to the Bulkley River. Others moved long distances to Gold Creek and Houston Tommy Creek during spawning season. Many of the long migrations observed were made while moving to and from spawning areas. For example, fish moved appreciable distances to reach redd sites in upper Gosnell Creek and Denys Creek, located 41.5 and 28.2 km respectively from the Thautil/Gosnell confluence. Although bull trout were categorized into five groups based on average movement per day, none of these groups varied significantly from each other in fork length ( $p=0.07$ ;  $F=2.28$ ;  $df=4,62$ ), weight ( $p=0.41$ ;  $F=1.00$ ;  $df=4,60$ ), age ( $p=0.125$ ; Chi-Square=7.205;  $df=4$ ) or condition factor ( $p=0.62$ ;  $F=0.67$ ;  $df=4,60$ ) (Table 2-1).

When spatial location and average distance moved per day are combined in a cluster analysis (Figure 2-4), bull trout can be divided into six groups (A-F). These groups are similar to those defined by spatial analysis; however, the addition of movement data reveals structure within some of the groups. Group A consists of only two fish that moved extensive distances per day and clustered into different groups based on spatial location; one from the Thautil/Gosnell group and one from the Nanika/Redslide group. Group B ( $n=8$ ) is a cluster of fish that moved both Large and Extensive distances per day and were spatially located in the middle Morice ( $n=6$ ) and the Thautil/Gosnell ( $n=2$ ) groups. Group C ( $n=22$ ) is a tight cluster based on spatial location, as it contains 21 fish from the Thautil/Gosnell group and one fish from the middle Morice group. This group displays a gradient in patterns of movement and includes fish that moved Moderate, Intermediate and Large distances per day. Group D is a tight cluster of five upper Morice fish and one Thautil/Gosnell fish. All upper Morice bull trout moved Small distances per day and the Thautil/Gosnell fish moved a moderate distance per day. Group E ( $n=22$ ) is also a tight cluster based on spatial location

(all fish were from the Nanika/Redslide group), but these fish showed variation in movement from Small to Moderate distances per day. Group F consisted of seven fish from the lower Morice (n=6) and middle Morice (n=1) groups that showed the greatest diversity in movement patterns. Fish in this group moved Moderate, Intermediate, Large and Extensive distances per day. A comparison of groups A-F indicated that none of the groups differed significantly in weight ( $p=0.12$ ;  $F=1.82$ ;  $df=5,59$ ) or age ( $p=0.170$ ;  $\text{Chi-Square}=7.765$ ;  $df=5$ ) (Table 2-1). However, significant differences in fork length ( $p<0.001$ ;  $F=3.55$ ;  $df=5,61$ ) and condition factor ( $p=0.02$ ;  $\text{Chi-Square}=14.285$ ;  $df=5$ ) were found. A Tukey test (Zar 1984) identified that the Thautil/Gosnell group (C) differed significantly in fork length from the Nanika/Redslide group (E). A multiple comparison test identified that group A and E fish differed in condition factor from all other groups. The middle Morice (B) and Thautil/Gosnell (C) groups did not differ significantly. Also, the upper Morice (D) and lower Morice (F) groups did not differ significantly.



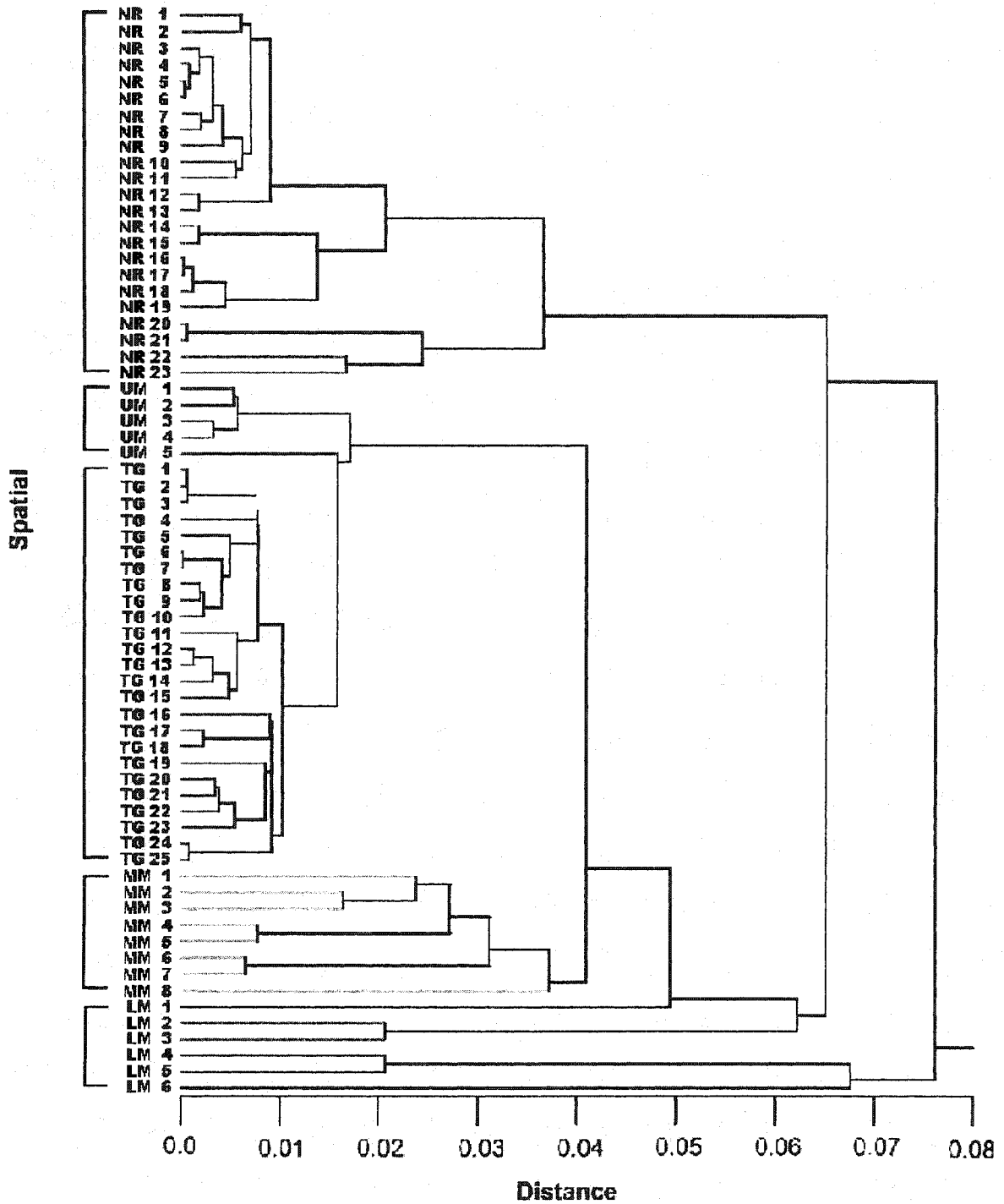


Figure 2-2. Hierarchical cluster analysis of 67 radio tagged bull trout in the Morice River watershed based on average spatial location (kilometre number) during June 4-September 23, 2000 and June 3-October 3, 2001. NR indicates Nanika/Redslide, UM indicates upper Morice, TG indicates Thautil/Gosnell, MM indicates middle Morice and LM indicates lower Morice.

**Table 2-1. Fork length, weight, condition factor and age of groups of bull trout in each cluster analysis. All values are given as means with standard error.**

Cluster	Group	Fork length (mm)	Weight (kg)	Condition Factor	Age
Spatial	NR (n=23 )	577 ± 14.4	1.82 ± 0.15	0.9 ± 0.02	8.6 ± 0.5
	UM (n=5)	517 ± 28.3	1.52 ± 0.16	1.1 ± 0.08	7.6 ± 0.5
	TG (n=25)	522 ± 8.4	1.47 ± 0.09	1.01 ± 0.04	7.5 ± 0.3
	MM (n=8)	512 ± 21.9	1.52 ± 0.15	1.05 ± 0.07	8.3 ± 0.7
	LM (n=6)	542 ± 14.2	1.85 ± 0.25	1.13 ± 0.09	7.5 ± 0.5
Movement	Small (n=12)	540 ± 14.6	1.55 ± 0.10	0.99 ± 0.05	7.8 ± 0.3
	Moderate (n=28)	561 ± 13.4	1.79 ± 0.13	0.98 ± 0.03	8.4 ± 0.4
	Intermediate (n=6)	518 ± 27.3	1.58 ± 0.23	0.99 ± 0.04	7.5 ± 0.5
	Large (n=13)	505 ± 11.1	1.41 ± 0.14	1.07 ± 0.06	7.0 ± 0.4
	Extensive (n=8)	548 ± 6.5	1.63 ± 0.11	0.97 ± 0.04	8.8 ± 0.4
Spatial and Movement	A (n=2)	555 ± 25.0	1.38 ± 0.18	0.8 ± 0.01	9.0 ± 2.0
	B (n=8)	529 ± 18.7	1.72 ± 0.22	1.04 ± 0.09	8.6 ± 0.6
	C (n=22)	510 ± 9.5	1.38 ± 0.08	1.03 ± 0.04	7.1 ± 0.3
	D (n=6)	524 ± 24.2	1.54 ± 0.14	1.08 ± 0.07	7.7 ± 0.4
	E (n=22)	579 ± 14.9	1.84 ± 0.16	0.91 ± 0.02	8.7 ± 0.5
	F (n=7)	543 ± 12.0	1.84 ± 0.21	1.12 ± 0.08	7.7 ± 0.5

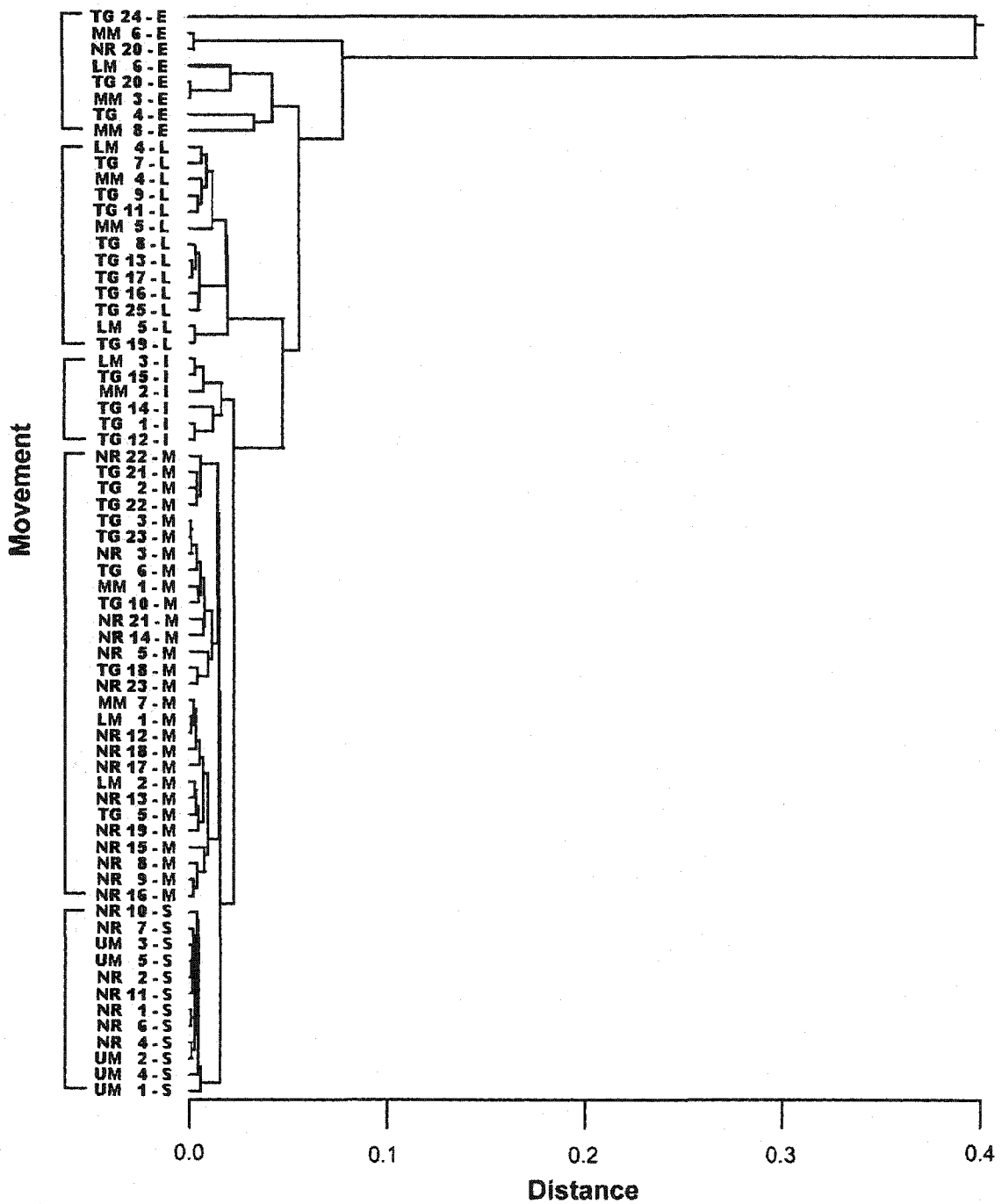


Figure 2-3. Hierarchical cluster analysis of 67 bull trout in the Morice River watershed based on average distance moved per day (movement) during June 4-September 23, 2000 and June 3-October 3, 2001. Movement is indicated by S for Small, M for Moderate, I for Intermediate, L for Large and E for Extensive.

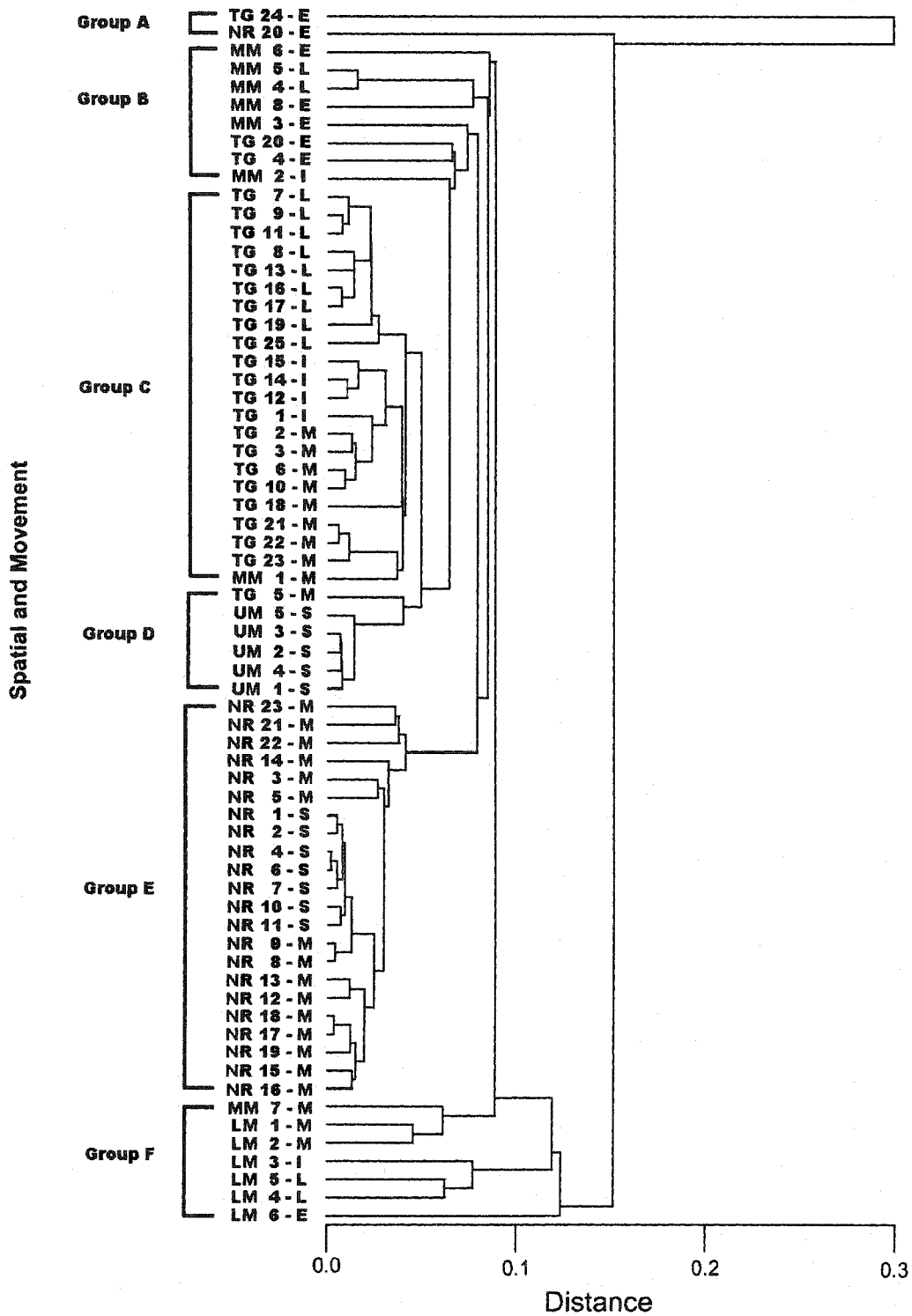


Figure 2-4. Hierarchical cluster analysis of 67 bull trout in the Morice River watershed based on spatial location (kilometre number) and average distance moved per day (movement) during June 4-September 23, 2000 and June 3-October 3, 2001. Abbreviations are as described in Figures 2-2 and 2-3.

## ***Discussion***

Bull trout in the Morice River watershed were observed to use specific areas of the watershed for spawning. Radio tagged bull trout spawned in five tributaries; however, redd distribution was clumped within each of the tributaries, and movement to the same areas was observed by many of the radio tagged fish. The distribution of redds in tributaries and the movement of bull trout to these areas was consistent in both study years. Other research has shown that bull trout migrate to tributaries to spawn, (Fraley and Shepard 1989; Rieman and McIntyre 1993, 1996; McPhail and Baxter 1996; Baxter and McPhail 1999) but may spawn in only a portion of the available stream reaches (Fraley and Shepard 1989; Baxter and McPhail 1999), similar to the population of bull trout in the Morice River watershed. Individuals in the spatially identified groups also showed variation in the average distance moved per day, and the combination of the spatial and movement data revealed that the spatial component had a strong influence on the structure of the groups of bull trout.

The radio telemetry data indicated that population structure based on geographical separation exists within bull trout from the Morice River watershed. Spatial analysis indicated that there are five groups representing five geographical regions within the watershed where bull trout are commonly found and/or observed to spawn. The variation in distances moved by some of the radio tagged fish suggests that there is movement between the spatially identified groups. For example, a fish captured and tagged in the Nanika River migrated to Thautil River system to spawn in 2000, and remained in the Nanika River during 2001. As well, six fish tagged in the upper Morice River migrated to Redslide Creek to spawn. Two fish were captured in the upper Morice River, spent time in the Nanika River, overwintered in Morice Lake and one was assumed to spawn in Redslide Creek. This is

confounded because bull trout are iteroparous, and may spawn intermittently once they reach sexual maturity (Goetz 1989; Ford et al. 1995).

Results from a cluster analysis that combined spatial and movement data showed that fish within all groups except the upper Morice group varied in average distances moved per day. Distances moved per day may be representative of the total distance to the location of spawning areas. For example, fish within the Thautil/Gosnell group moved Moderate to Large distances per day, which is expected since the majority of redds were found in the upper reaches of these systems. Bull trout in the Nanika/Redslide group moved Small to Moderate distances per day; areas for spawning in Redslide Creek are much closer to the Nanika River than those in Thautil/Gosnell are to the mainstem Morice River.

My analysis demonstrates that there was variation in spatial location and distance moved for bull trout in the Morice River watershed. However, the presence of multiple life history forms within this population was not evident. Bull trout were tracked primarily in mainstem rivers, but moved to tributaries to spawn. I found no evidence for spawning within the mainstem rivers and Morice Lake appeared to be used primarily as a corridor for moving between river systems. According to previous literature that defines life history forms based on movement patterns, bull trout in the Morice River system could be labeled as fluvial fish (Goetz 1989; Rieman and McIntyre 1993, 1996; Baxter and McPhail 1996; McPhail and Baxter 1996; Swanberg 1997a, 1997b). Although “fluvial” bull trout were numerically dominant in my study, bull trout that overwintered in Morice Lake and spawned in Redslide Creek could be classified as having either fluvial or adfluvial life history form based on movement patterns. These individuals were few in number in my study, but may be more prevalent if the behaviour of bull trout was monitored over the long-term.

Factors other than life history are more likely to account for the variation in movement and distribution observed in bull trout from the Morice River watershed. Bull trout clustered spatially into groups within the Morice River mainstem, likely due to presence of specific habitat and potentially high food availability. The Morice system has a number of fish species that bull trout may feed on, since they are aggressive piscivores and eat a variety of other species (Boag 1987; Goetz 1989; Donald and Alger 1993; McPhail and Baxter 1996; Beauchamp and Van Tassell 2001). Additionally, many salmonids spawn in the upper Nanika and upper Morice rivers in both the spring and fall. Bull trout were observed individually downstream of salmon redds in these areas during snorkel surveys, suggesting that bull trout may feed on eggs from other species. Salmon eggs may be an important component of the energy budget of bull trout (Boag 1987). As well, upper Nanika and upper Morice mainstem areas have deep sections (>3 metres) that provide cover from terrestrial and avian predators. Habitat in these two areas, therefore, may be preferred because of the available food and suitable cover. Areas in the lower Morice River have similar habitat characteristics; however, these fish moved larger distances comparatively. The lower Morice River is a long section of river (>50 km) with areas of suitable feeding and overwintering habitat throughout but there does not appear to be a small concentrated section of river that all lower Morice bull trout inhabit, as occurs in the upper Morice or Nanika rivers. Further, overwintering habitat may limit distributions of bull trout at this latitude since many of tributary streams freeze during the winter. Bull trout presumed to spawn in the Shelagoyote River watershed, a proximal watershed that has similar latitude to the Morice, appear to overwinter in the Babine River due to warmer winter water temperatures (Giroux 2001). Deep sections throughout the Morice and Nanika rivers also provide favourable

overwintering habitat. Additionally, Morice and Kidprice lakes moderate environmental factors in the adjacent Morice and Nanika rivers such as water temperature and flow.

Although mainstem habitat appears to be suitable for spawning in both the upper Morice and Nanika rivers, a number of observations suggest that mainstem fish did not spawn. When observed in the mainstem, bull trout were located individually downstream of chinook or sockeye redds, and were not observed to demonstrate spawning behaviour such as digging and quivering (Goetz 1989). The apparent lack of spawning in large rivers by bull trout may be an artifact of sampling since visual observations are difficult (McPhail and Baxter 1996). This is a concern in my study since the observations made during floating were limited to areas that were safe to float. All potential spawning habitat could not be assessed since observers could not always unobtrusively investigate areas with undercut banks, overhanging vegetation or debris jams. I cannot, therefore, conclude that spawning did not occur in the mainstem. Mainstem bull trout, however, did not display sexually dimorphic characteristics such as obvious colour differences between males and females and kype development (McPhail and Baxter 1996).

Bull trout were not observed to spend time during either migratory season in Morice Lake. Locations from radio tracking indicate that bull trout used Morice Lake as a corridor for movement to other river systems. Eight bull trout moved between the Nanika and Morice rivers, and two moved between the Atna and Morice rivers. Three bull trout potentially used Morice Lake for overwintering as they were detected in the fall during tracking flights, were not observed through the winter and returned past the fixed station to the upper Morice River in the spring. These fish were not detected over the winter potentially due to the inability of radio signals to penetrate through deep water in the lake. Morice Lake supports a population



of lake trout, and Donald and Alger (1993) found that lake trout displace bull trout. They indicated that niche overlap and the potential for competition between the two species was substantial within 34 lakes studied in Alberta, British Columbia and Montana. It is possible that a similar interaction between lake trout and bull trout may occur in Morice Lake. The overwinter location of these three fish therefore, is not known.

Migration to spawning grounds appears to be the principle motivation for movement to tributary systems, but movement to specific habitat for feeding is also probable. From the combined spatial and movement analysis, bull trout in the Thautil/Gosnell group showed a range of Moderate to Large average movements per day. Fish that migrated Large distances per day were often associated with directed migrations to spawning habitat. Some of the fish that moved Moderate distances per day moved only to the lower reaches of Gosnell Creek. Gosnell Creek was most heavily utilized by radio tagged bull trout and it contained the largest proportion of observed redds. Although bull trout utilized all reaches of Gosnell Creek, and habitat that appeared suitable for spawning was abundant, spawning was not assumed for bull trout that remained in lower Gosnell Creek. Redds and aggregations of bull trout were not identified during ground surveys and floating in this area. Bull trout located in lower Gosnell Creek held in deep pools and were found in areas where pink salmon were spawning. Non-spawning bull trout were likely feeding on eggs deposited by pink salmon. Swanberg (1997a) gave an alternate reason for migration to tributaries. He found that the majority of bull trout that migrated to tributaries or the upper Blackfoot River, Montana, did not spawn. He hypothesized that movement of bull trout to tributary habitat was not related to foraging (prey fish densities in tributaries were lower than in the mainstem), but occurred to avoid seasonally unfavorable temperatures in the mainstem. This hypothesis is unlikely in

this study since other bull trout remained in the Morice River mainstem during the spawning season and food sources, although not quantified, did not appear limited in Gosnell Creek. Further, bull trout that migrated up the other four tributaries to spawn were not observed holding in the tributaries for long periods of time prior to the spawning season as they did in Gosnell Creek. There was also no evidence of anadromous salmonids spawning in the other four systems during the study, although pink and coho salmon have been previously observed in Houston Tommy Creek (FISS 1991).

Habitat in the upper Morice and upper Nanika rivers was heavily used by bull trout at all times of the year. Some bull trout migrated to spawning locations from these areas and also returned there after spawning. Many of them remained there to overwinter or until migrating to other overwintering areas. Site fidelity to overwintering grounds is not without precedence in the literature. Bull trout in the Blackfoot River, Montana, returned to overwintering grounds suggesting a precise homing ability (Swanberg 1997a). A similar homing ability may be occurring in the Morice River population of bull trout.

Bull trout in the Morice River watershed may exhibit a biannual spawning cycle as shown in other watersheds (Fraley and Shepard 1989; Goetz 1989; Clayton 2001; Hvenegaard and Thera 2001). Fish tagged in this study and observed for only one year may have remained in the mainstem if it was not a year to spawn. Alternate year spawning was observed in the present study ( $n=10$ ), as was spawning in both years ( $n=3$ ) and no spawning in either year ( $n=10$ ). Swanberg (1997a) proposed that some of the bull trout in the Blackfoot River, Montana may have remained in the mainstem if they were not yet sexually mature. This is unlikely in the Morice River watershed as radio tagging was biased to include fish of large fork lengths that had the greatest likelihood of sexual maturity.

Alternate year spawning among many of the fish tagged in this study is possible, and emphasizes the need for long-term monitoring.

The groups of bull trout formed from cluster analyses of spatial location and movement correspond well with geographically separated habitat within the watershed. Six habitat locations are important for spawning bull trout; Redslide Creek, Gosnell Creek, Crystal Creek, Denys Creek, Houston Tommy Creek and Gold Creek. Although there is no evidence of multiple life history forms from the radio telemetry data, if site fidelity is strong and there is little migration between geographical regions, multiple stocks may exist within the Morice River watershed. Population structure based on genetics has been shown in populations with both small and large geographic scales. Population structure in bull trout exists in the Lightning Creek watershed in Idaho (Spruell et al. 2001) as well as across British Columbia and northern Washington (Taylor et al. 2001). It is expected therefore, that bull trout using geographically distinct areas for spawning in the Morice River system may also be distinct genetically. This finding would have significant implications for conservation. If genetic population structure is not evident, identifying different life history forms based on movement patterns of bull trout is meaningless. In this case, spatial segregation to geographically distinct spawning locations is not crucial to maintain population structure.

**Chapter 3 : Factors affecting timing of migration and spawning of bull trout (*Salvelinus confluentus*) in the Morice River watershed.**

## **Abstract**

My objective was to determine if migration timing of bull trout in the Morice River watershed, northwestern British Columbia was influenced by physical factors such as temperature and discharge. To accomplish this, temporal patterns of movement to and from tributary habitat were determined for bull trout using radio telemetry from April 2000 through November 2001. All radio tagged fish showed an increased rate of movement between June and September with the maximum average movement per day occurring in September. Movement decreased and remained at a low level after spawning, during late fall and winter. A direct relationship existed between movement and temperature; however, the correlation showed greater variation as water temperatures increased above 11°C. Although the onset of migration occurred on a variety of dates, directional movement to spawning habitat in tributaries spanned from early June to late July, with the latest migrants entering tributaries in late August and early September. A positive correlation existed between the date of entry into tributaries and water temperature. Dates of entry into one tributary, Gosnell Creek, were also negatively correlated to water depth. Bull trout spent between eight and 110 days (mean  $47.4 \pm 4.58$  days) residing in tributary habitat; however, bull trout remained in Gosnell Creek the longest (mean  $68 \pm 5.32$  days). Spawning began when water temperatures dropped below 8°C. Out-migration was complete by mid to late September, and was quicker than in-migration, lasting approximately three to seven days. The overall pattern of migration timing for bull trout in the Morice River watershed was similar to other populations of bull trout. The short duration of out-migration may be related to an increased risk of predation associated with spending time in small tributary streams.

## **Introduction**

Populations of bull trout (*Salvelinus confluentus*) have been declining (Rieman and McIntyre 1993), and they are now listed as a species of special concern in most of their Canadian range and as a threatened species in the United States under the Endangered Species Act (Haas and McPhail 2001). They are known to migrate long distances to spawn in small tributary streams and are often found to spawn only in a portion of the available stream habitat (Rieman and McIntyre 1993; Ford et al. 1995; Baxter and McPhail 1999; Baxter and Hauer 2000). Like other salmonids, the benefits of migrating must outweigh the costs, since it can be energetically expensive to change residence sites, compete for new habitat, and potentially contend with new predators, parasites, or diseases (Northcote 1992). An understanding of timing of spawning migration and the cycle of movement associated with migration provides knowledge of rearing, staging, spawning and overwintering habitat used by fish in a watershed. This information can be incorporated into resource exploitation plans to minimize potential impacts to fish populations (Jackson et al. 2001).

Timing of movement and habitat selection of bull trout have been documented through the use of radio telemetry in a number of watersheds and appear to be specific to each watershed examined. For example, upstream migration has been recorded anywhere between April and late August (Schill et al. 1994; Swanberg 1997a; Burrows et al. 2001; Hvenegaard and Thera 2001) with spawning occurring in the fall (Fraley and Shepard 1989; McPhail and Baxter 1996; Chandler et al. 2001). Evidence suggests that changes in water temperature, river discharge and photoperiod coincide with spawning migrations of bull trout (Fraley and Shepard 1989; Goetz 1989; Brenkman et al. 2001).

My objectives were to identify temporal patterns of movement within the Morice River watershed and determine when bull trout moved to spawning habitat. To accomplish this, I determined spawning location, and movement rates and timing of radio tagged bull trout in 2000 and 2001 in the Morice River watershed. Temporal differences in movement were identified by examining the overall movement patterns, onset of migration timing, tributary residence time and timing of out-migration. I also determined if migration timing was influenced by two physical factors; water temperature and discharge.

## **Methods**

### ***Study Location***

A detailed description of the study area is given in Chapter 2.

### ***Data collection***

A detailed outline of the methodology used for radio tagging bull trout and tracking their movements is given in Chapter 2. Temperature loggers were located at the fixed station site in Gosnell Creek, near the mouth of the Thautil River and approximately 7 km upstream of the mouth of Houston Tommy Creek.

### ***Statistical Analysis***

I examined patterns of movement for 82 radio tagged individuals throughout the duration of the study. Eleven of the 93 radio tagged bull trout were excluded from the analysis either because they disappeared immediately after tagging ( $n=3$ ) or had two or less tracking contacts prior to the transmitter becoming stationary ( $n=8$ ). Average movement per day for individual bull trout was calculated for each tracking flight by dividing the distance moved between tracking flights by the number of days between flights. To avoid introducing a bias as a result of the tagging procedure, the first two average distance measurements from the telemetry contacts for each individual were excluded from the analysis. As well, distance measurements were terminated for bull trout if fish movement ceased permanently. This resulted in a minimum of five and a maximum of 38 distance measurements for each fish. The average movement of bull trout for each tracking flight was calculated as the grand mean of the average distances moved by all individuals contacted during each flight. Regression



analysis of average movement against mean temperature in the upper Morice River was used to define the relationship between these variables. The effect of temperature has been reviewed in relation to active metabolic rate (Brett and Groves 1979), routine cruising speed (Brett 1995) and burst swimming speed (Randall and Brauner 1991) of salmonids. All of these models show that there is an optimum temperature above which these variables decrease. For this reason, a quadratic model was fitted to this relationship. Significance of the variables in the quadratic was determined from the regression analysis.

To examine when significant differences in distance moved per day occurred, I used a change point test (Siegal and Castellan 1988). The change point test determined whether there had been a change in the underlying process which generates the sequence of events (ie. rate of movement based on radio tracking locations) and identified the time at which the change occurred. The test assumes that the observations form an ordered sequence, and that initially, the distribution of responses has one median, and at some point there is a shift in the median of the distribution. The differences in movement between tracking flights were calculated for each fish, ranked and the sum of the ranks of the variables was calculated at each point. The difference between the observed and predicted sum of ranks at each point was calculated to determine where a significant difference occurred which corresponded to the change point date. Two time periods were considered; May 14, 2000 to October 11, 2000 and November 13, 2000 to November 5, 2001.

Bull trout were included in the change point analysis if the individual was identified on five or more telemetry flights during the specified time period used, and if the individual was considered alive during the time period of the analysis. Bull trout were considered alive if they made movements larger than the error margin estimated from telemetry flights ( $\pm 475$

m) or if they moved distances larger than the error margin after the time period considered in the analysis. If a transmitter became stationary during the time period considered, locations of that individual were included up to the first date recorded at the stationary location. When it appeared that a fish had died, the transmitter was not observed to float progressively downstream, but appeared to find a resting location and remain stationary. Of the 93 tagged bull trout, 67 were included in the change point analysis; three fish were never located after tagging, four were presumed dead and the remaining 19 were excluded as they were located on less than five telemetry flights during the sample period. If the change point date occurred prior to migration to the spawning grounds, the distance from the change point date to the most upstream location recorded for that fish was compared to day of the year by linear regression analysis. Thirty-three fish were included in this analysis.

The relationship between migration timing and water temperature was tested using Pearson's correlation (Zar 1984). Date of entry to Gosnell Creek, Thautil River and Houston Tommy Creek and water temperature were examined in 2000 and 2001. The date that bull trout entered Gosnell Creek in 2001 was determined from data collected at the fixed station located in the lower reach of the creek. The date of entry to Thautil River and Houston Tommy Creek was determined as the median day between the last pre-entrance and the first post-entrance radio-tracking contact. Dates were compared with average daily water temperatures. Pearson's correlation was also used to test the association of discharge and timing of migrants into Gosnell Creek in 2001. Discharge is the volume of water passing a point per unit time (Murphy and Willis 1996). I measured water depth as a surrogate of discharge as the two are directly related.

Residence time of bull trout was determined in Gosnell Creek, Thautil River and Redslide Creek watersheds as the length of time between entry and exit from tributaries. Difference in residence time among the three tributaries was compared using an analysis of variance (ANOVA) and a Tukey test (Zar 1984).

## **Results**

Patterns of movement were first considered for all radio tagged bull trout. An overall pattern of increased movement throughout the watershed was observed in summer and fall, with the greatest movement occurring during the spawning season, between mid August and mid September both years (Figure 3-1). Movement generally decreased after spawning and remained at low levels during winter. This trend was repeated in both years, and rate of movement generally corresponded to the mean water temperature in the upper Morice River. Interestingly, the greatest movement per day was observed after the maximum water temperature had been reached for both years.

When rate of movement was regressed against mean temperature, average daily movement increased with temperature in a curvilinear fashion ( $p < 0.001$ ;  $F = 22.5$ ;  $df = 2, 34$ ) (Figure 3-2). The significance of the temperature and temperature squared terms were  $p = 0.03$  and  $p = 0.04$ , respectively. Average daily movement was highly variable at temperatures above  $11^{\circ}\text{C}$ . Temperatures were generally greater than  $11^{\circ}\text{C}$  between June and September in the Morice mainstem. The greatest variability therefore, occurred during the spawning season and may have been attributed to pre and post spawning migrations.

There was no synchronous date when the change point was significant. In fact, migration timing to spawning areas was asynchronous and occurred over a number of months. Fish began to enter Gosnell Creek watershed in early June, Thautil River watershed in late June and Redslide Creek in late July. The change point test did not identify any specific time period when bull trout migrated to spawning grounds; when all 67 bull trout were considered, the test identified 13 different dates in 2000 and 18 different dates in 2001 when an underlying change of movement occurred. Change point dates were not always

identified at the onset of migration; some occurred when bull trout were at their spawning locations, or after they had made their migration out of the tributaries. There were also no groups in the spatial cluster analysis (Chapter 2, Figure 3-3) that demonstrated a synchronous change point date. However, a total of 33 bull trout (49%) had change point dates occur prior to migration to the spawning grounds. The total distance that each bull trout traveled from its location on this date to its most upstream location in the tributary was plotted against the change point date for these fish (Figure 3-3). A weak linear relationship was present between the change point date and the distance that bull trout traveled to spawning sites. Linear regression indicated a significant movement relationship between date and distance ( $p=0.01$ ;  $F=6.73$ ;  $df=1,31$ ) suggesting that fish that move farther to spawning areas begin their migration earlier in the season.

Bull trout migrated to spawning areas between June and August in both years (Table 3-1). Bull trout spawning in Redslide Creek traveled short distances to the spawning areas (generally less than 15 km), as most of them were rearing in the upper Nanika River prior to spawning. Fish that clustered into the Thautil/Gosnell group generally traveled longer distances to the spawning grounds (up to 67 kilometres). The observed spawning areas were in the upper reaches of Gosnell and Denys creeks. Bull trout gradually moved upstream in Gosnell Creek until they reached their destinations. In contrast to the bull trout in Gosnell Creek who held in pools for up to a month, bull trout did not hold in the Thautil River for more than 10-14 days. Specific dates of movement into Houston Tommy Creek and Gold Creek are not certain, but fish were located in these creeks during August and September of both field seasons.

Date of entrance to tributary streams was positively correlated with the average daily water temperature in Gosnell Creek, Thautil River, and Houston Tommy Creek. As water temperature increased, bull trout began moving into tributaries (Figure 3-4). Tributary name, correlation coefficients, and number of fish entering each tributary are given in Table 3-2. The positive correlations seen in Houston Tommy Creek (n=3) and Thautil River in 2001 (n=4) should be evaluated with caution due to small sample sizes. Nevertheless, bull trout using this tributary likely follow a pattern similar to those that use Gosnell Creek and Thautil River tributaries.

Timing of entrance of bull trout to Gosnell Creek was also negatively correlated with water depth ( $P < 0.001$ ;  $r = -0.984$ ;  $n = 10$ ; Figure 3-5), which is an indicator of discharge. Migration of bull trout in Gosnell Creek spanned June to September both years, during the peak and decline of discharge.

The spawning window for bull trout in the Morice River watershed was approximately 22 days in length and varied slightly for the different geographic locations. It ranged from August 23 through September 15<sup>th</sup>. Spawning began when water temperatures fell below 8°C and active spawning was observed when the temperature was near 6°C. Gosnell Creek was most heavily used for spawning; 75% (82 of 109) redds identified in the Morice River watershed were located in upper Gosnell Creek. Twenty-five radio tagged fish were tracked in Gosnell Creek and its tributaries; three migrated both years. Although 43.1% (25 of 58) of bull trout utilized habitat in Gosnell Creek, only 53.8% (14 of 25) of these were presumed to have spawned. Based on their location during the spawning season, the remaining 11 were presumed to utilize habitat but not spawn. No redds formed by bull trout or aggregations of adult bull trout were found where these fish were located in lower Gosnell

Creek, upstream of Shea Creek confluence for a distance of 12.8 km or downstream of Shea Creek confluence to the outlet.

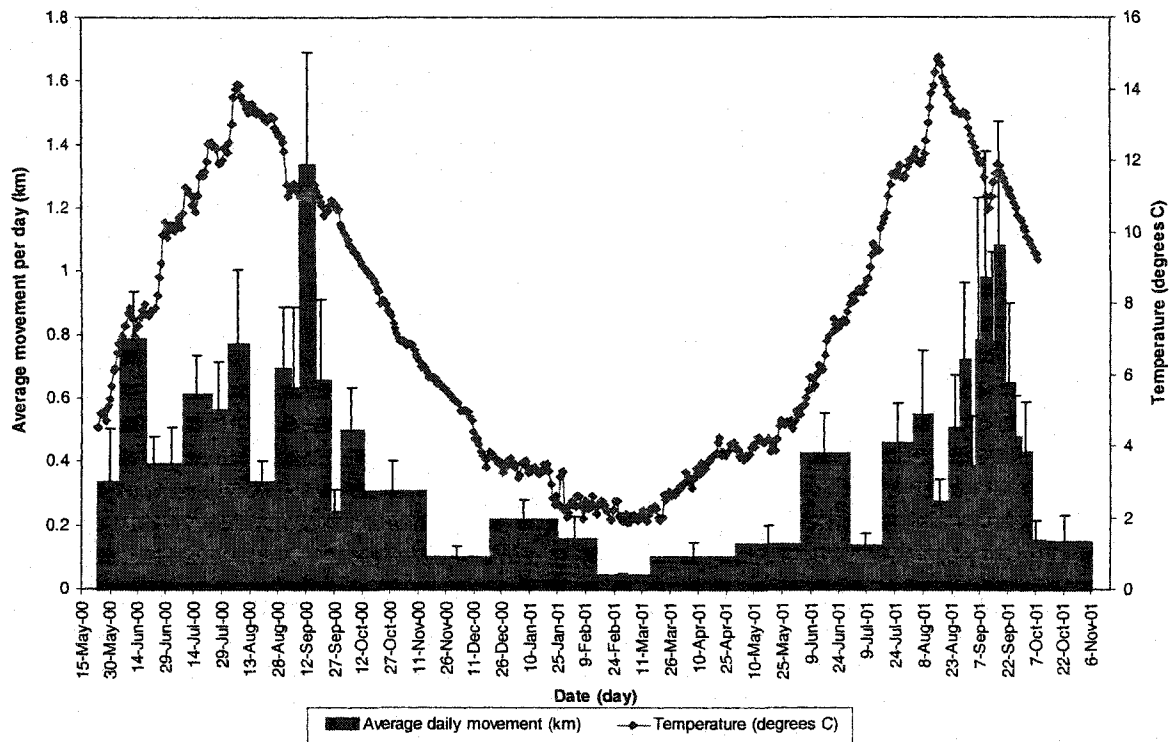
Eighteen radio tagged fish were tracked in Thautil River and its tributaries and nine of these fish (50%) were presumed to spawn in Denys Creek. The other nine bull trout were presumed to have died prior to spawning based on location of recovered transmitters and duration of time that bull trout remained stationary. Sixteen bull trout migrated to other tributaries and were presumed to spawn in Houston Tommy Creek (18.8%, one migrated both years), Gold Creek (25%) and Redslide Creek (36%).

Bull trout spent from 8 to 110 days utilizing tributary habitat and there is a significant difference between tributary watersheds in residence time ( $p < 0.001$ ;  $F = 13.2$ ;  $df = 2,31$ ) (Figure 3-6). A Tukey test (Zar 1984) indicated that bull trout spent significantly more time in Gosnell Creek watershed than in Thautil River watershed or Redslide Creek. Residence time may be linked to habitat quality or risk of predation by animals such as bears (*Ursus sp.*) or river otters (*Lutra canadensis*), since bull trout were observed to hold in pools in Gosnell Creek whereas they migrated more quickly up Thautil River to Denys Creek and Redslide Creek.

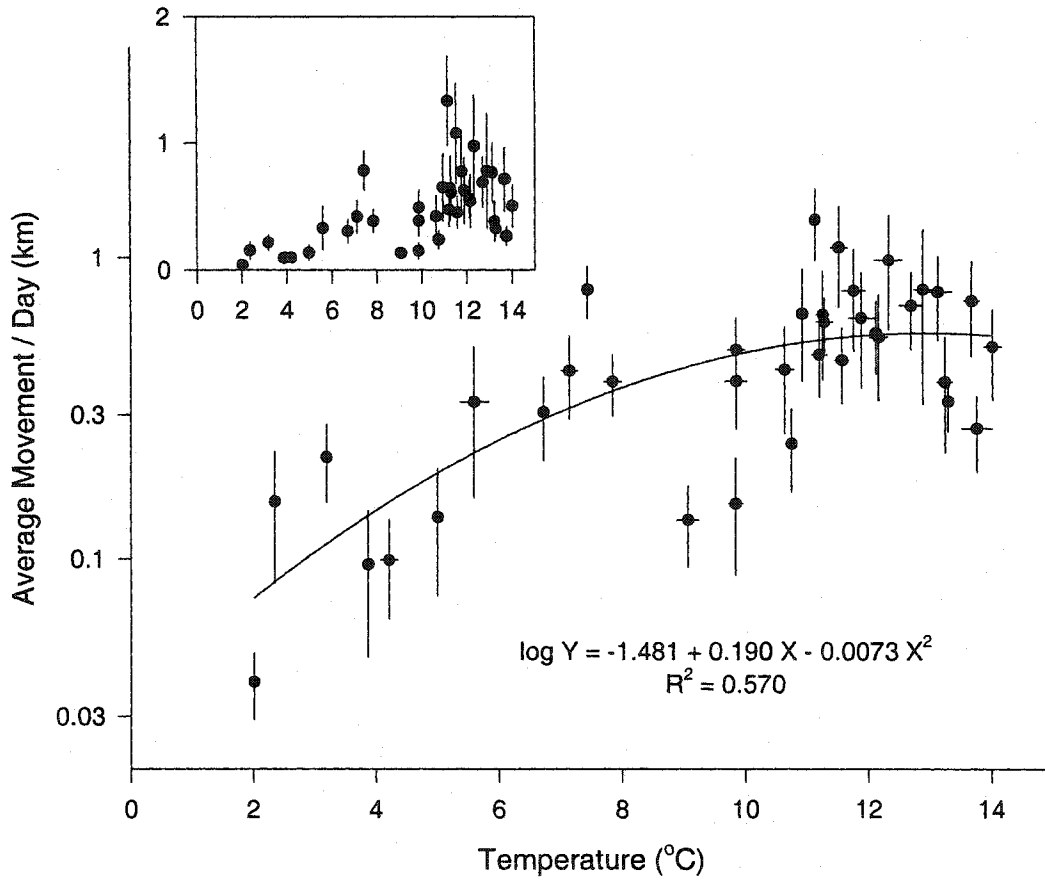
Although upstream migration and residence time occurred over a period of months, outward migration from the tributaries occurred quickly in mid to late September. Bull trout were tracked post-spawn in the upper Morice River by September 15 in both field seasons. All radio tagged bull trout had left tributaries to the Morice River by September 28 in both years, and had left Redslide Creek by October 3, 2001. When bull trout left tributaries they migrated back to the mainstem without staging, usually within three to seven days, and in as little as 39 hours. The majority of fish that migrated out of the tributaries moved to salmon

spawning areas in the upper Morice and upper Nanika rivers. For example, three of five bull trout that left the Thautil River drainage and eight of ten fish that left Gosnell Creek migrated to the upper Morice River after spawning. Additionally, six of seven bull trout in Redslide Creek migrated back to the upper Nanika River.

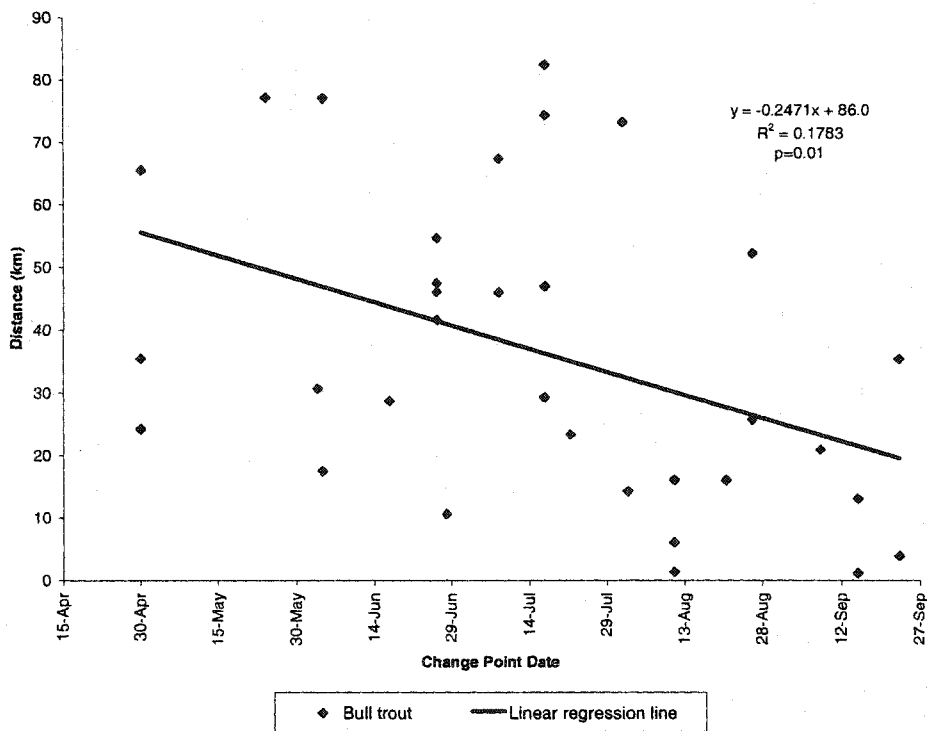




**Figure 3-1. Average daily movement (km) between tracking flights and average daily temperature vs. date for 82 radio tagged bull trout in the Morice River watershed. The first two movement calculations after tagging were omitted for each fish. Average daily temperature was logged in the upper Morice River. Error bars represent standard error.**



**Figure 3-2.** Average movement per day (km) between tracking flights was log transformed and plotted against temperature (°C) for 82 radio tagged bull trout in the Morice River watershed. The first two movement calculations after tagging were omitted for each fish. Movement was calculated on a daily basis and averaged over the number of days between tracking flights. Temperature is plotted as the average of hourly temperatures for 14 days prior to each tracking date to reflect duration between tracking flights as much as possible. Error bars represent standard error. Inset graph displays linear plotted data.



**Figure 3-3. Linear regression for bull trout (n=33) displaying change point date (date of onset of upstream migration to tributary habitat) against distance traveled from location on that date to the most upstream location in the tributary.**

**Table 3-1. Dates of entry and exit to tributary habitat in the Morice River watershed by radio-tagged bull trout in 2000 and 2001.**

Tributary	2000			2001		
	Beginning of Entry	Latest entry	Exit	Beginning of Entry	Latest entry	Exit
Gosnell Creek	Jun. 4-17	Aug. 11	Aug. 26-Sept. 23	Jun. 10-Jun. 28	Aug. 6	Sept. 6-24
Thautil River	Jun. 17-26	Sept. 2	Sept. 8-23	Apr. 30-Jun. 3	Aug. 21	Sept. 23
Redslide Creek	n/a	n/a	n/a	Jul. 17-Aug. 31	Aug. 31	Sept. 15-Oct. 3

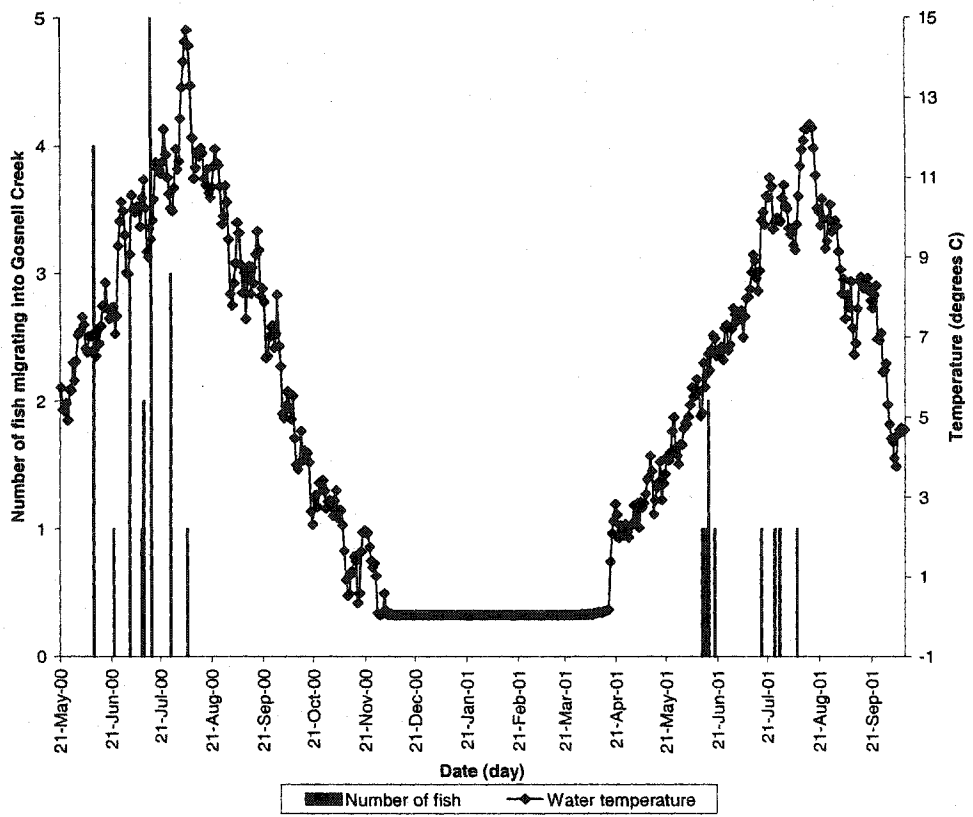


Figure 3-4. Plot of water temperature in Gosnell Creek to dates bull trout migrated into Gosnell Creek in 2000 and 2001.

**Table 3-2. Details of Pearson correlations of water temperature with entrance date to tributary, including year, correlation coefficient, significance and number of bull trout.**

Tributary	Year	Correlation coefficient (r)	P	Number of bull trout
Gosnell Creek	2000	0.876	0.000	20
Gosnell Creek	2001	0.956	0.000	10
Thautil River	2000	0.675	0.023	11
Thautil River	2001	0.969	0.031	4
Houston Tommy Creek	2000	1.000	0.000	3

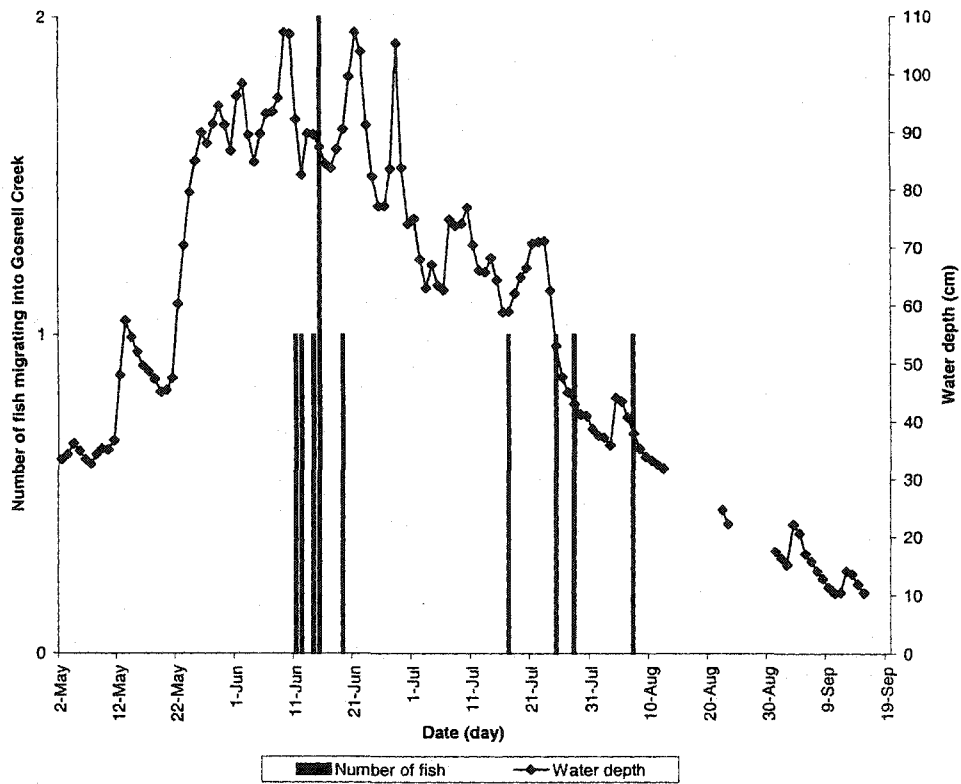
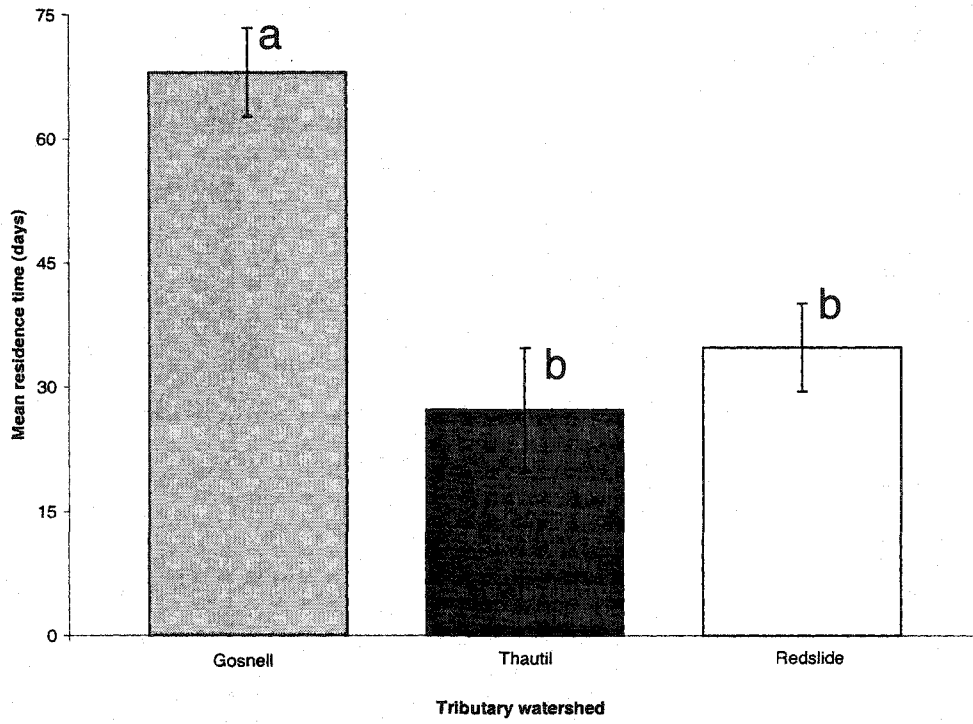


Figure 3-5. Plot of Gosnell Creek water depth from May 2-September 15, 2001 to dates bull trout entered Gosnell Creek during upstream migration.



**Figure 3-6. Mean residence time (days) of bull trout in tributary watersheds of the Morice River system during spawning season. Error bars represent one standard error of the mean. Values with a common letter do not differ significantly.**



## ***Discussion***

The overall pattern of movement by bull trout observed in the Morice River watershed is comparable to other populations of bull trout (Ford et al. 1995; McPhail and Baxter 1996). Movement rates were low during the winter, increased over the summer months and maximum movement rates occurred in the spawning season. The increase in movement rates during the summer may be related to a number of factors including competition, increased foraging opportunities, habitat selection, or temperature. Bull trout are known to be aggressive piscivores (Ford et al. 1995) that grow well under conditions with abundant food (Swanberg 1997a) and temperatures less than 15°C (Rieman and McIntyre 1993). Additionally, bull trout in the Blackfoot River, Montana used habitat of coldwater confluences as a thermal refuge when mainstem temperatures were unfavourable (Swanberg 1997a).

Average movement rates per day increased to their highest levels during August and September, which corresponded to the spawning migration. These movements were primarily associated with migration up tributaries, and there was no evidence of spawning in the mainstem Morice or Nanika rivers. Fluvial bull trout are known to migrate from large rivers into smaller rivers to spawn, and these migrations generally occur in late summer when water temperatures are high and water levels are low (McPhail and Baxter 1996). Timing of migrations, ground surveys of locations of redds, and condition of recaptured fish during late summer indicated that bull trout were moving to and from spawning locations in the Morice system.

Bull trout utilized the same locations in each of the five tributaries in the Morice River watershed for spawning in both study years. Use of the same spawning sites in

multiple years has also been observed in other watersheds despite the availability of what appears to be suitable alternate spawning habitat (Baxter and McPhail 1999; Hvenegaard and Thera 2001). Bull trout spent the longest amount of time in Gosnell Creek; this may be due to a combination of factors such as an abundance of food (eggs or juveniles of salmonids known to spawn in Gosnell Creek; Bustard 1999), habitat availability, or temperature. Differences in discharge likely do not account for differences in residence time in the Morice River watershed, since discharge in Gosnell Creek was similar to that in Thautil River and bull trout spent less time in Thautil River watershed, an area that has different habitat features such as fewer holding pools and less large woody debris. Habitat quality was similar in Redslide Creek watershed to Thautil River watershed, and bull trout using this tributary also had a significantly lower residence time than those in Gosnell Creek.

Onset of migration was asynchronous and occurred gradually over a wide time interval. The change point test indicated that migration began at different dates for individual fish. Migration timing to spawning areas in the Morice River watershed was similar to migration timing of bull trout in the Flathead River, northwest Montana (Fraley and Shepard 1989), Rapid River, Idaho (Schill et al. 1994) and the Blackfoot River, western Montana (Swanberg 1997a). Swanberg (1997a) and Schill et al. (1994) showed that bull trout began upstream migrations in June and July. Fraley and Shepard (1989) observed most bull trout to enter tributary streams in August. I did find, however, that the date for change in rate of movement was correlated to distance fish were from their spawning locations. Consequently, fish that were further away from the spawning locations began migrations earlier. Additionally, three bull trout returned to the same spawning locations in both years. These findings suggest that bull trout are homing to specific regions in the watershed.

Entrance to tributaries by bull trout occurred up to three months in advance of the spawning window, and a cue for migration may be physical factors such as temperature and discharge. Entrance timing to Gosnell Creek by bull trout was positively correlated with water temperature and negatively correlated to water depth. The majority of bull trout moving past a weir in Rapid River, Idaho did so through the period of time when temperatures were rising in late May through June (Schill et al. 1994). Swanberg (1997a) reported that most bull trout from the Blackfoot River, Montana began migrations during peaks in temperature and during declines in discharge. In the North Fork Skokomish River, Washington, however, spawning migration was correlated with increased river discharge (Brenkman et al. 2001). Although bull trout in the Morice system show similarities to some bull trout in other watersheds by demonstrating a general migratory pattern related to increasing temperature and decreasing discharge, it seems that populations of bull trout may have migratory patterns that are locally adapted to their watersheds (Rieman and McIntyre 1993; Brenkman et al. 2001; Haas and McPhail 2001) or they may not use temperature or discharge as cues for migration.

Bull trout in the Morice River watershed spawned from late August to late September similar to bull trout in other watersheds (Fraley and Shepard 1989; Kitano et al. 1994; Swanberg 1997a, 1997b; Jakober et al. 1998; Baxter and McPhail 1999). Gosnell/Thautil spawners had completed most spawning activity by September 15; however, the spawning activity in Redslide Creek extended into late September. Fraley and Shepard (1989) observed that spawning began when water temperatures dropped below 9°C in the Flathead River system, Montana, and Schill et al. (1994) observed pairing behaviour of bull trout when average water temperature dropped from 10 to 6.5°C in Rapid River, Idaho. Water

temperatures in Gosnell and Denys creeks were decreasing below 8°C when bull trout were spawning, following a similar pattern to bull trout in these other watersheds.

Out-migration from spawning areas was much quicker than in-migration, and was complete in less than one month, with most downstream migrations lasting between three and seven days. These findings are similar to the bull trout monitored by fixed station at the confluence of the Chowade and Halfway rivers; bull trout remained 35 times as long within reception of the receiver during their upstream movements than when moving downstream (Burrows et al. 2001). Risk of predation associated with spending time in smaller tributary streams has been identified by others (see Schill et al. 1994; McPhail and Baxter 1996; Chandler et al. 2001) and was also likely in the Morice River watershed. Twenty-five of sixty (41.7%) bull trout that migrated up tributaries during this study did not migrate back downstream, and their transmitters were retrieved or were unrecoverable in tributary habitat. Only one partial carcass of a bull trout was found, and the location of recovered transmitters indicated that fish were predated and likely scavenged. Transmitters were recovered from animal trails, under brush on the banks, on gravel bars and under log jams. As well, some transmitters also had teeth marks on them. Bull trout that did migrate downstream from the spawning area did so quickly, perhaps to minimize risk of predation or to quickly move to better feeding areas prior to overwintering. A large proportion of bull trout that migrated successfully out of tributary habitat returned to the upper Nanika and upper Morice rivers where chinook and sockeye salmon were actively spawning. Snorkel surveys documented bull trout located individually downstream of other salmonids redds.

Migration timing of bull trout in the Morice River watershed appears similar to observations in other watersheds (Fraley and Shepard 1989; Kitano et al. 1994; Swanberg

1997a, 1997b; Jakober et al. 1998; Baxter and McPhail 1999); however, small differences may be related to adaptations to local environmental conditions (Brenkman et al. 2001), particularly temperature and discharge. The overall migratory pattern includes the use of a range of habitat; tributary habitat for spawning and mainstem habitat for overwintering and rearing during other times of the year. Bull trout in the Morice system demonstrate that specific habitat is necessary for each part of the life cycle. The diversity in use of these habitats must be incorporated into conservation plans to effectively manage populations of bull trout (Swanberg 1997a).

**Chapter 4 : Determination of population structure for bull trout (*Salvelinus confluentus*) in the Morice River watershed using two methods: microsatellite analysis vs. radio telemetry data.**

## **Abstract**

Population structure of bull trout (*Salvelinus confluentus*) within the Morice River watershed, northwestern British Columbia was assessed and compared using two methods: first, fish were radio tagged and tracked over approximately two years and second, genetic analysis of population structure was determined from eight polymorphic microsatellite loci. Based on spatial location and distance moved per day, radio tagged fish were assigned to six clusters. The analysis of microsatellite data indicated low numbers of alleles at all loci. Using the software program *STRUCTURE*, three clades were evident. The three *STRUCTURE* clades, however, did not group together on an unrooted neighbour joining tree. Additionally, direct correspondence between the spatial clusters defined by the radio telemetry data and the genetic clades were not evident. Comparison of the clusters and clades indicated that genetic clades were composed of individuals that spawned in numerous tributaries throughout the watershed. Although genetic subpopulations were evident in the Morice River watershed, there is gene flow among them. Due to the weak population structure seen in the Morice River watershed, it should be viewed as a panmictic breeding population. Consequently, the results of this study indicate that bull trout in the Morice River watershed should be managed at the watershed level.

## ***Introduction***

Bull trout are char native to the Pacific northwest that originally ranged south to approximately 41°N; however, most of the southern populations are extinct and they presently extend south to approximately 42°N latitude (Haas and McPhail 1991). Numbers of bull trout have been declining over much of their geographic range due to a combination of factors including habitat degradation through logging and road construction, poor fisheries management practices, introduction of non-native salmonids, (Fraley and Shepard 1989; Donald and Alger 1993; Williams et al. 1997; Baxter et al. 1999; Kanda and Allendorf 2001), and obstruction to migratory corridors by hydroelectric dams (Swanberg 1997b). To ensure effective conservation of remaining populations of bull trout, it is necessary to understand their population structure. The Morice River watershed provides an opportunity to examine population structure of bull trout since they have previously been identified to spawn in a number of discrete regions in this watershed (Bustard 1997, 1999; Chapter 2).

There is evidence that bull trout show spawning site fidelity (Schill et al. 1994; Baxter and McPhail 1996; Hvenegaard and Thera 2001, Chapter 2). Bull trout that segregate by spawning area may be genetically distinct and therefore may represent more than one stock. Conservation management of this species, however, may incur loss of genetic diversity due to treatment of multiple reproductively isolated populations as one breeding unit (Spruell et al. 1999). Genetic differentiation among populations may have evolved through adaptation to local environments, therefore treatment of multiple populations as one breeding unit may cause loss of genetic variation and disrupt adaptation to local environments (Kanda and Allendorf 2001). Low levels of genetic variation are generally considered to reduce the ability of a species to respond to threats of disease, predators,



parasites or environmental change (Amos and Harwood 1998). As a result, conservation efforts often focus on preserving stock structure in an effort to conserve genetic diversity.

In Chapter 2, I showed that bull trout in the Morice River watershed separated into distinct groups based on spatial location/movement data from radio telemetry. This analysis strongly suggests multiple breeding populations within the Morice system. Homing and patterns of movement have been used previously to identify population structure of stocks (Larkin 1972; Behnke 1993). An alternate approach is to use genetic markers to identify stock structure. Population structure can be identified by studying variation at microsatellite loci; use of this approach has increased in recent years (Wright and Bentzen 1994; Angers et al. 1995). Microsatellites are short sections of DNA (tens to hundreds of base pairs), composed of tandem nucleotide repeats usually less than five base pairs in length (Bruford and Wayne 1993; Wright 1993; Wright and Bentzen 1994; Scribner et al. 1996; Angers and Bernatchez 1997; Hancock 1999). The use of microsatellites as genetic markers has been increasing because they are very abundant in almost all eukaryote organisms (Bruford and Wayne 1993; Wright and Bentzen 1994; Jarne and Lagoda 1996; Dimsoski and Toth 2001), are generally highly polymorphic (Bruford and Wayne 1993; Wright 1993; Wright and Bentzen 1994; Jarne and Lagoda 1996; Olsen et al. 1996; Scribner et al. 1996; Wenburg et al. 1996; Nelson et al. 1998; Dimsoski and Toth 2001), and are assayed using polymerase chain reaction (PCR) which requires only small amounts of DNA.

The objective of this chapter was to examine if population structure of bull trout in the Morice River watershed corresponded to movement and spawning locations identified from radio telemetry. To accomplish this, I examined whether genetically distinct subpopulations of bull trout exist within the Morice River watershed, and if those

subpopulations use geographically distinct spawning areas. Genetic clades resulting from the analysis using eight polymorphic microsatellite loci and 67 radio tagged bull trout were compared to clusters of the same individuals generated by a hierarchical cluster analysis of their average spatial location and average distance moved per day.

Although researchers have previously described movement patterns of radio tagged bull trout (Schill et al. 1994; Swanberg 1997a, 1997b; O'Brien 1999; Wilcox 1999; O'Brien and Zimmerman 2000) and genetic population structure using microsatellite loci (Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001; Taylor et al. 2001), I am unaware of any studies that have compared geographical separation of spawning sites and genetic population structure of radio tagged individuals in a single watershed. The results of my analysis provide valuable information on population structure for bull trout in the Morice River watershed and compare two methods used previously to identify stocks in fish populations.

## **Methods**

### ***Study Location and Samples***

A detailed description of the study area is given in Chapter 1. Bull trout were captured by angling in the upper Nanika River, Morice Lake, Gosnell Creek, and all sections of the Morice River during 2000-2001. Adipose fin tissue was collected from individuals and stored in 95% ethanol.

### ***Genetic Analysis***

DNA was extracted by following a modified proteinase K digestion procedure. Adipose tissue was digested overnight at 37 °C by gentle rocking in 200  $\mu$ L of solution of proteinase K buffer (composed of 10 mM Tris (pH 8.0), 10 mM ethylenediamine-tetraacetic acid (EDTA), and 0.5% sodium dodecylsulphate (SDS)) and proteinase K (50  $\mu$ g/mL). Digested samples were extracted twice with phenol:chloroform:isoamyl alcohol (24:24:1) and precipitated using sodium acetate (2.5M NaOAc (pH 5.5)) and isopropanol. Precipitated DNA was washed with 70% ethanol, dried and redissolved in 100  $\mu$ L of TE (10mM Tris, 1mM EDTA (pH 8.0)) or distilled water and kept as a stock solution.

To examine variation at microsatellite loci (Table 4-1), diluted DNA (1 mL:10 mL distilled water) was used in polymerase chain reactions (PCR) that were run through a fragment analysis procedure on a VisGen automated sequencer. PCRs were run using reactions consisting of 10.95  $\mu$ L double-distilled water, 0.05  $\mu$ L Taq polymerase (Gibco-BRL), 1.5  $\mu$ L of 10x reaction buffer (Gibco-BRL), 0.9  $\mu$ L magnesium chloride (25 mM), 0.3  $\mu$ L dNTPs (10 mM each dNTP), 0.3  $\mu$ L reverse primer (100 ng/ $\mu$ L), 0.2  $\mu$ L forward primer (100 ng/ $\mu$ L) and 0.8-1.1  $\mu$ L diluted DNA. The forward primer was dye-labeled for all

primers except One $\mu$  18. Fragment analysis with 101, 166, 200 and 351 bp size standards allowed size determination of amplified dye-labeled microsatellite fragments which were rounded to the nearest whole repeat number.

### *Statistical analysis*

Of the 10 microsatellite loci amplified, two were excluded from analysis. Ogo2 was fixed at allele 209 in greater than 98% of the samples and was therefore eliminated from the statistical analysis. Amplification was low for the Sco19 locus; nine of 67 individuals did not amplify, and there was a large deficiency of heterozygotes (observed heterozygosity was 0.38 and expected heterozygosity was 0.73). Beaumont and Bruford (1999) suggest that certain alleles will not amplify due to insertions, deletions, or substitutions within priming sites leading to the appearance of null alleles. As well, heterozygous individuals may be mistyped as homozygotes if there is non-inheritance of parental alleles in some offspring (Bruford and Wayne 1993). Although Sco19 has been used successfully and results have conformed to Hardy-Weinberg equilibrium in other populations of bull trout (see Spruell et al. 1999; Neraas and Spruell 2001; Taylor et al. 2001), those populations may have been unaffected by the presence of the null allele, even though it may be prevalent in the Morice River population of bull trout. As a result, Sco19 was eliminated from the analysis.

Heterozygosity was calculated as an estimate of genetic variation using *Tools for Population Genetic Analyses* (TFPGA 1.3) software by Mark Miller (Biology Department; Arizona State University, PO Box 5640, Flagstaff, AZ 86011-5640, USA). An exact test for Hardy-Weinberg equilibrium using 20 000 permutations was calculated (using TFPGA) at each locus and corrected for multiple simultaneous comparisons using the sequential Bonferonni correction (Rice 1989).

The software program *STRUCTURE* was used to analyse the microsatellite data for population structure (Pritchard et al. 2000). This program uses a Bayesian clustering approach that includes a model for identifying subpopulations and assigning individuals probabilistically to these populations. It attempts to assign individuals to populations based on their genotypes while estimating population allele frequencies at the same time. It assumes that markers used are unlinked and at linkage equilibrium with one another within populations. Populations are also considered in Hardy-Weinberg equilibrium (Pritchard et al. 2000). For this reason, *STRUCTURE* was run twice; once with all eight primers, and once with the seven primers that were in Hardy-Weinberg equilibrium.

Estimating the number of populations ( $K$ ) was done by an ad hoc approximation to the Bayesian paradigm of placing a prior distribution on  $K$  and base inference for  $K$  on the posterior distribution. This approach is suggested by Pritchard et al. (2000) and has given reasonable results in practice. Estimations of  $K$  were made by running *STRUCTURE* for 100 000 steps, with a *burnin* of 50 000 steps for each of the values of  $k$  between 1 and 7. Once  $K$  was decided, *STRUCTURE* was run 100 times for 1 million steps with a *burnin* of 50 000 steps. In every run, *STRUCTURE* estimated the fraction of each individual's genome that belonged to each of five possible clusters. Individuals were placed after each run into the cluster with the highest fraction of its genome. The number of times individuals were placed in the same cluster was tallied for the 100 runs; individuals were assigned to the cluster into which they fell the most times during the 100 replications.

An unrooted neighbour-joining cluster analysis using Cavalli-Sforza & Edwards (1967) chord distance was performed using *Populations Version 1.2.24* (O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Genetique et Evolution, Gif

sur Yvettev; <http://www.cnrs-gif.fr/pge/bioinfo/populations>) and *Treeview* (Page 1996) software.

## Results

All eight loci examined were polymorphic (2-13 alleles) for the bull trout used in this analysis from the Morice River watershed (Table 4-2). After Bonferroni correction, Sfo23 was the only locus that showed significant deviation from Hardy-Weinberg equilibrium as fewer heterozygotes were observed than expected (Table 4-2).

The ad-hoc approximation used by *STRUCTURE* to estimate the number of populations in the radio-tagged sample yielded a K value of three. This value was used during all structure runs and radio tagged bull trout were assigned to Clades A, B, or C. Assignment to clades varied considerably for some of the fish during the 100 repeated runs of *STRUCTURE*; individual assignment to the same clade ranged from 52 to 98 times. Clade A was the only group in which individuals were assigned 95 or more of the trials. Twenty-one fish using seven loci, and four fish using eight loci were assigned to Clade A 95 or more times out of the 100 trials. Overall, the results differed slightly depending on whether *STRUCTURE* was run using the microsatellite data from seven or eight loci; six individuals changed assignment between Clades A and B when the results were compared (Figure 4-1). Four of those individuals belonged to the Thautil/Gosnell spatial group, one belonged to the middle Morice group and one belonged to the upper Morice group.

In Chapter 2, I showed a hierarchical cluster analysis based on average spatial location and average distance moved per day that revealed six clusters of bull trout. These clusters moved variable distances within the watershed and were composed of five potential spatially distinct populations. The results of this analysis are shown in Figure 4-2, overlaid with the three genetic clades determined by *STRUCTURE* (indicated as A, B, or C) for eight loci. The results from *STRUCTURE* do not directly correlate with the clusters, and there is

considerable overlap of the three genetic clades with the six clusters identified using the radio telemetry data (Figure 4-3). Genetic clades were composed of bull trout from a minimum of three to a maximum of six clusters. As well, each clade was composed of individuals that showed a range of average movement per day (Figure 4-4). Two patterns were evident from this analysis. First, Clade A was the dominant genetic clade found in the Nanika/Redslide group and the presence of this group decreased in abundance in the lower reaches of the Morice River watershed. Second, fish found below the outlet of Morice Lake were most abundant in genetic clade B. Genetic Clade C contains only three individuals and little pattern existed; two fish were from the Thautil/Gosnell group and the third belonged to the Nanika/Redslide group. The first two fish migrated to Denys Creek to spawn, and the third fish remained in the Nanika River mainstem during both years.

Radio-tagged individuals are displayed on an unrooted neighbour joining tree (Figure 4-5). Each individual was labeled with the letter of the genetic cluster designated by *STRUCTURE*. The figure shows that the genetic clusters were mixed, and there was no evidence of distinct genetic groups.



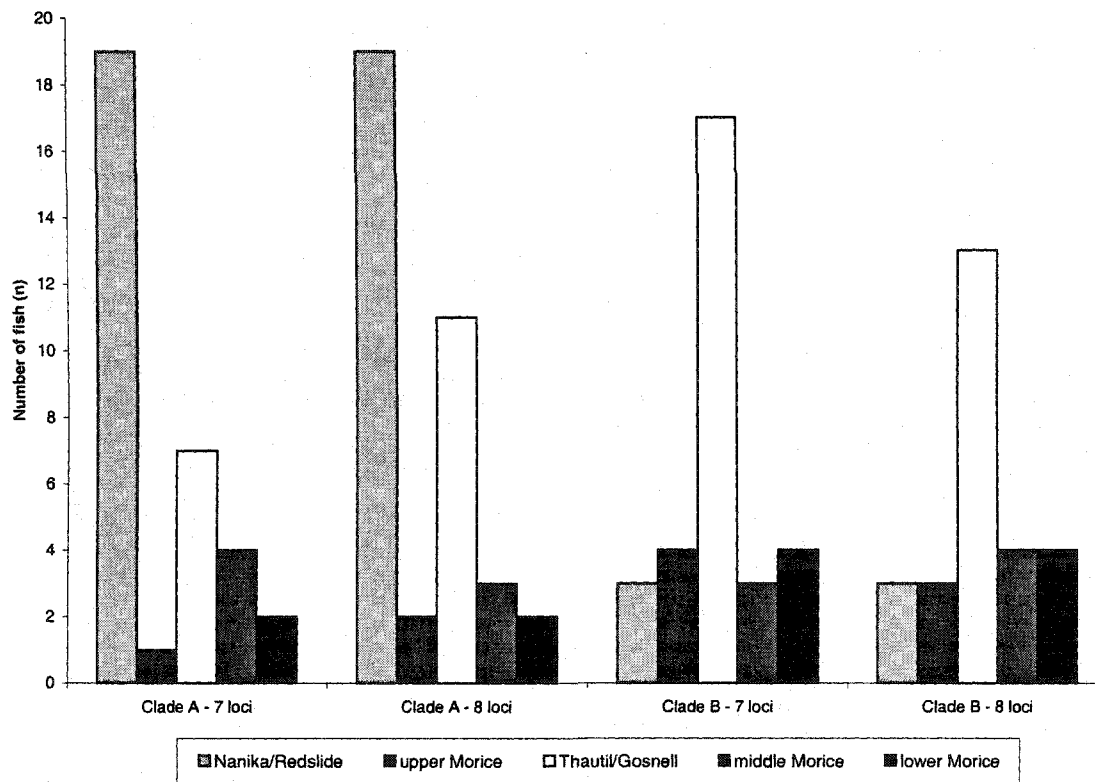
**Table 4-1. Molecular size range (base pairs), sequence, annealing temperatures and reference for primers used to analyse bull trout in the Morice River watershed.**

Locus	Sequence	Allele range (bp)	Annealing temp. (°C)	Reference
<i>μSat15</i>	F: TGCAGGCAGACGGATCAGGC R: AATCCTCTACGTAAGGGATTTGC	177-257	50	Estoup et al. 1993
<i>μSat60</i>	F: CGGTGTGCTTGTTCAGGTTTC R: GTCAAGTCAGCAAGCCTCAC	174-276	50	Estoup et al. 1993
<i>Fgt3</i>	F: CAAGAAATTTGTGGAGCGG R: GAAGCCCTGTTTGACTTTTAGC	139-347	50	Sakamoto et al. 1994
<i>Ogo2</i>	F: ACATCGCACACCATAAGCAT R: GTTTCTTCGACTGTTTCCTCTGTGTTGAG	145-209	58	Olsen et al. 1998
<i>Oneμ10</i>	F: ATGGGGAACAGAAGAGGAAT R: CTGTAGGTGTGAAATGTATTTAAA	132-159	50	Scribner et al. 1996
<i>Oneμ18</i>	F: ATGGCTGCATCTAATGGAGAGTAA R: AAACCACACACACTGTACGCCAA	132-211	50	Scribner et al. 1996
<i>Sco19</i>	F: CTTGAAATTAGTTAAACAGC R: CCAAAC TACCAATAATC	103-289	50*	Taylor et al. 2001
<i>Sfo23</i>	F: GTGTTCTTTTCTCAGCCC R: AATGAGCGTTACGAGAGG	187-299	50*	Angers et al. 1995
<i>SfoD75</i>	unpublished	174-343	58	TL King, personal communication
<i>SfoD105</i>	unpublished	125-231	58	TL King, personal communication

\*Optimized conditions included a touchdown PCR protocol: Use an annealing temperature of 54°C; drop 1°C for four cycles prior to using listed annealing temperature

**Table 4-2. Microsatellite loci, sample size (N), number of alleles (A), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and significance value (p) found in bull trout in the Morice River watershed. \* indicates that the observed value deviates significantly from the expected value of Hardy-Weinberg Equilibrium after Bonferonni correction.**

Locus	N	A	$H_o$	$H_e$	p
<i>μSat15</i>	67	7	0.54	0.44	0.01
<i>μSat60</i>	45	11	0.69	0.79	0.11
<i>Fgt3</i>	66	2	0.05	0.07	0.08
<i>Oneu10</i>	67	4	0.06	0.06	1.00
<i>Oneu18</i>	67	3	0.49	0.38	0.03
<i>Sfo23</i>	59	13	0.61*	0.77	0.00
<i>SfoD75</i>	56	11	0.91	0.85	0.01
<i>SfoD105</i>	67	6	0.37	0.38	0.23



**Figure 4-1. Difference in spatial group composition of genetic clades A and B compared to groups using seven or eight loci. Clade C did not differ using 7 or 8 loci.**

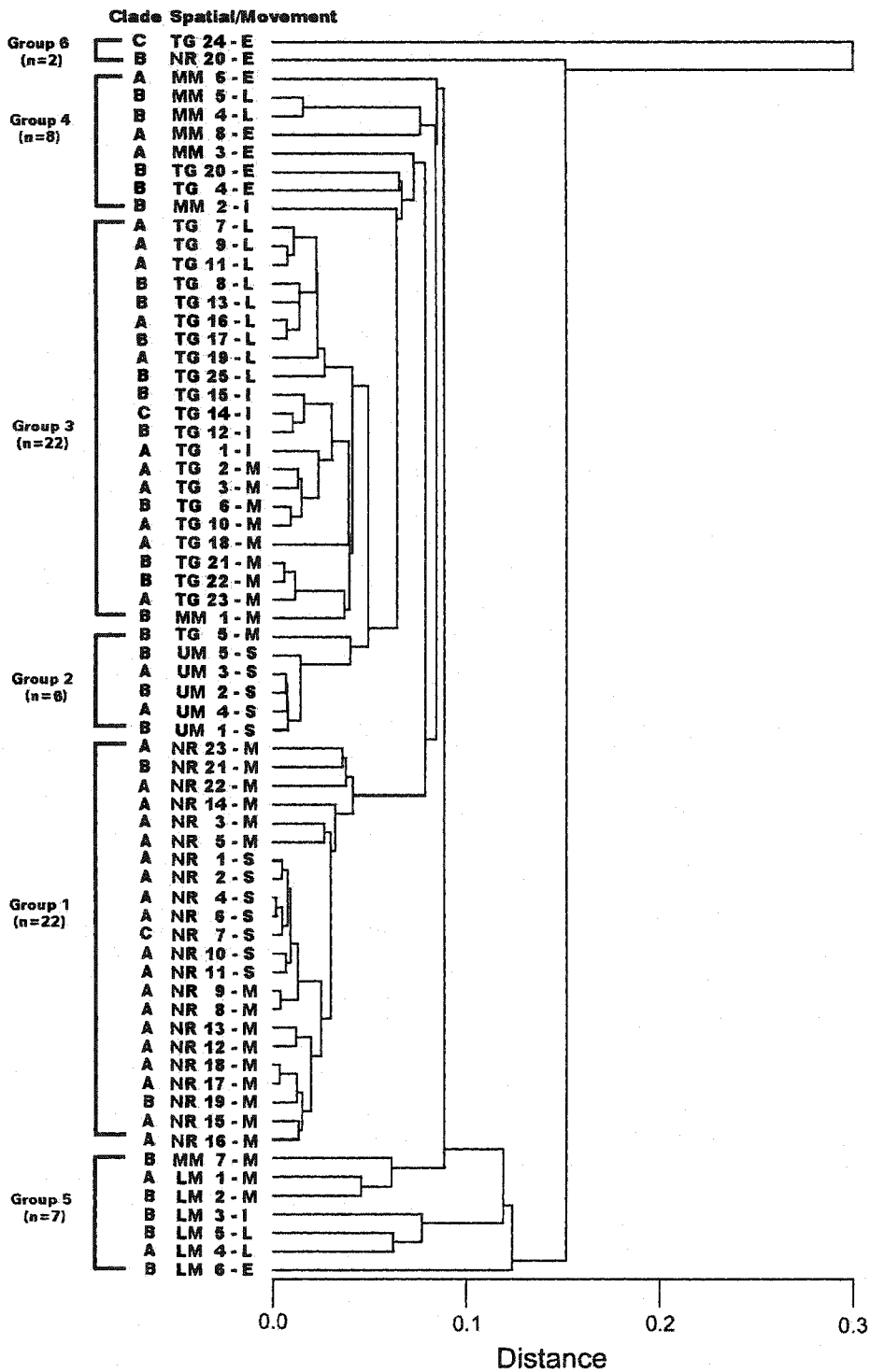
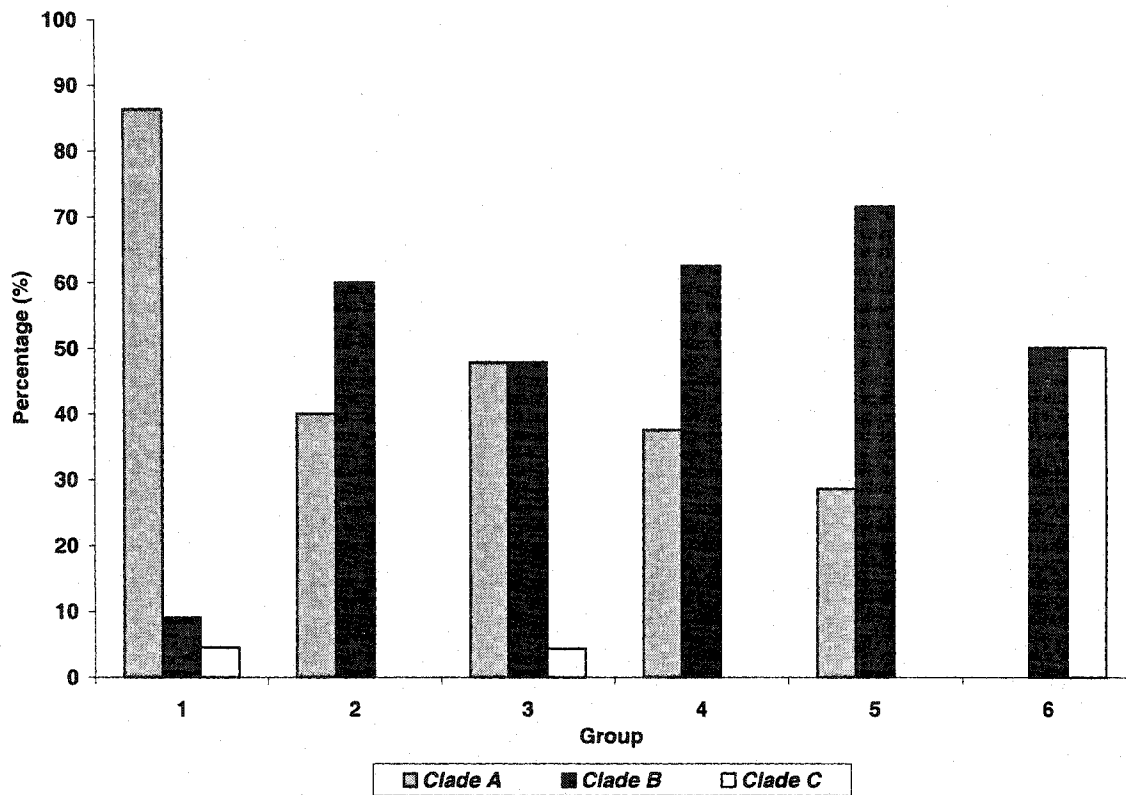


Figure 4-2. Hierarchical cluster analysis of spatial location of radio tagged bull trout overlaid with genetic clusters A, B, and C determined from genetic population analysis using *STRUCTURE* software. NR indicates Nanika/Redslide, UM indicates upper Morice, TG indicates Thaultil/Gosnell, MM indicates middle Morice and LM indicates lower Morice. Average movement per day is indicated by S for Small, M for Moderate, I for Intermediate, L for Large and E for Extensive.



**Figure 4-3. Genetic composition of groups of bull trout based on a hierarchical cluster analysis of average spatial location and average distance moved per day. Groups are identified in Figure 4-2.**

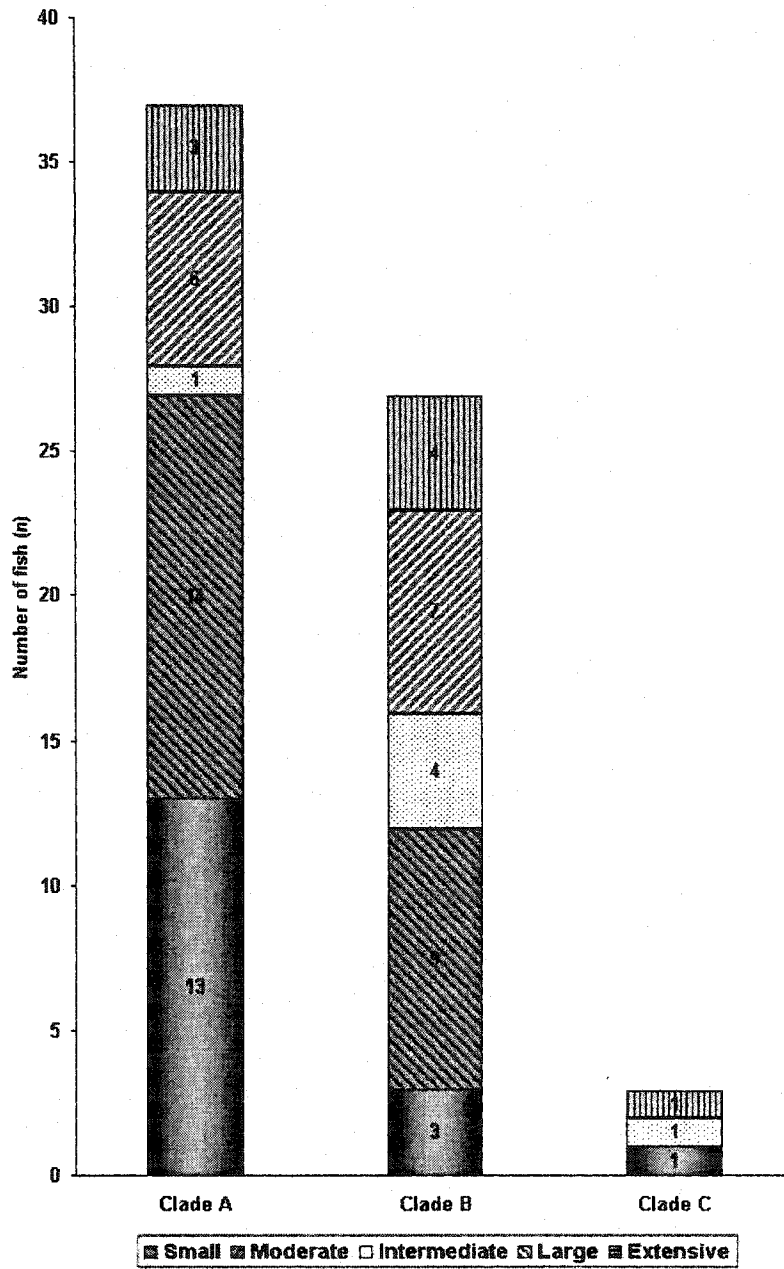
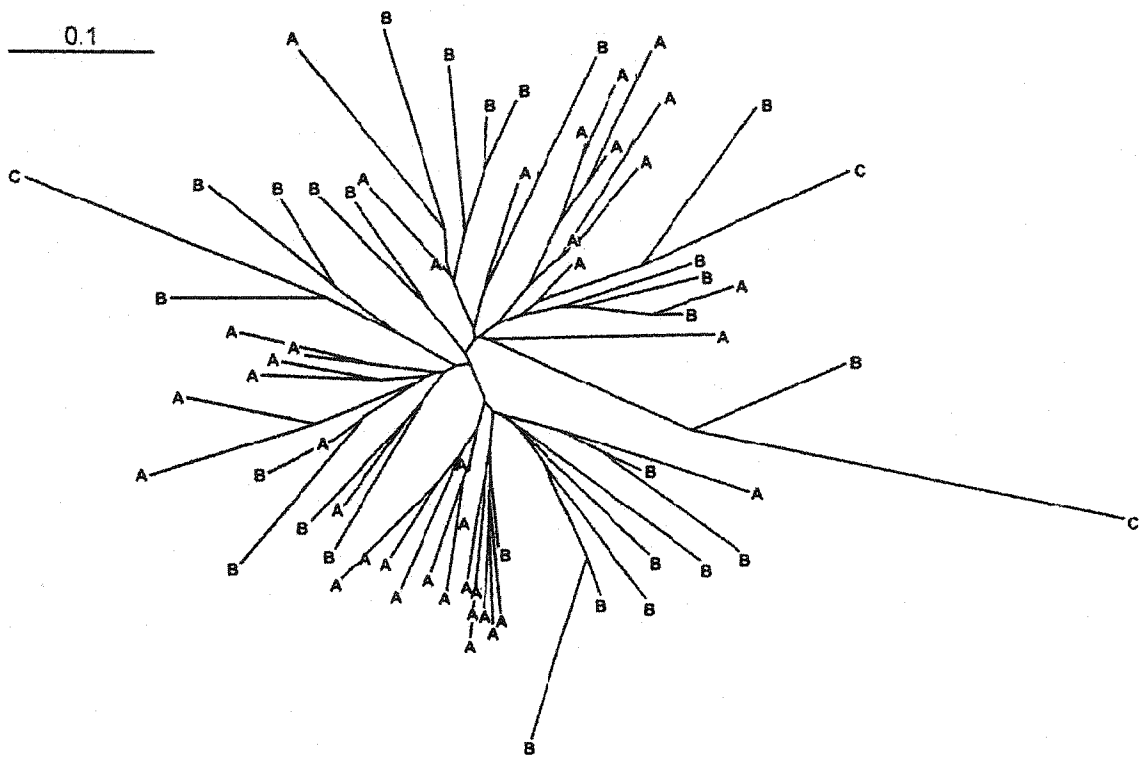


Figure 4-4. Composition of genetic clades based on average movement per day of radio tagged bull trout.



**Figure 4-5. Unrooted neighbour joining tree of radio tagged bull trout using 8 microsatellite markers and Cavalli-Sforza genetic distance. Each bull trout is represented by the genetic clade (A, B, or C) it was assigned to using *STRUCTURE*.**

## **Discussion**

Analysis of spatial and movement data (Chapter 2) suggests that bull trout within the Morice River watershed can be split into six groups. Bull trout in three of these groups (Thautil/Gosnell, Nanika/Redslide, lower Morice) migrated to the five main spawning areas identified in the watershed; Gosnell Creek, Denys Creek in the Thautil River watershed, Redslide Creek, Houston Tommy Creek and Gold Creek. The potential for a high level of spawning site fidelity in bull trout (Schill et al. 1994; Baxter and McPhail 1996; Hvenegaard and Thera 2001) and strong homing tendency in salmonids in general (Larkin 1972; Behnke 1993) suggests that identification of discrete spawning grounds and knowledge of fish movements should be a useful tool to discriminate stocks. The genetic clades resulting from the statistical analysis using *STRUCTURE*, however, were not uniquely related to geographical spawning locations. Genetic clades were composed of individuals that spawned in many different locations within the watershed and individuals from each genetic clade were not grouped together on the neighbour joining tree. This suggests that there is weak subpopulation structure within the Morice River watershed and that gene flow exists among these subpopulations.

Distribution of bull trout among watersheds and river basins represents spatial separation that may lead to genetic difference (Rieman and McIntyre 1993) and radio telemetry is a useful tool to investigate spatial distribution (Winter 1983; Ward and Miller 1988). Genetic discrimination among fish at the population level is also a powerful tool that can resolve issues of stock identification (Wirgin and Waldman 1994). Knowledge of stock structure allows fisheries managers to restrict harvest levels to protect the weakest stock, and genetic markers may distinguish individuals from different stocks for monitoring of variables



such as fecundity and age structure (Wirgin and Waldman 1994). Leary et al. (1993) found large genetic divergence among populations from different streams, indicating that reproductive isolation would allow for evolution of local adaptations. Conservation efforts to protect stocks can then be focused on protection of various spawning locations.

Evidence for distinct populations of bull trout was previously provided by Spruell et al. (1999) in a small geographic area (570 km<sup>2</sup>). Although the Morice River drainage is considerably larger than the Lightning Creek drainage studied by Spruell et al. (1999), there appears to be only weak levels of population structure in the Morice River watershed with three clades evident from the genetic analysis. Clade A was dominant in the headwaters of the system, particularly the Nanika/Redslide area. Clade B was found primarily downstream of Morice Lake and encompassed fish that used all extents of the watershed. Although the comparison of telemetry and genetic results suggests a lack of correspondence between the radio telemetry and genetic data, there is some commonality in the two approaches. For example, many of the Nanika fish did not make long migrations and never moved below the mouth of the Nanika River. The Nanika River bull trout were dominated by Clade A; however, telemetry data also suggests that mixing occurs. Some bull trout were found to migrate between the Nanika, Morice and tributary drainages. Clades resulting from the genetic analysis confirm this mixing, and the gene flow between clades parallels the movement among tributary watersheds observed in the telemetry data. Gene flow among clades is also evident in the unrooted neighbour joining tree. Clades did not exclusively group together on the tree, also emphasizing the mixing observed in the telemetry data. The weak population structure may be a result of the migration and mixing in the population, even though bull trout use geographically separated spawning areas.

Results of the genetic analysis indicate that the population of bull trout in the Morice system should be managed at a watershed level. The five areas important for spawning of bull trout represent a very small proportion of the available habitat in the watershed. Although the population should be managed at a watershed level, bull trout are known to be selective in their spawning locations and are sensitive to habitat disturbance (Rieman and McIntyre 1993; Baxter and McPhail 1996; Cross and Everest 1997; Baxter and McPhail 1999). These ecological considerations also need to be addressed in conservation plans. Anthropogenic changes such as road building and logging may have considerable impacts on the spawning areas that bull trout utilize in the Morice River watershed. As well, gene flow between Morice River fish and bull trout nearby in the Bulkley watershed may occur, but this is not known and the geographic scale of the breeding population is also unknown. Further genetic analysis of bull trout in other watersheds proximal to the Morice River watershed, therefore, would enhance our understanding of the population structure that exists in this area of northwestern British Columbia. This additional information could be used to effectively conserve populations of bull trout on a larger scale.

The number of alleles per microsatellite locus in the Morice River population was generally lower than that observed in other fish species. For example, others found variations of five to eight alleles at the FGT3 locus in comparison to the two alleles observed in the Morice population (Sakamoto et al. 1994; Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001). Ogo2 was eliminated from our analysis as it was fixed at one allele; however, Spruell et al. (1999) found four alleles in Lightning Creek bull trout and Olsen et al. (1998) found eight alleles in pink salmon (*Oncorhynchus gorbuscha*) at the same locus. Although the greatest number of alleles (13) was detected at the Sfo23 locus in bull

trout in the Morice River watershed, others found 16 in brook charr (*Salvelinus fontinalis*) and 49 in Arctic charr (*Salvelinus alpinus*) (Angers et al. 1995; Brunner et al. 1998). The low microsatellite variability observed in the Morice population is consistent with other studies of bull trout using isozyme loci and mitochondrial DNA (Leary et al. 1993; Taylor et al. 1999). Even though other studies found greater numbers of alleles at microsatellite loci than were found in the Morice River population of bull trout, low microsatellite variability is probably a characteristic of bull trout (Kanda and Allendorf 2001) and may suggest a historically small effective population size, or a low mutation rate (Taylor et al. 1999).

Many studies throughout the geographical range of bull trout indicate low levels of genetic variation within populations and higher levels of variation among populations (Leary et al. 1993; Kanda et al. 1997; Taylor et al. 1999; Kanda and Allendorf 2001; Taylor et al. 2001). However, Spruell et al. (1999) was able to identify five genetically distinct populations within the Lightning Creek drainage area, a small watershed, but found little correlation between geographic distance and genetic differentiation. Bull trout in the Lightning Creek drainage had more genetic differentiation than expected for populations connected by frequent migration (Spruell et al. 1999). The genetic distinctions evident among bull trout from geographically close populations indicate low levels of migration between populations (Hartl 1988; Hartl and Clark 1997) and hence little gene flow, therefore those populations of bull trout may adapt to their local environments over time (Kanda and Allendorf 2001).

Both Kanda and Allendorf (2001) and Spruell et al. (1999) found genetic difference in populations of bull trout that inhabit smaller geographical areas than the Morice River watershed. Genetic distinction could be expected in the Morice drainage due to the large

geographical separation of spawning areas. Migration has a homogenizing effect on the genetic structure (Hartl 1988; Hartl and Clark 1997) however, and bull trout are able to migrate throughout the Morice system since there are no barriers. In the upper Flathead and Lake Pend Oreille drainages, the construction of hydroelectric dams has restricted fish passage for 50 to 90 years (see Kanda et al. 1997; Neraas and Spruell 2001) and this may contribute to the genetic difference observed in these areas. In addition, the approach to genetic analysis of population structure was completely different between Spruell et al.'s (2001) study in the Flathead and Lake Pend Oreille drainages and my study in the Morice River watershed. Spruell et al. (2001) identified populations of fish based on five sample locations and then tested differences between them using an exact probability of population differentiation and the likelihood ratio estimator of genetic divergence ( $D_{LR}$ ) from Paetkau et al. (1997). My approach did not categorize populations based on location of fish capture or any other variable prior to analysis, but used the program *STRUCTURE* to assign individuals to populations based on their genotypes. The difference in the two approaches may also contribute to the reason Spruell et al. (2001) found genetic structure in bull trout within a smaller watershed than the Morice River watershed.

Results from *STRUCTURE* suggest a panmictic population of bull trout in the Morice River watershed that contains weak subpopulation structure assigned to three clades. These clades do not directly correspond with the six spatial/movement clusters or the five geographical areas important for spawning (Redslide Creek, Gosnell Creek, Denys Creek, Houston Tommy Creek, and Gold Creek) within the watershed. If management was based on the results from the radio telemetry data, six distinct stocks would be present, which contradicts the genetic results. Some mixing was observed in the radio telemetry data when

bull trout migrated among geographically separated locations, however the amount was remarkably low in comparison to the mixing observed in the genetic clades. The analysis of the genetic data presents the most compelling evidence that bull trout in the Morice River watershed are one large population with weak subpopulation structure. Conservation management of this population should reflect the genetic evidence, but include maintenance of critical tributary habitat for spawning.

The apparent lack of correspondence between the genetic and radio telemetry data is likely due to a difference in temporal scale. Separation of groups by movement data may happen in a short time frame, perhaps even in a single generation. Additionally, anthropogenic change and natural events may impose change on geographical spawning locations within a season. Genetic separation of populations, however, may not reflect recent behavioural or ecological change. The lack of agreement between the two approaches emphasizes the complexity of the genetic/ecological relationship. Although the two approaches do not correspond, each approach increases the level of understanding of bull trout in the Morice River watershed and their integration provides knowledge that can be incorporated into specific management recommendations to conserve the population of bull trout.

## **Chapter 5 : Epilogue**

Homing and patterns of movement have been used previously to identify population structure of stocks (Larkin 1972; Behnke 1993); however, an alternate approach to identify population structure is to use genetic markers. My analysis of genetic variation at eight polymorphic loci using the program *STRUCTURE*, included a model for identifying subpopulations and assigning individuals probabilistically to these populations. Three weak subpopulations or clades were evident from the analysis and were mixed on an unrooted neighbour joining tree. These findings are in contrast to those of Spruell et al. (1999) who found five significantly differentiated populations within a small watershed (570 km<sup>2</sup>) and to those of Neraas and Spruell (2001) who found genetically distinct populations above and below a hydroelectric dam in the Clark Fork River system, Montana/Idaho.

When compared to the radio telemetry data, there was considerable overlap of the genetic clades with the five spatially located clusters in the Morice River watershed. These results suggested that gene flow existed between the clades. The commonality between the genetic and radio telemetry approaches was the mixing observed in each; some bull trout migrated to various mainstem locations and more than one tributary location within the watershed, while others remained in a small part of the watershed. The clades resulting from the genetic analysis paralleled the movement among tributary watersheds observed in the telemetry data, although the genetic analysis suggested much greater gene movement than was inferred from the radio telemetry data. The level of mixing evident in the Morice population was higher than that observed in the Lightning Creek drainage (Spruell et al. 1999) or the Clark Fork drainage (Neraas and Spruell 2001), and may be linked to the migratory patterns in these watersheds. The Morice River system has no barriers to fish migration; however, the systems described in Montana and Idaho both have hydroelectric

dams that prevent fish passage (Spruell et al. 1999; Neraas and Spruell 2001). Since migration has a homogenizing effect on the genetic population structure (Hartl 1988; Hartl and Clark 1997), populations with migratory barriers may show increased levels of genetic differentiation over time. Owing to the high level of mixing observed in the Morice River watershed, the genetic analysis suggested a panmictic population of bull trout that should be managed on a watershed level. Panmictic infers random mating, and therefore the population in the Morice system is a homogenous group.

The apparent lack of correlation between the genetic approach and the radio telemetry approach is likely due to a difference in scale as discussed in Chapter 4. Although the radio telemetry data did not correlate directly with the genetic clades, the lack of agreement between the two approaches emphasizes the complexity of the genetic/ecological relationship. For example, two life history forms of *O. nerka* (sockeye salmon and kokanee) spawn in close proximity but remain genetically distinct (Wood et al. 1999). Microsatellite analysis provides resolution for life history forms and stocks; both important considerations for fisheries management. However, limiting analysis only to a genetic approach does not reveal where critical habitat is located for spawning, rearing or overwintering. The integration of knowledge from both approaches provides a comprehensive overview of the population dynamics of the Morice River watershed population of bull trout and can facilitate informed management decisions for conservation.

It is my recommendation that bull trout in the Morice River system be managed at the watershed level. This means that all habitats are important for viability of this population. My reasons for this are that genetic diversity appears low within this population. The number of alleles per microsatellite locus in the Morice River population was generally lower



than that observed in other fish species (ie. Sakamoto et al. 1994) and also in bull trout (ie. Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001). It is possible that low microsatellite variability is a characteristic of bull trout (Kanda and Allendorf 2001) and may suggest a historically small effective population size, or a low mutation rate (Taylor et al. 1999). Measures of genetic diversity are linked to population fitness and low levels of genetic variation are thought to limit a species' ability to respond to threats of disease, predators, parasites as well as environmental change (Amos and Harwood 1998). Loss of genetic diversity and heterozygosity is linked to reduced individual fitness (Heath et al. 2002).

The level of genetic mixing that currently exists in the Morice River population must be maintained to preserve genetic diversity. Anthropogenic changes that impact fish populations include road building, logging, dams, mining, agriculture and water abstraction, as well as dykes and stream channelization (Riddell 1993). Bull trout in the Morice system will be affected by any of these changes as well as by fishing harvest. For example, if a portion of the population is lost due to degradation of spawning habitat or stochastic events, there will be fewer breeding adults and the effective population size may decrease.

The question becomes "what to conserve"? In the Morice system, genetic variation should be maintained by maximizing the spatial and temporal distribution of localized spawning groups. The likelihood of maintaining genetic variation and adaptability in Pacific salmon increases as the number of salmon reproducing per group increases and as the number of groups increase (Riddell 1993). This principle can also be applied to bull trout. If the spatial and temporal distribution of spawning populations is maximized, numbers of spawners would increase, exchange between the spawning populations would be facilitated

and new spawning populations may develop as spawners disperse from more productive habitats (Riddell 1993). In the Morice system, bull trout use a very small percentage of the watershed for spawning, therefore these areas should be protected.

If the population of bull trout in the Morice system is homogenous, why is it necessary to conserve all spawning areas? One may argue that if random mating occurs and one spawning area is lost, the remainder of the population should still maintain a level of genetic diversity through mixing. In the Morice system, the highest density of spawning occurred in Gosnell Creek watershed, with pockets of spawning habitat located in other areas throughout the watershed. The importance of protecting habitat in Gosnell Creek seems obvious, however bull trout inhabiting other areas of undisturbed habitat may be “nature’s in situ ‘gene bank’” (Riddell 1993). The problem with accepting a loss of spawning habitat is that no one knows how much habitat can be lost before too much genetic diversity has been lost.

Effective population size ( $N_e$ ) has been assessed for Pacific salmonids (*Oncorhynchus* sp.) (Heath et al. 2001; Shrimpton and Heath 2003) and bull trout (Rieman and Allendorf 2001).  $N_e$  indicates the size of an ideal population that has a rate of genetic drift equal to that of the actual population, but has nonoverlapping generations, constant size, an even sex ratio, random mating and random survival of offspring (Kalinowski and Waples 2001).  $N_e$  decreases with decreasing numbers of animals in a population ( $N$ ) and results in a loss of genetic variation through inbreeding depression (Rieman and Allendorf 2001). Since  $N_e$  is difficult to calculate due to the amount of demographic or genetic data required for an accurate estimate, it is often calculated from the harmonic mean of census data (Kalinowski and Waples 2001). However, Shrimpton and Heath (2003) found that census data had no

predictive power for their measures of genetic diversity, but they did find that  $N_e$  was positively correlated with available spawning area in five populations of chinook salmon (*O. tshawytscha*). A similar finding was also reported by Jorde and Ryman (1996) for brown trout (*Salmo trutta*) in Sweden;  $N_e$  corresponded to available habitat for spawning. Managers should attempt to conserve  $N_e$ , however due to the difficulty in accurately estimating it, a general 50/500 rule has become prominent in conservation management (Rieman and Allendorf 2001). This rule implies that an  $N_e$  of 50 is at high risk of inbreeding depression and an  $N_e$  of 500 will maintain adaptive genetic variation for long periods of time. Small population sizes will face greater threats than large population sizes, because mortality or competition may compound the effects of reduced genetic variation (Rieman and Allendorf 2001).

In the Morice system, it is not possible to accurately estimate  $N_e$  because the size of the population of bull trout is unknown. Alternately, Rieman and Allendorf (2001) simulated the relationship of  $N_e/N$  with hypothetical bull trout populations and found that the best estimate of  $N_e$  was 0.5 to 1.0 times the mean number of adults observed annually through typical monitoring programs such as counts of spawning adults. If the 50/500 rule is followed, this means that a minimum of 100 (100 individuals x 0.5 number of adults observed =  $N_e$  of 50) and an average of 1000 spawning bull trout would be necessary to maintain genetic variation. Without knowledge of the number of spawning adults observed annually, even Rieman and Allendorf's (2001) approximation of  $N_e$  cannot be calculated. The presence of these information gaps makes it imperative to conserve all spawning sites within the Morice River system. This is the best attempt to conserve population fitness of bull trout in the watershed.

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