Simpson River Westslope Cutthroat Trout Population Assessment

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Research Notes

Peter J. Corbett, 2003

Abstract

We estimated that there was 20.8 westslope cutthroat trout per kilometer in the Simpson River using a mark and recapture sampling technique with an upper confidence level of 40.6 fish/km and a lower confidence level of 10.4 fish/km. This is considered to be a small population in comparison to other rivers in the region. Tissue samples were collected at the time of marking and molecular analysis was completed to determine the levels of hybridization between the native cutthroat trout and introduced rainbow trout. We found 4% hybridization. This is considered to be relatively low compared to other tributaries of the upper Kootenay River that have no barriers to fish passage. These results lead one to believe that the Simpson River should be considered a high priority for conservation/protection.

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1.0 Introduction

Westslope cutthroat trout (*Oncorhynchus clarki lewisi*) are considered threatened (blue listed) in British Columbia and are currently under federal review by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Present distribution has been seriously reduced across most of its historic range (Liknes and Graham, 1988; Behnke, 1992; Young et al, 1995; Duff et al, 1996; Shepard et al, 1997; Thurow, Lee and Rieman, 1997). Westslope cutthroat trout (WCT) are thought to occupy less than 28% of their historic range in Montana with less than 3% of their home range occupied by genetically pure populations. Allendorf and Leary (1988) suggest that genetic introgression (hybridization) with rainbow trout (*Oncorhynchus mykiss*) is the most important factor responsible for the loss of native cutthroat trout. In British Columbia, the last stronghold of WCT (Rubidge et al), our research has found an increase in hybridization throughout the Kootenay River. Of the 20 sites sampled across their range, we found only 5 (25%) pure populations. Hilderbrand and Kershner (2000) suggest that small isolated populations are at the greatest risk of extirpation.

The intent of this study was to determine the incidence of hybridization in the Simpson River and determine the population size as a measure of risk to extirpation. During the 2002 summer field season, tissue samples (caudal fin clip) were collected from 32 trout from the Simpson River for molecular DNA analysis to determine the levels of hybridization in the trout population. All fish captured were clipped and therefore in essence they have been "marked", facilitating a mark and recapture survey to estimate the population size.

2.0 Study Site

The Simpson River is an order 5 stream. Its headwater's commence along the continental divide and flows westward until its meets the Vermillion River in Kootenay National Park. Approximately 3.8 km of river was sampled, staring at the confluence with the Kootenay River.

3.0 Methods

The initial tissue collection and marking survey was conducted on August 28th, 2002. Angling using a barbless dry fly was employed to capture all fish and subsequently

released unharmed back into the habitat (pool) from which they were captured. The caudal fins from all trout captured were clipped and therefore distinguishable from the non-captured fish within the population. Fin clips were stored in individual vials containing a 95% solution of ethanol. The fork-length of all marked fish was recorded. A follow-up recapture survey was conducted on September 9th, 2002. The sampling area and capture techniques were identical to the initial marking survey. The total number of fish was recorded along with fork-length and all marked fish were identified.

To determine population size the Petersen Estimate was used, which employs the following proportionality argument:

$$N/M = C/R \quad \text{or stated} \quad N = CM/R$$

Where N = estimate of population size
M = the number of fish captured and marked in the 1st pass
C = the number of fish captured in the second pass
R = the number of fish in the second sample that are marked

To reduce the bias produced in the above argument Seber (1982) recommends the estimator:

$$N = \frac{(M+1)(C+1)}{R+1} - 1$$

This equation provides an estimate for the sample area. In order to determine the population estimate per km within the study area, the product of the estimator must be divided by the length of river sampled.

The upper and lower 95% confidence levels where then determined using the standard Poisson methods (Krebs, 1989).

To determine the genetic status of each fish, genomic DNA was extracted from the tissue samples and then a polymerase chain reaction (PCR) was completed using the Ikaros (IK) marker. The IK marker consistently distinguishes westslope cutthroat trout from rainbow trout (Baker 2002, Rubidge 2001, Taylor and Stamford 2000). A more detailed description of lab techniques can be found in the appendices.

The results of the genetic assessment are expressed as a percentage of pure cutthroat trout, pure rainbow trout and percentage of hybrids within the sampled population.

4.0 Results

The following table shows the surveys results for both passes.

Table 1. Fish captured during survey.

	1st pass	2nd pass	marked
Number of Fish	32	16	6

Using these values above, the following estimate is produced:

$$N = \frac{(32+1)(16+1)}{6+1} - 1 \qquad N = 79.1$$

Approximately 3.8 km of stream was sampled, therefore the average number of fish per stream was 20.8 fish per km. Using the values from the Poisson table to determine the upper and lower 95% confidence levels, the following estimates where determined:

Where R = 6 Upper confidence level R = 2.613
Lower confidence level R = 12.817

Therefore the upper confidence level when R = 6 is

$$N = \frac{(32+1)(16+1)}{2.613+1} - 1 \qquad N = 154.273 \text{ or } 40.6 \text{ fish/km}$$

and the lower confidence level is

$$N = \frac{(32+1)(16+1)}{12.817+1} - 1 \qquad N = 39.600 \text{ or } 10.4 \text{ fish/km}$$

Fish distribution by size is presented in Figure 1 in the form of a fork-length histogram. The three spikes at 16, 22 and 30 cm represent the 1+, 2+ and 3+ age cohorts. An additional age class clustered around the 34 cm likely exists representing the 4+ or greater cohort.

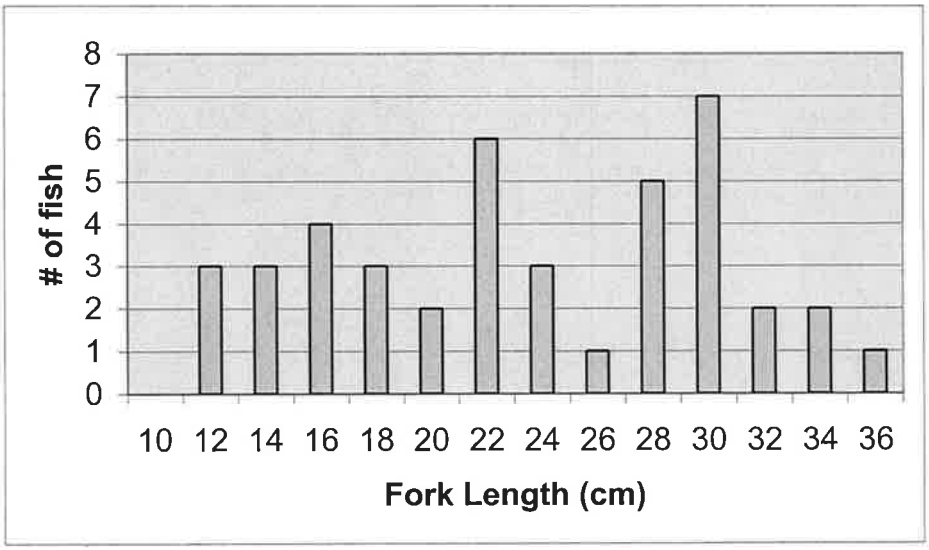

Figure 1. Fork-length distribution of cutthroat trout in the Simpson River. Fish were captured over a two-pass sampling, with marked fish captured during the second pass excluded from the table to prevent duplication (n = 42).

The results of the genetic assessments are presented in Table 2. The results are based on 24 of the original 32 samples. The remaining 8 samples could not be amplified in the lab and therefore analysis could not be completed.

Table 2. Incidence of hybridization in the Simpson River based on molecular analysis using the IK marker to determine trout species.

Life Stage	Cutthroat	Rainbow	Hybrid
fingerling	5 (21%)	0 (0%)	0 (0%)
juvenile	6 (25%)	0 (0%)	0 (0%)
adult	12 (50%)	0 (0%)	1 (4%)
total	23 (96%)	0 (0%)	1 (4%)

5.0 Discussion

The genetic analysis indicates that 1 out of 24 samples or approximately 4% of the sampled population was a hybrid based on molecular analysis using the IK marker. In other studies a second marker (HSC) was employed. The results increased the ability for detection by approximately 50% (Corbett et al, 2001). Therefore incidence of hybridization in the Simpson River is likely higher than 4%. The hybrid was an adult, meaning that the spawning event that created this progeny was approximately 4 years ago (1998). The fact that none of the younger age classes were hybrids is a strong indication that the level of hybridization is not getting worse. There were also no rainbow trout captured which would lead one to believe that there are very few rainbow trout in the

system and that their numbers are too low to establish a naturalized, self sustaining population. Compared to other tributaries in the Kootenay River (see Table 3), the Simpson River would be considered to have a low level of hybridization (< 10%). The tributaries of the Kootenay River with no evidence of hybridization exist above barriers preventing rainbow trout to access these waters. Most of the other tributaries with low levels of hybridization do not have a barrier but some form of a restriction preventing large numbers of rainbow trout to moving into these systems. Hector Gorge on the Vermillion River down stream of the Simpson, likely poses a restriction to rainbow trout movement. In addition, we have seen lower levels of hybridization in tributaries at higher elevations. It is thought that the colder water temperatures do not favour rainbow trout.

Table 3. Incidence of hybridization for tributaries throughout the Kootenay River. Samples from 1999 have been adjusted to reflect predicted results based on the uses of both the IK and HSC markers to make comparison between these and recent results. These results are based on Corbett et al (2001) and Rubidge et al (2001).

Location	% wct	% rbt	% hybrids	n	year sampled
Lussier R.	80	0	20	30	2000
Mather Cr.	60	0	40	30	2000
Wildhorse Cr.	80	0	20	45	2000
Lodgepole Cr.	44	23	33	30	2000
Michel Cr.	75	11	14	28	2000
Coal Cr.	93	0	7	40	2000
Palliser R.	100	0	0	21	2000
Cross R.	90	0	10	30	2000
Finley Cr.	100	0	0	29	2000
Fording R.	100	0	0	18	2000
St Mary Upper	99	0	1	320	2000
St Mary Lower	79	2	19	362	2000
Bull R	100	0	0	36	1999
Wigwam R.	100	0	0	34	1999
Upper Kootenay R.	58	0	42	15	1999
White R	82	0	18	33	1999
Gold Cr.	64	8	28	36	1999
Upper Skook	94	0	6	39	1999
Lower Skook	83	3	14	33	1999
Upper Elk R.	100	0	0	38	1999
Morrissey Cr.	94	0	6	30	1999

The population estimate for the Simpson River would be considered relatively low compared to more productive cutthroat trout streams at lower elevation and more southerly latitudes. This is especially true when considering our study was conducted in the most productive habitat (i.e. deep pools). It is likely that the population size will decrease further upstream as the stream decreases in size and becomes more cascading and colder. The Simpson River has approximately 21 fish/km. In order to compare this value to other streams of different size, it is best to express the population estimate in term of fish/ha. We visually estimated the average width of the Simpson River as 4 m wide; therefore we sampled approximately 15.2 ha. which equates to a population

estimate of 5.2 fish/ha. The following table compares this result with other streams in the Rocky Mountain Region.

Table 4. Comparison of fish population densities between similar streams in the Rocky Mountain Region of Canada (from Courtney and Lightle, 1999).

Stream	Fish/km	Fish/ha
Sheep River	465	297
Racehorse Creek	211	289
Corral Creek	52	261
Bow River (above Bow Falls, Site 1)	666	201
Bow River (above Bow Falls, Site 2)	473	129
Little Elbow River	83	73
Pipestone River	35	23
Simpson River	21	5

The Simpson River has a relatively high Catch Per Unit (CPU) of 4.57 fish/hr. compared to other more productive trout streams giving the illusion of a much larger population than indicated by our results. Therefore CPU is a poor estimator for population size.

The age class distribution as seen in Figure 1 is skewed towards age class 3+ fish. A more typical scenario would be for the reverse of this demographic. These results likely reflect the fact that angling is more successful at capturing larger fish and that the best habitat often supports the dominant fish and the smaller fish are relegated to rearing habitat found in more cascading and cooler waters up stream and in smaller tributaries. The dominance of the 3+ age class is however a good indicator of spawning productivity. This age class will be the primary spawning cohort in the 2003 spring spawning event.

Of the total number of fish captured, approximately 29% (12/42) were over 30 cm and only 12% (5/42) were greater than 30 cm. Of the six marked fish recaptured, all were over 27 cm and 2 of them were 30 cm or greater. This is a good indication of how susceptible the older adult cohort is to angling capture.

6.0 Conservation Implications

There are many issues to consider when assessing the conservation risk of a population. The factors that may favour the Simpson River population are:

- there is a relatively low incidence of hybridization,
- there is no evidence of a naturalized rainbow trout population,
- younger age classes are showing no signs of hybridization (this however may be an artifact of our sample size of smaller age classes),
- rainbow trout are potentially restricted from entering the upper Vermillion watershed at Hector Gorge, and

- the Simpson River is considered a cold water habitat.

However, the Simpson River population is at risk as hybrids are present. High levels of hybridization have been found downstream of the Simpson River in the upper Kootenay River (Taylor and Stamford 2000). While there is evidence that rainbow trout are less likely to colonize small, high elevation streams, the movement of hybridization up stream is well documented (Hitt 2002, Corbett 2001). The restriction at Hector Gorge may exist, however it does not stop bull trout from moving up into the Simpson River and may not keep rainbow trout from accessing the Simpson in greater numbers in the future. Because of the small population size, very few rainbow trout are required to cause excessive hybridization within the population and could create a hybrid swarm and the loss of any pure cutthroat trout in the population.

If rainbow trout continue to expand their range up stream, the Simpson River is vulnerable to extirpation due to the isolated nature and the small size of the population. Hilderbrand and Kershner (2000) suggest that in order to maintain a target of 2,500 individuals with an effective population size of 500, that 25 km of stream habitat are required to maintain a low population (100 fish/km). Our results estimate the population of the Simpson River at 21 fish/km (with a potential high of 41 and low of 10 fish/km). Therefore, the population would require in excess of 25 km of stream habitat in order to remain viable. This is assuming that the Simpson River population is relatively isolated and that little immigration and/or emigration occurs.

It could be argued that due to the unique nature of each wct populations within the Kootenay River watershed (Leary et al 1987), that effective population size may vary through the process of co-evolution of a population with its environment. Theoretical arguments aside, it is safe to say that the Simpson River is likely close to its population threshold. Excessive fish harvest and the continued threat of increasing hybridization put the Simpson River at risk.

At present, fish regulations permit the harvest of 2 cutthroat over 30 cm each day. Considering the low populations size of the Simpson River, the fact that many of the 30 cm. or great individuals fall within the 3+ age cohort and have not spawned yet, and the susceptibility to this age cohort to angling, I recommend that the harvest of cutthroat trout be eliminated.

I recommended that regular monitoring be conducted to determine any changes in population size, demographics and levels of hybridization and that appropriate conservation measures be taken. Once hybridization begins within a population, it can be very persistent and can often lead to the development of a complete hybrid swarm. Conservation strategies employed elsewhere to address these concerns included:

- Rainbow trout removal
- Barrier construction to stop up stream gene flow, and
- Stocking pure wct to augment the contribution of pure fish to the population.

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Appendices

A. Tissue Collection and DNA Extraction

Tissue samples were collected from all ages classes except fry. Fry were collected whole. A small (2-10 mg) fin clip was removed from the bottom of the caudal fin with surgical scissors. All samples were stored in 10-15 ml vials in 95% ethanol. Sample vials were stored at room temperature and kept out of direct light.

DNA was extracted from tissue samples using a generic Proteinase/K extraction procedure. Each fin clip was removed from the storage vial with tweezers and cut in half. One half was returned to the original vial for long-term storage. Between 2-8 mg of tissue was then transferred to a 1.5 mL reaction tube after any excess ethanol was blotted off. To each 1.5 mL reaction tube 755 uL of 1 x STE (buffer), 10 uL of 20% SDS (detergent), and 5 uL of 20 mg/mL Proteinase/K (enzyme) was added. Tweezers and scissors were flame sterilized between each sample with a Bunsen burner for 10-15 seconds. The reaction vials were then incubated in a water bath at 55 °C for a minimum of two hours and gently hand mixed every 15 mins for the first hour to aid DNA digestion. Following digestion samples were washed with equal volumes of Phenol-Isoamyl alcohol-Chloroform (PCI) at 25:24:1 and spun in a centrifuge for 3 mins at 14,000 rpm. The top, clear aqueous layer was transferred to a new vial and washed again with PCI (the remaining liquid was discarded). After two PCI washes samples were washed twice with equal volumes of Chloroform-Isoamyl alcohol (CI) at 24:1 in the same manner as the PCI wash. The DNA was then precipitated by adding 1 mL of 100% ethanol (stored at -20 ° C) and 40 uL 2M NaCl and storing the samples at -20 ° C for 40 mins.

After precipitation of the DNA the vials were removed from the freezer and the samples were washed once with 1 mL 100% ethanol and then spun for 3 min as above. A small white DNA pellet could usually be observed at the bottom of the vial at this point. The 100% ethanol was then poured off and 1 mL of 70% ethanol was added and the same procedure applied. This is to remove any left over PCI or CI. Once the 70% ethanol was poured off the vials were carefully blotted to remove excess ethanol and then dried for 15 mins at 45 ° C in a Savant© Speed Vac. Once all the ethanol had been evaporated off, the remaining DNA pellet was re-suspended in 100uL of 1 x TBE and stored in a -20 ° C freezer. The concentration of all DNA samples was assessed using a TKO 100 model Fluorometer. The fluorometer was calibrated using 2 uL of ctDNA (100 ng/uL).

B. Molecular Techniques

I followed as closely as possible the techniques used by Rubidge et al. (2001) to genetically determine westslope cutthroat trout, rainbow trout, and their hybrids. I amplified two intron regions of genomic DNA with the Ikaros (IK) and Heat shock cognate (HSC) primers using the polymerase chain reaction (PCR). IK and HSC are species specific markers that consistently distinguish westslope cutthroat trout from rainbow trout (Baker et al. 2002, Rubidge et al. 2001, Taylor and Stamford 2000). Corbett et al. (2001) showed that IK alone detected on average half the number of hybrids detected with both IK and HSC (Table 1)

Table 1. Percent of hybrids detected with IK and HSC markers in population of St Mary River fish (Corbett et al. 2001).

Life Stage	IK (% detection)	IK and HSC (% detection)
Fry	8.4	17.8
Fingerling	16.2	24.7
Juvenile	15.6	18.8
Adult	14.0	23.5
Totals	**13.2**	**24.5**

The PCR reaction was optimized by conducting several tests. First, the thermal profile for the PCR reaction was developed using a generic profile and published data (Baker et al. 2002, Rubidge et al. 2001). Second, samples were run with varying concentrations of BSA and $MgCl_2$ until an 800 base pair (bp) PCR product

was consistently produced. Finally, a temperature gradient was set up using six different annealing temperatures: 46, 48, 50, 52, 54, 56 ° C. Results of the gradient test suggested that the PCR was not sensitive to the range of temperatures selected, as all samples produced an 800 bp fragment regardless of temperature. A PE Applied Biosystems© Gene Amp PCR system (9700 and 2400 model) was used for all PCR reactions except the temperature gradient test for which I used a MJ Research PTC-2000 Peltier Thermal Cycler© was used. The thermal profile for all PCR reactions was as follows: 1 cycle at 94 °C for 2 mins to denature the DNA; 40 cycles at 94 ° for 1 min, 48 °C for 1.5 mins, and 72 ° C for 2 mins for amplification; 1 cycle at 72 ° C for 7 mins for extension. Samples were held at 4 ° C once the profile was completed. The recipe for both PCR reactions are listed in Table #.

Table #. Polymerase chain reaction recipe for IK and HSC markers (25 uL reaction).

Agent	Stock Concentration	IK – Volume/ reaction (uL)	HSC – Volume/ reaction (uL)
dH2O	-	14.50	17.2
10 x PCR buffer	-	2.50	2.0
dNTP	5mM	4.00	3.2
IK-forward	10uM	0.50	0.4
IK-reverse	10uM	0.50	0.4
Taq DNA polymerase	5 units/uL	0.30	0.2
BSA	25 mM	0.25	-
$MgCl_2$	10mg/mL	1.00	0.6
DNA	5-50 ng/uL	1.5	1.0

Species-specific sequence variants were assayed with restriction enzymes to cut the amplified DNA sequences into a series of smaller fragments (Table #). PCR products for IK and HSC were digested with Hinf 1 and Taq 1 respectively (Baker et al. 2002). A 50 uL restriction reaction was set up in a 1.5 mL reaction tube as follows: 24.8 uL dH2O, 5.0 uL Buffer, 0.2 uL Hinf 1, and 20 uL PCR product. The restriction reaction was incubated for 1 hr at 37 ° C. The species-specific fragments were then separated by electrophoresis on a 2% agarose gel at 115-118 volts. Gels were stained with a mixture of 30uL of ethidium bromide dye and 250 mL of double-distilled water for 10-15 mins to visualize fragment bands. The IK has one restriction site for Hinf 1 in westslope cutthroat trout which results in two DNA fragments, 519 and 294 bp, whereas the rainbow trout lack the restriction site. F1 hybrids produce both the westslope cutthroat trout bands and the rainbow trout 813 bp band.

Table #. Primer sequences, polymerase chain reaction conditions, PCR product size, and restriction enzymes for each locus (Baker et al 2002, Rubidge et al. 2001).

Locus and Primer Pair	Primer Sequence	Annealing Temp. (°C) and Cycles	Product Size	Restriction Enzyme and Fragment Size
Ikaros IK-F1/IK-R1	5'-CTTCGAGTGCAACCTCTG-3 5'-ATTTTCTTTGCCACCGAGG-3	48/40	813	*HinF 1* 519/294
Heat Shock Cognate HSC 71-F/HSC 71-R	5'-TACTCAGACAACCAGCCTGG-'3 5'-GATCGAGACGGTCATGAC-'3	60/8, 56/32	1,184	*Taq 1* 352, 216, 367, 249