

Evaluation of Current Westslope Cutthroat Trout Hybridization Levels in the Upper Kootenay Drainage

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Prepared by: Heather M Lamson

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Executive Summary

This report summarizes the findings of the first year of a multi-year project studying hybridization within the BC distribution of westslope cutthroat trout (WCT). Through partnership funding from the Fish and Wildlife Compensation Program (FWCP) and the Ministry of Forests, Lands and Natural Resource Operations (FLNRO) Land Based Investment Strategy (LBIS), hybridization resulting from interbreeding between introduced rainbow trout (RBT) and native westslope cutthroat trout (WCT) was assessed. This project will be ongoing with future partnership funding from FWCP, FLNRO, and the Department of Fisheries and Oceans.

A total of 271 samples collected in 2012 and 2014 were genotyped using SNPs in 2015. Over 750 genetic samples were collected from June 1, 2015 to October 1, 2015 throughout the BC WCT distribution. Of these 631 were sent to the Montana Genetics Conservation Lab (MGCL) for genotyping using Single Nucleotide Polymorphic Loci (SNPs). Full genetic results from samples collected in 2015 were not available when writing this report and will be included in the 2016/17 report.

Of the genotyped populations, the Upper St Mary River and Skookumchuck River populations showed no evidence of hybridization. The North Fork of the White River and Middle Fork of the White showed low rainbow trout (RBT) population allele frequencies of 1% and 3% respectively. However, results indicate that the only population sampled that represented a hybrid swarm was the North Fork of the White River. Perry Creek samples were genotyped at 5% and the Elk River mainstem below Elko dam was 10% RBT allele frequency. The Lower St Mary River reported a RBT allele frequency of 12%. The source of RBT for these populations is likely Kooicanusa Reservoir.

Samples collected downstream of Whiteswan Lake, verified that it is a source of rainbow trout. Outlet Creek drains Whiteswan Lake to the White River and has a falls that is impassible to upstream migration. In Outlet Creek, almost all samples were pure RBT (RBT allele frequency of 99%). Downstream of Outlet Creek in the White River mainstem, RBT frequency was 59% and in the Kootenay River mainstem, near the White River confluence, RBT allele frequencies were 15%. Upstream of Outlet Creek in the White River mainstem, the RBT allele frequency was 10%. The results of the genetic sampling emphasize the need to focus effort on reducing the outmigration of RBT from Whiteswan Lake and the need for more sampling to inventory hybridization throughout the BC WCT distribution and identify current sources of RBT genes.

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Introduction

The Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*; WCT) is the most widely distributed subspecies of cutthroat trout in western North America, historically occupying portions of the Columbia, Fraser, Missouri, and Hudson Bay River drainages of the United States and Canada (Allendorf and Leary 1988; Behnke 1992; Shepard et al. 2005; Trotter 2008). However, native populations have dramatically declined due to non-native species introductions, habitat degradation, fragmentation, overexploitation, and climate change (Shepard et al. 2005; Muhlfeld et al. 2014). Human-induced hybridization with non-native salmonids stemming from widespread stocking and subsequent spread of introgression has been especially detrimental to WCT (Allendorf and Leary 1988; Allendorf et al. 2005), particularly hybridization with introduced rainbow trout (*O. mykiss*, RBT). Non-hybridized WCT populations now persist in less than 10% of their historic range in the United States (Shepard et al. 2005) and less than 20% of their historic range in Canada (Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006).

The spread of introgressive hybridization between non-native RBT and native WCT is of conservation concern for several reasons. First, anthropogenic introgression disrupts distinct genotypic combinations among genetically divergent WCT populations (Allendorf and Leary 1988), resulting in genomic extinction of native taxon (Allendorf et al. 2001). Second, hybridization between native WCT and non-native RBT can reduce fitness through outbreeding depression (Muhlfeld et al. 2009; Kovach et al. 2015), and can spread rapidly across river networks (Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2004; Bennett et al. 2010; Yau and Taylor 2013; Muhlfeld et al. 2014). Third, hybridization is exasperated by habitat modification (Allendorf et al. 2001) and climate change (Muhlfeld et al. 2014), so this problem will probably become more serious with increasing land use and global climate change. Finally, native WCT have enormous ecological, socioeconomic, and cultural value in the Rocky Mountain Region, including the upper Kootenay River system and Elk River Valley. Therefore, loss of this highly prized native trout through invasive hybridization will have long-lasting, irreversible consequences for both native biodiversity and human services.

Westslope cutthroat trout populations in southeastern British Columbia, southwest Alberta, and adjacent areas of Montana, Idaho, and Washington State and are threatened by hybridization with introduced rainbow trout (Yau and Taylor 2012). The population that persists in British Columbia is listed under the Species at Risk Act as a species of special concern, while the Albertan population is considered threatened. The BC Ministry of Environment published a Management Plan for WCT in British Columbia in 2013 (MOE 2013) that identifies management actions deemed necessary to prevent WCT from becoming endangered or threatened. Priority management objectives identified in that plan include maintaining the native distribution and genetic diversity of populations. Population groups within the WCT core range reflect the extent to which native populations occur, and overlapping watersheds within the geographic scope of the Upper Kootenay Ecosystem Enhancement Plan (UKEEP) include the Elk River (Elk Lakes to Elko Dam, including all tributaries) and Upper Kootenay watershed (Kootenay River and its tributaries from headwaters to Koochanusa Reservoir, including the Elk River and tributaries below the Elko Dam). Consequently, protecting the genetic integrity of native WCT is a high priority for conservation and management programs in BC and the upper Kootenay River

system. Understanding the genetic status and distribution of these WCT populations is needed to make informed management decisions, provide recreational opportunities, and to conserve the evolutionary heritage of this native trout for future generations.

UKEEP identifies actions targeted at understanding and limiting hybridization between WCT and RBT a high priority in the lakes, streams and species of interest action plans. The first management objective of the WCT Management Plan defines maintaining the native distribution and genetic diversity of populations, and sets a target of less than 10% of each population group introgressed at levels greater than 1%. The plan also identifies that baseline genetic analysis of BC WCT populations are limited and data gaps exist. This genetic information in the WCT plan was based on the following studies in the Upper Kootenay watershed:

- Leary *et al.* in 1986 collected 219 fish in eleven sample sites in the upper Kootenay watershed,
- Rubidge *et al.* in 1999 and 2000 collected 981 fish at 23 sample sites in 12 different river systems,
- Bennett *et al.* from 2001 to 2006 collected 1065 additional fish at 26 sample sites.

More recently, to address concerns from stakeholders on limited data on Whiteswan Lake being a source of rainbow trout to downstream water, the following work was completed:

- McPherson and Lamson in 2013 collected 78 samples from Outlet Creek below a known source of RBT, Whiteswan Lake. Of the 78 samples analyzed, all but one were rainbow trout and one sample was a WCT.

Goals and Objectives

The objectives of this project are to: (1) define the current distribution and genetic status of WCT populations in the upper Kootenay River system using a spatially-explicit analysis; (2) identify populations that managers can conserve for genetic integrity; and (3) evaluate current and future threats to population persistence to inform conservation management of existing populations throughout the ecosystem.

Study Area

The foundation of British Columbia's westslope cutthroat trout distribution occurs within the Upper Kootenay watershed, which encompasses drainages that ultimately flow into the Kooconusa Reservoir upstream of Libby Dam. Prior to the construction of the Libby Dam in 1972, the reservoir consisted entirely of the Upper Kootenay River with a natural barrier at the location of the Libby dam. This project focusses on the Canadian portion of the Upper Kootenay watershed which covers approximately 20,000 km².

Bordering westslope cutthroat trout populations in British Columbia also are known to occur in the Upper Columbia watershed, Flathead drainage, west Kootenays, Kettle and South

Thompson. It is unknown, however, the extent to what these populations were native or colonized from hatchery stocking. Genetic analysis will subsequently occur throughout the BC WCT range through partnership funding (FLNRO, DFO).

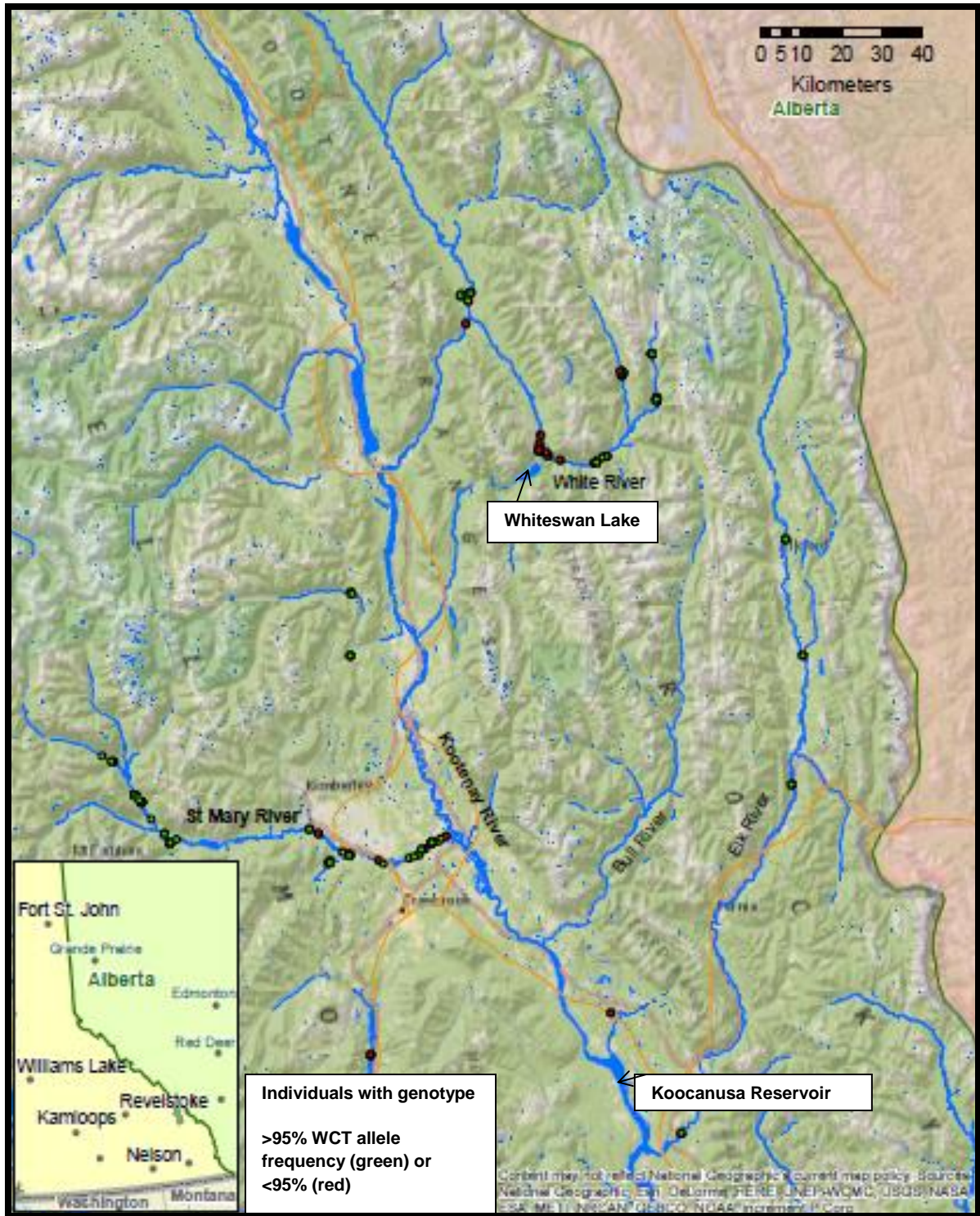


Figure 1. Map of trout samples genotyped in the Upper Kootenay Drainage genotyped in 2015.

Methods

Site locations to monitor hybridization throughout the BC WCT distribution were primarily selected to sample distinct populations and secondarily to evenly sample populations. For instance, the upper St Mary River and lower St Mary River are each considered separate populations and therefore separate sites. With SNPs, sample sizes can be lower than when using microsatellite markers for genotyping and 24 samples from each site was aimed for. Sites included those previously sampled for hybridization to monitor temporal changes, sites with a known rainbow source to and sites lacking information on hybridization.

The majority of samples were collected by angling, though at some (smaller) sites electrofishing was employed. A small piece of adipose fin was clipped from trout identified as WCT, RBT or hybrid. Upon collection, tissue was immediately preserved in 1.5 mL 97% anhydrous ethyl alcohol. The visual species identification was recorded for each fish as was the length, and the GPS location.

Samples were genotyped by the Montana Conservation Genetics Lab (MGCL) at the University of Montana Flathead Lake Biological Station. The level of westslope cutthroat trout (WCT), rainbow trout (RBT), and Yellowstone cutthroat trout (YCT) hybridization was determined by using a set of 95 single nucleotide polymorphic loci (SNPs). SNP loci included 19 that differentiate rainbow from westslope cutthroat and Yellowstone cutthroat trout (rainbow diagnostic markers), 20 that distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope diagnostic markers), and 20 loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone diagnostic markers). Thirty-four loci that are generally polymorphic within westslope cutthroat trout populations and two mitochondrial DNA (mtDNA) loci that differentiate cutthroat and rainbow trout were also genotyped. Hybridization analysis used the SNP loci and methods outlined in Leary 2013.

To compare hybridization rates to those sampled between 1999 and 2006, raw genotype data of individual fish at sites within the Upper Kootenay watershed were provided to the project by Dr. Stephen Bennett. These data included genotypes from Emily Rubidge's Master's thesis (samples collected in 1999 and 2000) and Dr. Bennett's PhD thesis (samples collected 2001 to 2006).

Hybridization is reported here as % RBT alleles, calculated as the # of RBT alleles in a population (site) / total alleles in a population (site).

Results

Genetic samples were opportunistically collected from 20 fish in the Elk River in 2012 (5 from below the Elko Dam and 15 above) under another study that lethally sampled WCT for contaminant monitoring. In 2014, samples were collected from sites within the upper Kootenay drainage including locations close to Whiteswan Lake, a known source of rainbow trout. These include Outlet Creek (drains Whiteswan Lake), the White River mainstem, Middle Fork of the White, North Fork of the White and the mainstem of the upper Kootenay River near to the White River confluence. In 2014, samples were also collected from several sites previously assessed

for hybridization, including the Upper and Lower St Mary River, Perry Creek, and the Skookumchuck River. Five samples were collected (two genotyped) from Little Sand Creek, a tributary to Koochanusa. Samples from two lakes were genotyped to gain understanding of the genetics of stocked WCT in BC. North McNair Lake has been stocked with RB and WCT and 5 samples from trout captured here were genotyped. Moyie Lake has been stocked with both RB and WCT in the past, though is not currently stocked with WCT. Nine WCT captured in Moyie Lake in 2014 were genotyped. Fish sampled in 2012 and 2014 were genotyped by MGCL in 2015 and results are detailed below.

Genotypes were successfully generated for 271 fish collected in 2012 and 2014 from WCT, RBT or hybrids from the upper Kootenay drainage at 15 sampling locations and analyzed for hybridization using SNPs. Raw SNP genotypes have three possible genotypes being homozygous for one allele (11 or 22) or heterozygous (12). Three of the 15 sample collections had 5 or fewer fin clips and will require additional fin clips to confirm the absence of admixture with RBT or YCT and to accurately estimate admixture levels where it exists.

Tissue samples collected from the Elk watershed in 2012 and from the other sites in the Upper Kootenay watershed in 2014 were genotyped in 2015. The 271 samples analyzed showed no evidence of hybridization with Yellowstone cutthroat trout. Power to detect 0.5% hybridization with YCT ranged from 33 to 99% probability assuming each sample collection represented a randomly mating population. The samples showed a wide range of hybridization with RBT. Two collections had RBT allele frequencies greater than 93% (Little Sand Creek and Outlet Creek) and appear to be composed of a few fish with 1-3 WCT alleles and fish with only RBT alleles. In the remaining sample collections moderate to low admixture with RBT (0 - 15%) was detected, but only the North Fork of the White River sample appears to be a hybrid swarm. Two F1 (first generation hybrids) were detected, one in each of the "White River Mainstem Above Whiteswan Lake" and "White River Mainstem Below Whiteswan Lake" samples and suggest ongoing hybridization in the White River. Finally, in the Upper St. Mary's River and the Skookumchuck River collections no RBT alleles were detected despite having good power to detect low levels of admixture (0.5% RBT; 88 to 92% probability). To test whether a sample is a hybrid swarm, the collection of hybrid indices in the sample is compared to the expected distribution given the observed frequency of RBT alleles in the sample. One reason a sample may not meet this expectation is if it does not represent a single randomly mating population. In this case, it is not possible to accurately estimate the percentage of admixture with rainbow trout accurately. In these cases, it may be more informative to look at the number of fish in different hybrid index classes (e.g. WCT, WCTxRBT, F1, RBTxWCT, RBT).

Upper St. Mary's River

No RBT alleles were detected in any fish from this sample. With a sample of 35, the probability of detecting 0.5% RBT or YCT admixture was greater than 99%. Approximately 71% (24 of 34) of the variable SNP loci were polymorphic and the expected heterozygosity corrected for sample size (uHe) was 0.218.

Genetic sampling on the St. Mary River occurred in 1999 (n=31) and 2000 (n=100) and no hybridization was detected (Rubidge 2003).

Lower St. Mary's River

With a sample of 37, the probability of detecting 0.5% YCT admixture was greater than 99%. Individuals with only RBT alleles, predominantly RBT admixture (RBTxWCT), predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were homogeneous ($X_{238}=14.66$, $P=1.00$) among the rainbow and westslope markers. However, the distribution of hybrid indices did not conform ($X_{210}=499.27$, $P<0.001$) to the expected random distribution. The hybrid indices were highly variable among the individuals ranging from 0 to 78. Based on the chi-squared hybrid swarm test, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

The RBT allele frequency of 0.12 for the Lower St Mary population was higher than that found by Rubidge in 2000 of 0.05 ($n=195$) and Bennett in 2007 of 0.005 ($n=31$). Differences in these estimates may be due to differences in the samples (date, location, etc.), the loci used to estimate admixture, or both.

Upper Skookumchuck River

No RBT alleles were detected in any fish from this sample. With a sample of 9, the probability of detecting 0.5% RBT or YCT admixture was 97%. Additional samples are necessary to determine whether low levels of YCT or RBT admixture are absent with high certainty.

Samples collected in 1999 and 2003 (pooled) estimated admixture at 0.07 in the Lower Skookumchuck River and 0.03 in the Upper Skookumchuck River. Differences in these estimates to the lack of hybrid alleles found in the 2014 samples may be due to differences in the samples (date, location, etc.), the loci used to estimate admixture, or both. Approximately 65% (22 of 34) of the variable SNP loci were polymorphic and the expected heterozygosity corrected for sample size (uHe) was 0.226.

Upper Kootenay River (Mainstem)

The Upper Kootenay River mainstem sampling site was located near the confluence of the White River to detect hybridization originated from rainbow trout escaping from Whiteswan Lake.

With a sample of 14, the probability of detecting 0.5% YCT admixture was 94%. Individuals with predominantly RBT admixture (RBTxWCT), predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were homogeneous ($X_{238}=12.38$, $P=1.00$) among the rainbow and westslope markers. However, the distribution of hybrid indices did not conform ($X_{26}=32.41$, $P<0.001$) to the expected random distribution. The hybrid indices were highly variable among the individuals ranging from 0 to 41. Based on the chi-squared hybrid swarm test, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

North Fork White River

DNA could not be successfully extracted from two fin clips in this sample. With a sample of 18, the probability of detecting 0.5% YCT admixture was 97%. Individuals with predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were heterogeneous ($X_{238}=107.00$, $P<0.001$) among the rainbow and westslope markers. However, the distribution of hybrid indices did conform ($X_{21}=0.83$, $P=0.36$) to the expected random distribution. The hybrid indices ranging from 0 to 5 were observed. Although the RBT allele frequencies varied significantly among loci, this sample conforms to the expected distribution of hybrid indices and likely did come from a hybrid swarm.

Middle Fork White River

With a sample of 32, the probability of detecting 0.5% YCT admixture was greater than 99%. Individuals with predominantly RBT admixture (RBTxWCT), predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were heterogeneous ($X_{238}=115.14$, $P<0.001$) among the rainbow and westslope markers. The distribution of hybrid indices also did not conform ($X_{24}=56.70$, $P<0.001$) to the expected random distribution. The hybrid indices were highly variable among the individuals ranging from 0 to 51. Based on these tests, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

White River Mainstem upstream of Whiteswan Lake

With a sample of 25, the probability of detecting 0.5% YCT admixture was greater than 99%. Individuals with only RBT alleles, predominantly RBT admixture (RBTxWCT), F1 (first generation hybrid), predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were homogeneous ($X_{238}=13.05$, $P=1.00$) among the rainbow and westslope markers. However, the distribution of hybrid indices did not conform ($X_{29}=281.39$, $P<0.001$) to the expected random distribution. The hybrid indices were highly variable among the individuals ranging from 0 to 78. Based on the chi-squared hybrid swarm test, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

White River Mainstem below Whiteswan Lake

With a sample of 12, the probability of detecting 0.5% YCT admixture was 91%. Individuals with only RBT alleles, predominantly RBT admixture (RBTxWCT), F1 (first generation hybrid), predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were homogeneous ($X_{238}=1.92$, $P=1.00$) among the rainbow and westslope markers. However, the distribution of hybrid indices did not conform ($X_{25}=22.40$, $P<0.001$) to the expected random distribution. The hybrid indices were highly variable among the individuals ranging from 0 to 78. Based on the chi-squared hybrid swarm test, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

Outlet Creek

With a sample of 25, the probability of detecting 0.5% YCT admixture was greater than 99%. Only individuals with RBT alleles, and predominantly RBT admixture (RBTxWCT) were observed.

Perry Creek

With a sample of 26, the probability of detecting 0.5% YCT admixture was greater than 99%. Individuals with predominantly WCT admixture (WCTxRBT), and only WCT alleles were observed.

RBT allele frequencies were heterogeneous ($X_{238}=57.04$, $P=0.02$) among the rainbow and westslope markers. The distribution of hybrid indices also did not conform ($X_{25}=71.65$, $P<0.001$) to the expected random distribution. The hybrid indices were variable among the individuals ranging from 0 to 24 (Figure 8). Based on these tests, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

Little Sand Creek

With a sample of 2, the probability of detecting 0.5% YCT admixture was only 33%. Only individuals with predominantly RBT admixture (RBTxWCT) were observed.

Elk River (Lower and Mid)

The 2012 samples collected from the Lower Elk River found 10% RBT admixture ($n=5$), while the previous estimate from a 2004 sample detected 17% rainbow trout alleles (Bennett 2006). More samples from the Lower Elk are needed to compare temporal admixture levels and are planned for 2016 sampling year.

Moyie Lake

With a sample of 9, the probability of detecting 0.5% YCT admixture was 84%. Only individuals with predominantly WCT admixture (WCTxRBT) were observed. Additional samples are necessary to determine whether low levels of YCT admixture are absent with high certainty and to accurately estimate the level of RBT admixture in the sample.

RBT allele frequencies were heterogeneous ($X_{238}=57.31$, $P=0.02$) among the rainbow and westslope markers. The distribution of hybrid indices also did not conform ($X_{24}=7.28$, $P<0.001$) to the expected random distribution. The hybrid indices were variable among the individuals ranging from 1 to 15. Based on these tests, the sample did not come from a hybrid swarm and given the distribution of hybrid indices it is uncertain whether it contained any non-hybridized westslope cutthroat trout.

McNair South Lake

With a sample of 5, the probability of detecting 0.5% YCT admixture was 63%. Individuals with only RBT alleles, and only WCT alleles were observed. Additional samples are necessary to determine whether low levels of YCT admixture are absent with high certainty and to accurately estimate the level of RBT admixture in the sample.

Table 1. Summary of hybridization analysis for westslope cutthroat trout samples collected 2014 from the Kootenay River drainage, BC. Summary data from genetic analysis of sample collections using 95 SNP loci marker panel for hybridization analysis included 19RBT diagnostic loci, 20 WCT diagnostic loci and 20 YCT diagnostic loci.

Site	N	WCT Alleles only	Predominantly WCT alleles (WCT x RBT)	F1 (First Generation hybrid)	Predominantly RBT alleles	RBT	RBT allele frequency	Bennett RBT allele frequency	Rubidge/ Bennett sample year
Upper St Mary River	35	35	0	0	0	0	0.00	0.00	1999/2000
Lower St Mary River	37	15	19	0	2	1	0.12	0.04	2000/2007
Skookumchuck River	9	9	0	0	0	0	0.00	0.06	2003
Upper Kootenay Mainstem	14	6	7	0	1	0	0.15		
North Fork White River	18	12	8	0	0	0	0.01	0.02	1999/ 2004
Middle Fork White River	32	15	16	0	1	0	0.03	NA	NA
White River Mainstem upstream of Whiteswan Lake	25	12	8	1	3	1	0.11	NA	NA
White River Mainstem near Outlet Creek	12	1	3	1	4	3	0.59	NA	NA
Perry Creek	26	11	15	0	0	0	0.05	0.06	1999 to 2006
Outlet Creek	25	0	0	0	14	11	0.99	NA	NA
Lower Elk River (below Elko dam)	5	3	2	0	0	0	0.10	0.17	2004
Mid Elk River	15	14	15	0	0	0	0.00	0	2000

Field collection of WCT for hybridization screening continued in 2015, from June to October at sites in the Upper Kootenay watershed and also included sites in the West Kootenays (through partnership funding). A total of 751 samples were collected. Where sufficient sample sizes were met at a site, samples were shipped and are presently being genotyped at MGCL (Table 2. Samples collected in 2015 and sent to MGCL (December 2015) for hybridization genotyping. Table 2). Samples that were not sent to MGCL due to low sample sizes included samples from sites in the Upper Kootenay River in Kootenay National Park from Parks Canada, the Cross River, Wigwam River, Michel Creek, Elk River, Dutch Creek, Moyie River, and from sites in the West Kootenays. These samples will be pooled with samples collected in 2016 to reach sufficient sample sizes.

Table 2. Samples collected in 2015 and sent to MGCL (December 2015) for hybridization genotyping.

Sample Year	Site	Receiving Water	Samples Sent to MGCL
2015	Templeton Creek	Upper Columbia	17
2015	Fell Creek	Kootenay Lake	12
2015	Corn Creek	Lower Kootenay	13
2015	Italy Creek	Kettle River	24
2015	Sutherland Creek	Kettle River	24
2015	Bloom Creek	Koocanusa	20
2015	Caven Creek	Koocanusa	22
2015	Gold Creek	Koocanusa	24
2015	Sand Creek	Koocanusa	32
2015	Tepee Creek	Koocanusa	24
2015	Wickman Creek	Koocanusa	22
2015	Fenwick Creek	Upper Kootenay	23
2015	Findlay Creek	Upper Kootenay	28
2015	Lower Bull	Upper Kootenay	24
2015	Lower Lussier	Upper Kootenay	23
2015	Mather Creek	Upper Kootenay	22
2015	Palliser	Upper Kootenay	25
2015	Skookumchuck River	Upper Kootenay	20
2015	Upper Bull	Upper Kootenay	20
2015	Upper Lussier	Upper Kootenay	24
2015	White River mainstem	Upper Kootenay	11
2015	Wildhorse River	Upper Kootenay	24
2015	East Fork White River	White	8
2015	Middle Fork White River	White	24
2015	North Fork White River	White	24
2015	Upper Fording	Elk River	29
2015	Greenhills Creek	Elk River	30
2015	Lower Lodgepole	Elk River	24
2015	Upper Lodgepole	Elk River	24

Discussion

Admixture levels from populations sampled in 2012 (Elk) and 2014 (Upper Kootenay) were similar to admixture results using 4 microsatellite loci (Occ34, Occ35, Occ37, Occ42) on samples collected between 1999 and 2007 (Bennett 2007) at the same locations. RBT allele frequency was 5% in the 2014 Perry Creek sample, while admixture results from samples collected from 1999 to 2006 were estimated to be 6%. A 2009 paper reported the proportion of the sample that was hybrid (fish with ≥ 1 RBT allele) annually from 1997 to 2006 and predicted that the proportion of hybrids would be 77.9% in 2020 (Bennett and Kershner 2009). Proportions ranged from 16.7% in 1997 to 43.3% in 2006. The proportion of the sample that had fish with one or more RBT alleles in 2014 was 61.5%. However, with the ability of SNPs to detect admixture at a finer level, using more alleles, these results may not be comparable to those reported previously. Given that Perry Creek has been a known spawning tributary for WCT from the Lower St Mary River, hybridization rates should be addressed and Perry Creek would be a good candidate stream for a focus on restoring native species. Consideration should be given to the presence of introduced eastern brook trout in Perry Creek, which make up the majority of the fish composition in the Creek.

In the Upper St Mary River, admixture levels remained at zero, with only pure WCT in the sample as was reported previously. These results have always been noteworthy as there is no physical barrier between the Upper and Lower St Mary River to exclude rainbow trout from migrating upstream. A telemetry project on the St Mary River from 2001 to 2004 identified that 25% of the tagged fish overwintered in St Mary Lake (lacustrine-adfluvial life history) (Morris and Prince 2004). The Upper St Mary fish also displayed fluvial-adfluvial (moving between mainstem and tributary habitats) and resident (adhering stream) life histories. Upper St Mary fish spawned at the Redding Creek confluence, within the mainstem, and up tributaries. Tagged fish from the Upper St Mary River did not move downstream of St Mary Lake.

The Upper and Lower St Mary River, divided by St Mary Lake, showed differing admixture levels, with the Lower St Mary River RBT allele frequency at 12%, an increase from the frequency in Rubidge (sampled in 2000) and Bennett's (sampled in 2007) pooled samples of 5%. The telemetry work identified resident and fluvial-adfluvial life histories in Lower St Mary River tagged WCT with some fish overwintering in the Upper Kootenay River. Twenty percent of the Lower River tagged fish spawned at the Perry Creek confluence and fifteen percent migrated up and spawned in tributaries. These life histories paired with genetic results show that the Upper and Lower River WCT are distinct populations. The Lower St Mary's River hybridization rates are most likely influenced by Kooocanusa, which was stocked with diploid RBT extensively in the 80's.

Whiteswan Lake was stocked with over 1.5 million fertile rainbow trout from 1964 to 2003 and outmigrating rainbow trout serve as the source for hybridization to the White River drainage. Outlet Creek drains Whiteswan Lake to the White River system and has a falls that precludes upstream fish migration. Samples collected in 2012 (McPherson and Lamson 2013) from below the falls were genotyped using microsatellite markers and only RBT alleles were detected. In samples genotyped using SNPs in this study, the RBT allele frequency was 99%. Samples collected in the White River mainstem near the confluence of Outlet Creek (approximately 3 km

upstream to 5 km downstream of Outlet Creek) had 59% RBT allele frequency. Samples collected in the White River mainstem above Outlet Creek confluence (approximately 4 km to 20km upstream of the confluence) had a pooled RBT allele frequency of 11%. The Middle Fork of the White (RBT allele frequency of 3%), and North Fork of the White (RBT allele frequency of 1%) both displayed hybridization. These sample sites were both approximately 40 km upstream of Whiteswan Lake. Genetic data has shown hybrid presence in the North Fork since the first sampling in 1986 (5.3% Leary et al. 1897a) and in 1999 (3.8% Rubidge 2003). The genetic results validate that the source of rainbow trout from Whiteswan Lake into the White River is ongoing and extends into the North Fork and the Middle Fork. The Middle Fork even had one fish that predominantly had rainbow trout genes. A fish barrier designed to limit RBT outmigration from Whiteswan Lake has been constructed on Outlet Creek upstream of the falls. The barrier will be operated starting in the Spring of 2016 and ongoing monitoring of hybridization will aid in determining the efficacy of the barrier to limit hybridization in the White River.

Samples from the Elk River in 2012 were collected from the mainstem above and below the Elko Dam. The dam serves as a barrier to upstream migration and limits any upward movement of RBT from Kooconusa Reservoir. Below the dam, in the lower river, the 5 samples showed 10% RBT allele frequency, lower than the RBT frequency of 17% estimated from Bennett's raw genetic data. Low sample size of five in the lower may explain differences and field collection in 2016 will focus on the Elk watershed. The twenty samples from above the dam showed no RBT allele frequency. Samples collected in 2015 from the Upper Fording, Greenhills Creek (near the Upper Fording) and the Lodgepole River (enters the Elk below Elko dam) are currently being genotyped at MGCL. Field collection in 2016 will focus around areas with past hybridization and downstream of areas with RBT stockings (Summit Lake, Grave Lake) that receive 3N genotypes but may still have a small percentage that are not sterile.

No evidence of RBT admixture was detected in samples from the Skookumchuck River, which previously reported to have low admixture (6%). Small sample size may have limited the ability to accurately estimate admixture in the Skookumchuck sample, but even with a sample size of nine, there is a high probability of detecting low levels of admixture. More samples have been collected from the Skookumchuck River in 2015 to confirm current admixture rates.

We sampled two populations from lakes to aid our understanding of the genetics of stocked WCT in the region. McNair Lake has been stocked with diploid WCT since 1978 (stocked every two years) and 1000 triploid RBT once in 2010. Samples taken from the 2014 captured fish were either RBT or WCT; no hybridization was evident. Moyie Lake, sampled in 2012 as part of a small lake gillnet assessment captured diploid WCT. WCT were stocked into Moyie Lake in 2005 and on various other occasions throughout the past century. WCT are most likely also native in the drainages surrounding Moyie Lake. The WCT stocking program currently sources brood from Connor Lake, which was once stocked with 50,000 WCT from Kiakho Lake in 1950. Connor Lake is located at the head of Forsyth Creek within the Elk River Watershed. Prior to the 1950 stocking, Connor Lake was considered barren. Brood for the WCT stocking program was also sourced from Kiahko Lake in the 1950's. The results of the PCA analysis lumped both Moyie Lake and McNair Lake together, suggesting that the fish captured in Moyie were from, or

similar to those stocked in the Kootenays. A goal of the WCT genetics in the future will be to discern stocked WCT and native populations within their current distribution.

Differences in admixture estimates from the data genotyped at MGCL and that previously genotyped may be due to differences in the samples including temporal shifts in allele frequencies (e.g. change in admixture), differences in the number of fish analyzed, and differences in the age of the fish collected, or the presence of migrants in the sample. Small differences in RBT allele frequencies are possible even when re-sampling the same population due to random chance. In addition, the use of different loci may have contributed to the observed differences as estimates may vary based on the number of loci used and their distribution across the genome.

Principal component analysis (PCA) was performed using the 34 variable SNPs on the seven sample collections where RBT admixture was estimated to be less than 10% using only individuals with less than 5% RBT alleles (Appendix 1, Figure A1.1). Additionally, the three fish where only WCT alleles were detected from McNair South Lake were also included. The purpose of the PCA was mainly for quality control, as it can be used to determine whether any fish may have been mislabelled or contaminated based how well they cluster with other fish from the same collection. First, the fish from the two lake collections appear to be differentiated from the river collections. Second, the Elk River collection is differentiated from the other river collections. An increased sample size, especially for the two lake collections, would improve the confidence in these differences. However, because some of the fish included in this analysis came from collections where RBT alleles were detected, some of the differentiation observed may be due to admixture even if the individual had a hybrid index of 0.

Only two sample collections showed no RBT introgression, seven had less than 10%, and no YCT alleles were detected in any fish. In general, admixture levels with RBT varied widely among sample collections, most of these samples are not hybrid swarms, and contain fish with a wide range of hybrid indices. Diversity in the two samples (Upper St. Mary's River and Skookumchuck River) where no RBT alleles were detected was moderately high. Between 65-71% of the SNP loci were variable, and the expected heterozygosity ranged from 0.218 to 0.226.

Recommendations

Genetic results from the 2015 sampling season, which were not available at the time of this report, will aid in filling the many data gaps that exist on current hybridization throughout the Upper Kootenay drainage. Sampling in 2016 will focus on the Elk River watershed. As well, at some sites, sample sizes were not met in 2015 for building population genetic data and 2016 will be pooled with 2015 samples for genotyping. As well genotyping using SNPs of archived DNA from previous hybridization studies in the Upper Kootenay will aid in understanding temporal trends in hybridization.

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References

Amish SJ, Hohenlohe PA, Painter S, Leary RF, Muhlfeld C, Allendorf FW, Luikart G. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. *Molecular Ecology Resources*. 2012; 12:653–660.

Bennett SN. 2007. Assessing the extent of hybridization between westslope cutthroat trout and introduced rainbow trout in the upper Kootenay River, British Columbia. A dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in fisheries biology. Utah State University. Logan, Utah. USA.

Bennett, S N & J L Kershner 2009. Levels of Introgression in Westslope Cutthroat Trout Populations Nine Years after Changes to Rainbow Trout Stocking Programs in Southeastern British Columbia, *North American Journal of Fisheries Management*, 29:5, 1271-1282, DOI: 10.1577/M08-048.1

Finger AJ, Stephens MR, Clipperton NW, May B. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trout. *Molecular Ecology Resources*. 9:759–763.

Kalinowski ST, Novak BJ, Drinan DP, Jennings deM, Vu NV. 2011. Diagnostic single nucleotide polymorphisms for identifying westslope cutthroat trout (*Oncorhynchus clarki lewisi*), Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) and rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology Resources*. 11:389–393.

- Leary, R. 2013. Report on the genetic status of westslope cutthroat trout in the Kootenai River drainage, Montana. Montana Fish, Wildlife & Parks. Report number 4392.
- Leary RF, FW Allendorf and KL Knudsen. 1987. Genetic divergence among populations of westslope cutthroat trout in the upper Kootenay River drainage, BC. Dept. Zoology University of Montana Laboratory.
- McGlaufflin MT, Smith MJ, Wang JT. 2010. High-resolution melting analysis for the discovery of novel single-nucleotide polymorphisms in rainbow and cutthroat trout for species identification. Transactions of the American Fisheries Society. 139:676–684.
- McPherson SJ. and Lamson, HM. 2014. 2013. Outlet Creek, fish genetics monitoring. Lotic Environmental Ltd and Ministry of Forests, Lands and Natural Resource Operations, Kootenay Region. 24 pgs.
- McPherson SJ and MD Robinson. 2013. Whiteswan Lake Provincial Park Fisheries Management Plan. Prepared by Lotic Environmental Ltd. for the Ministry of Forests, Lands and Natural Resource Operations.
- Ministry of Environment. 2013. Management Plan for the Westslope Cutthroat Trout (*Oncorhynchus clarki lewisii*) in British Columbia. BC Freshwater Fisheries Program. Prepared by BC Ministry of Environment. Victoria, BC
- Morris, K.J. and A. Prince. 2004. St. Mary River Westslope Cutthroat Trout Radio Telemetry Study 2001-2004. Report prepared by Westslope Fisheries Ltd., Cranbrook, B.C. Report prepared for Columbia-Kootenay Fisheries Renewal Partnership, Cranbrook B.C. 39 pp. + 5 App.
- Rubidge E. 2003. Molecular analysis of hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisii*), and introduced rainbow trout (*O. mykiss*) in southeastern British Columbia. Master's thesis, Dept. Zoology UBC Vancouver.
- Yau M and EB Taylor. 2013. Environmental and anthropogenic correlates of hybridization between westslope cutthroat trout (*Oncorhynchus clarki lewisii*), and introduced rainbow trout (*O. mykiss*). Conservation Genetics 14:4 885-900.

Appendix 1. PCA of low admixture WCT collections

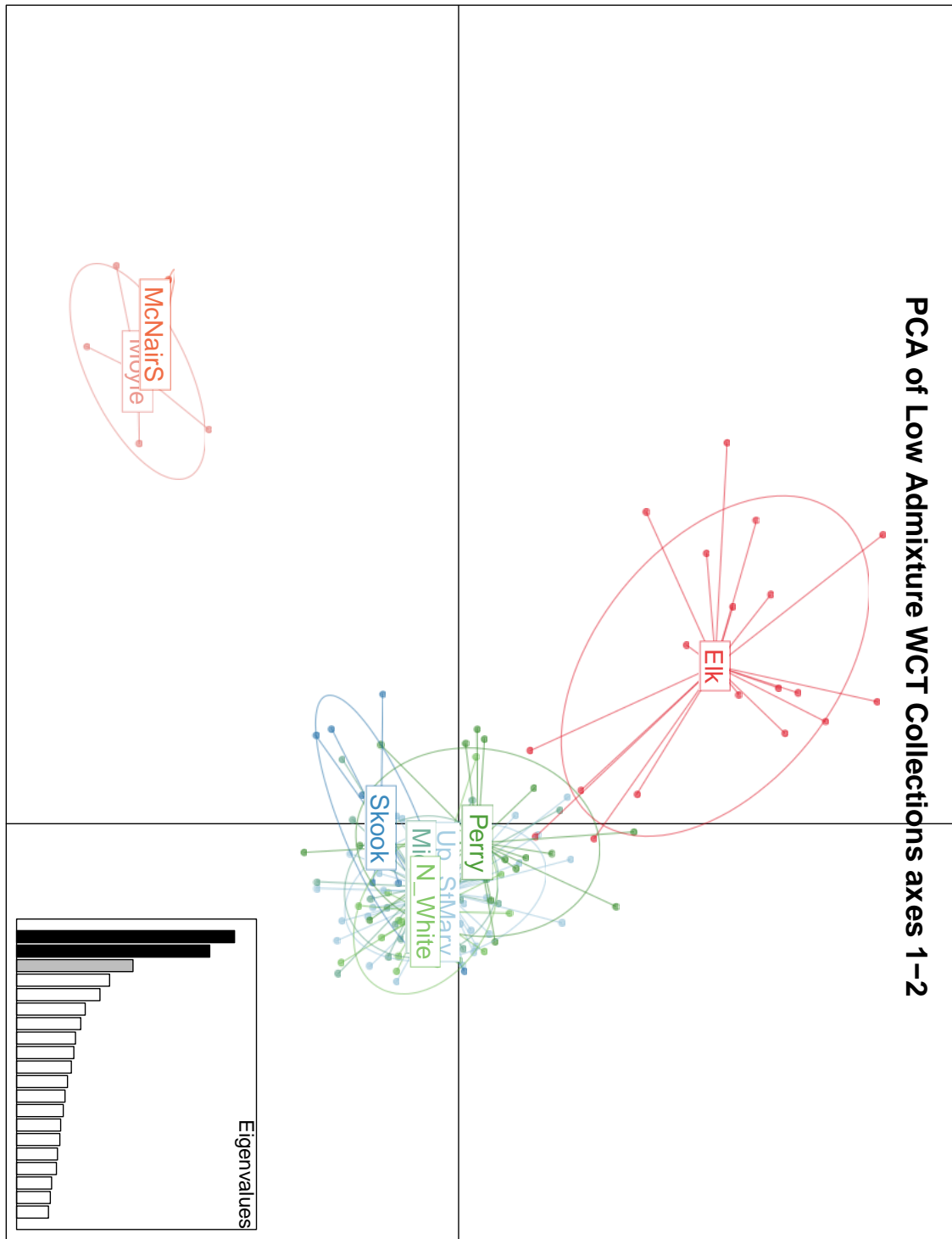


Figure A1.1 Preliminary principal component analysis of allele frequencies among fish from sample collections with low levels or no RBT admixture using 34 polymorphic WCT SNPs. Each dot represents a fish color-coded by sample collection. The main structuring is between the fish from the 2 lake collections and the river collections. The Elk River sample is also differentiated from the other river collections.

Appendix 2. Observed hybrid indices for sampled populations.

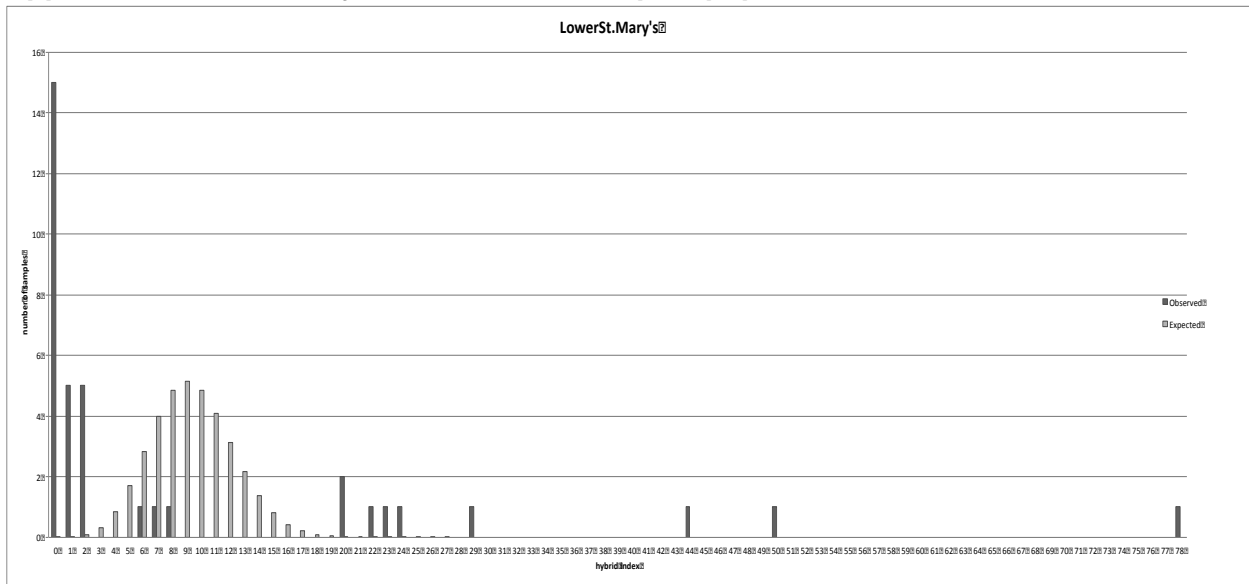


Figure A2.1. Observed hybrid indexes for fish in the Lower St. Mary's sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

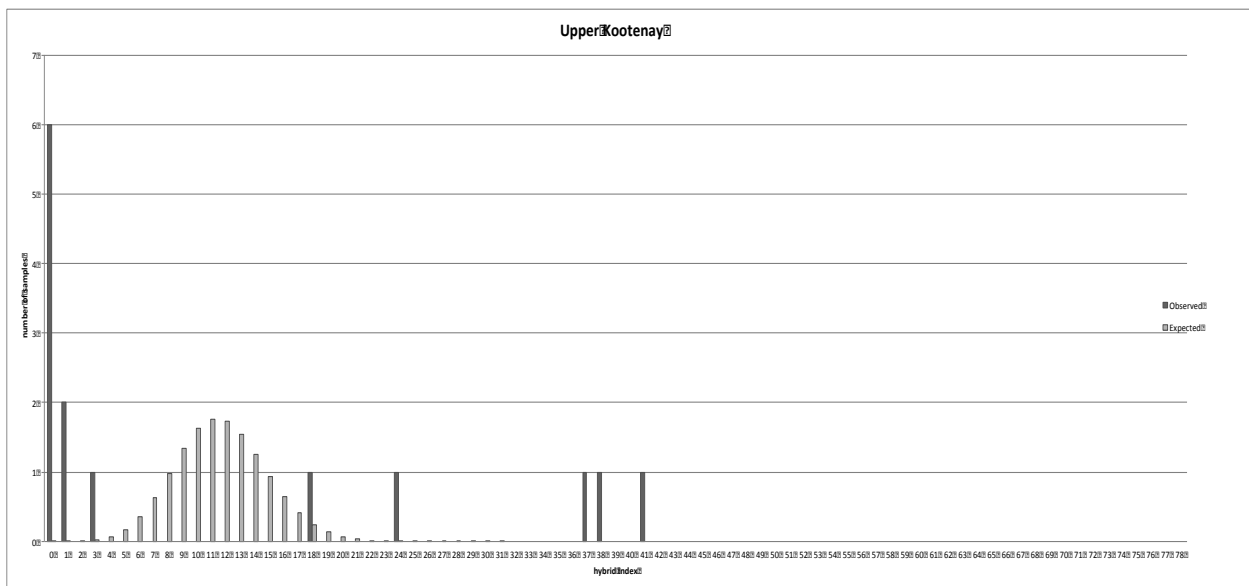


Figure A2.2. Observed hybrid indexes for fish in the Upper Kootenay River sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

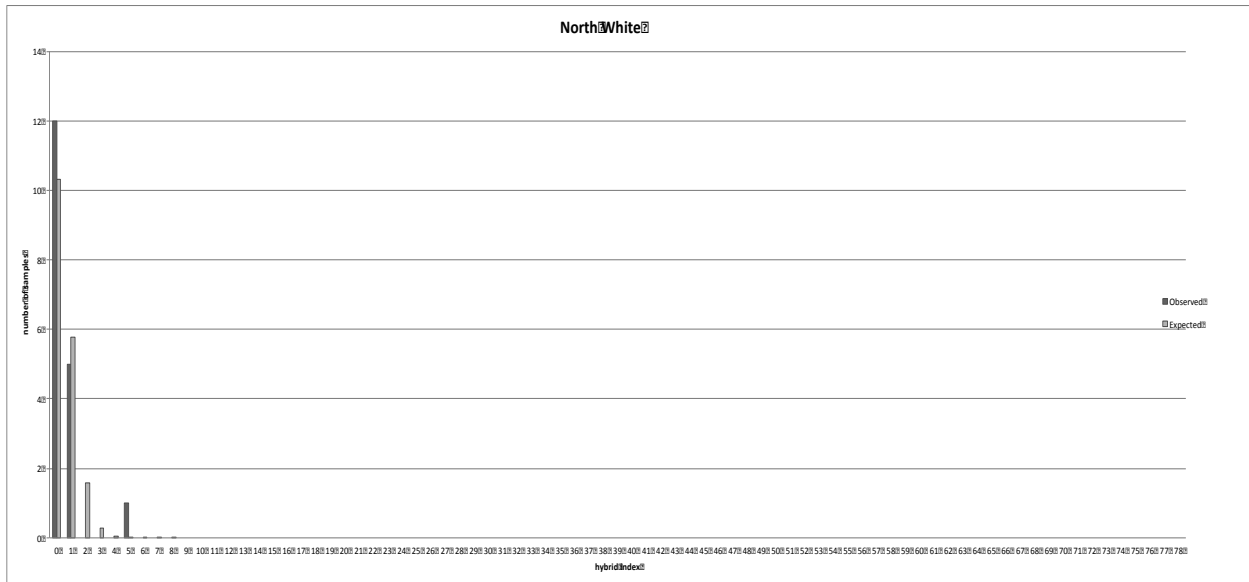


Figure A2.3. Observed hybrid indexes for fish in the North Fork of the White River sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution does not significantly ($P=0.36$) differ from the expected random one indicating the fish represent a hybrid swarm.

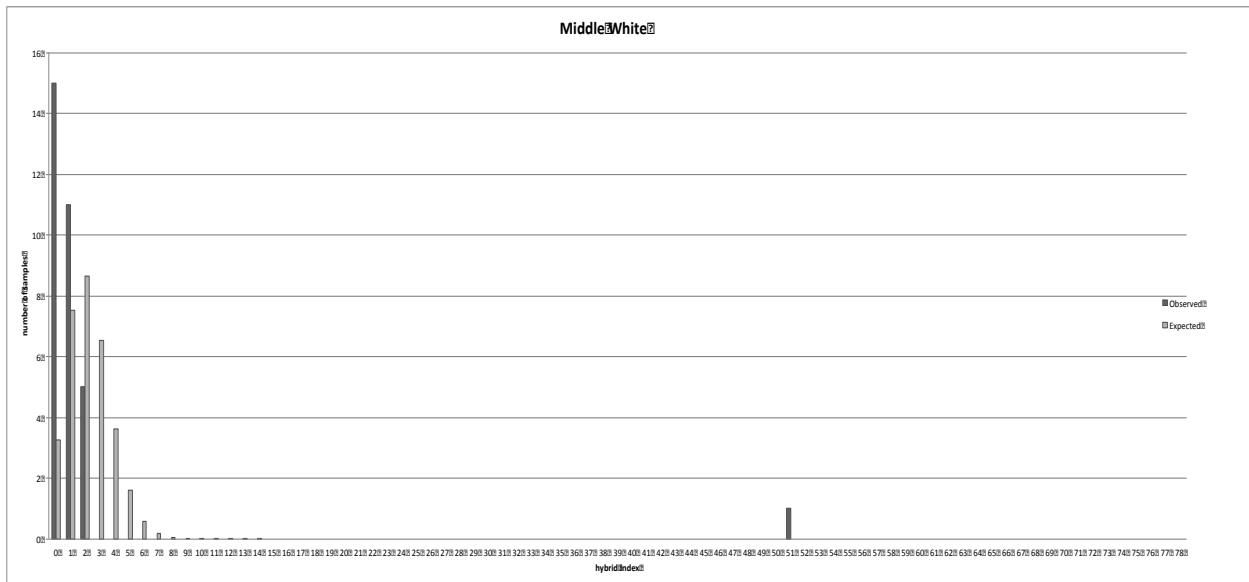


Figure A2.4. Observed hybrid indexes for fish in the North Fork of the White River sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P<0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

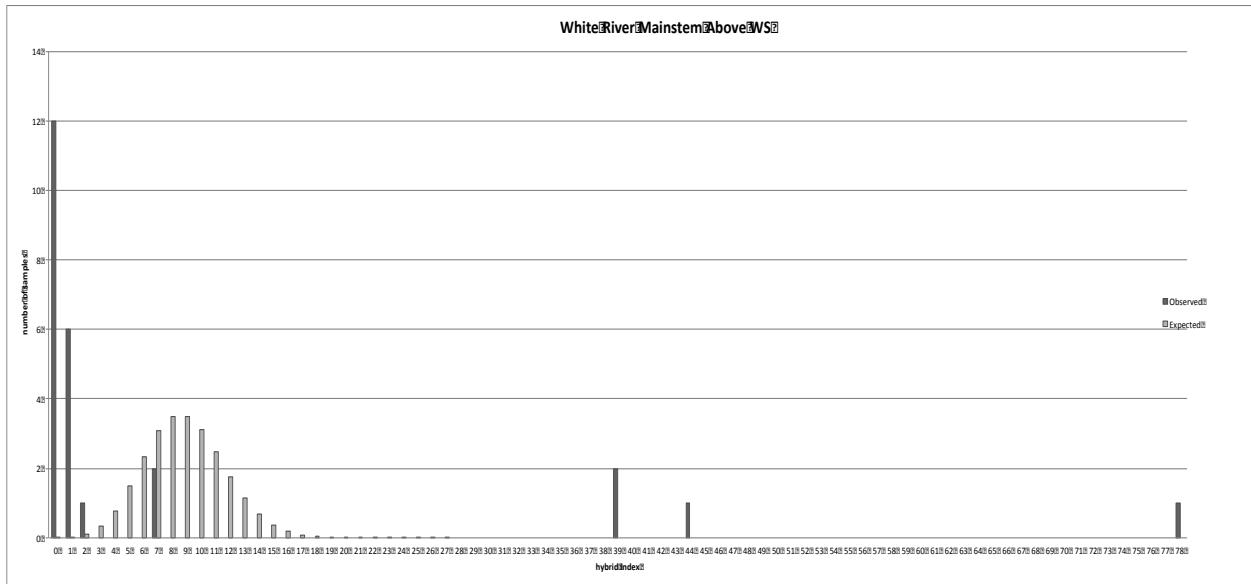


Figure A2.5. Observed hybrid indexes for fish in the White River Mainstem above WS sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

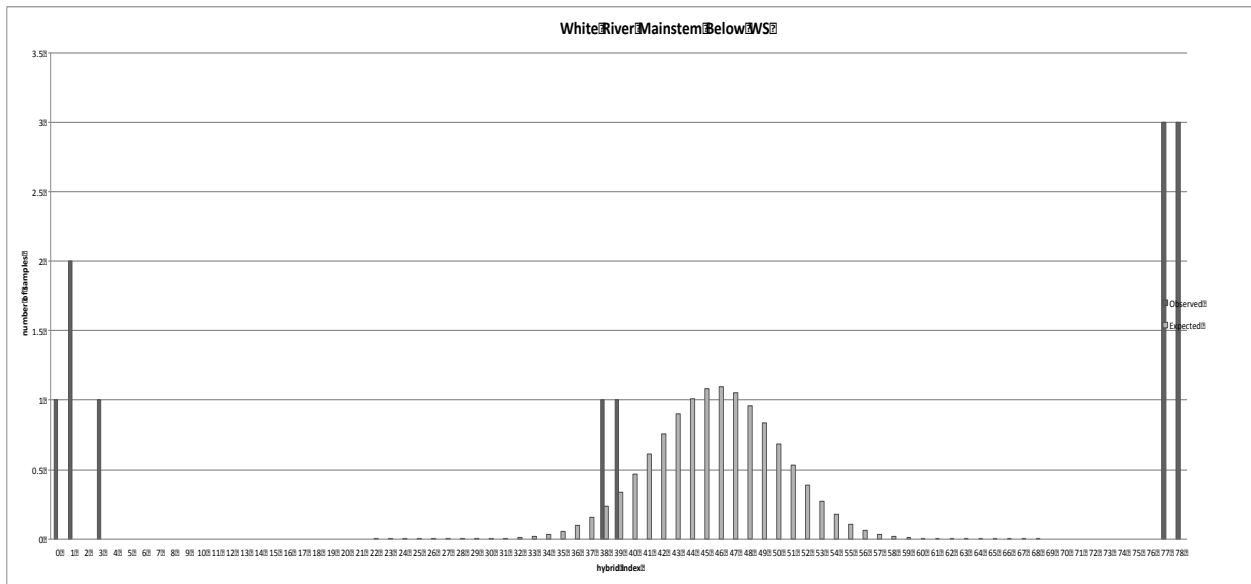


Figure A2.6. Observed hybrid indexes for fish in the White River Mainstem below WS sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

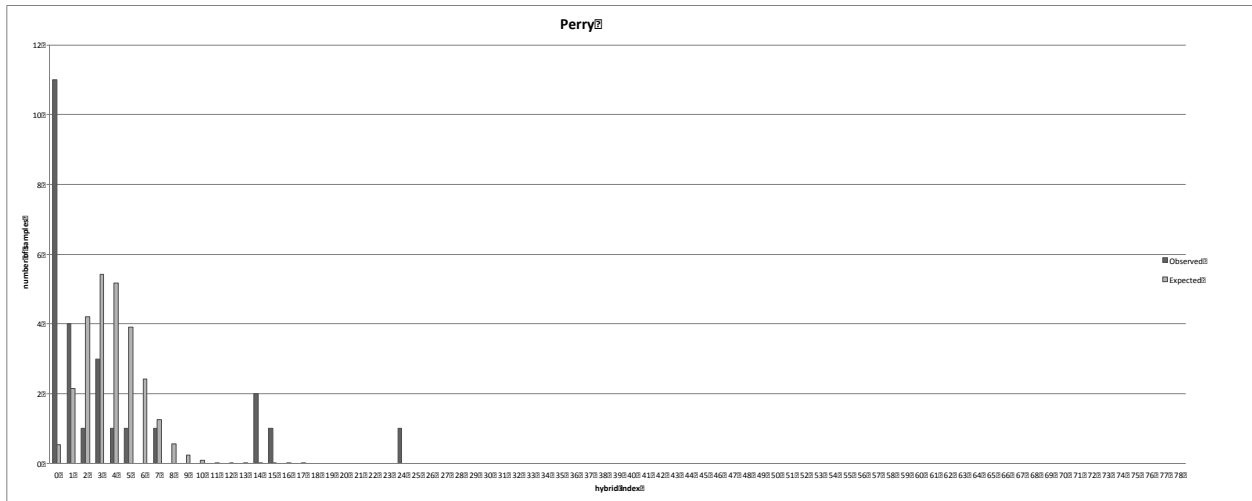


Figure A2.7. Observed hybrid indexes for fish in the Perry Creek sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.

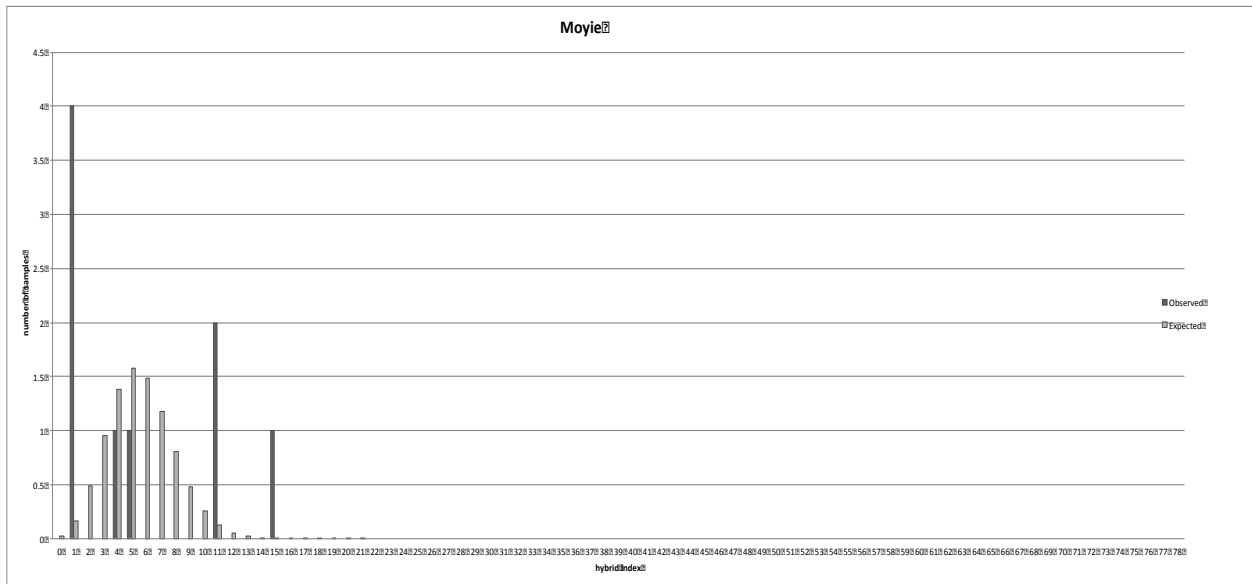


Figure A2.8. Observed hybrid indexes for fish in the Moyie Lake sample. Observed and expected random distribution of hybrid indices among the fish analyzed. Note the observed distribution significantly ($P < 0.001$) differs from the expected random one indicating the fish do not represent a hybrid swarm.