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

Final Report

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The Columbia Basin Trust and **Fish and Wildlife Compensation Program** on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and public stakeholders and;

The **Ministry of Forests, Lands and Natural Resource Operations and Rural Development,**

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Executive Summary

This report summarizes the findings of the multi-year (2014 to 2019) project studying hybridization between westslope cutthroat trout (WCT) and rainbow trout (RBT) in the Upper Kootenay watershed. Through partnership funding from the Columbia Basin Trust (the Trust), Fish and Wildlife Compensation Program (FWCP), Fisheries and Oceans Canada (DFO) and the Ministry of Forests, Lands and Natural Resource Operations (FLNRORD) this project focussed on determining current hybridization resulting from interbreeding between introduced rainbow trout and native WCT in the Upper Kootenay watershed.

A total of 2,549 tissue samples from trout captured within the BC distribution of WCT were genotyped using Single Nucleotide Polymorphic loci (SNPs) or Rad Capture (Rapture) techniques. Of these, 1,711 were within the Upper Kootenay Watershed. Samples were analyzed at the Montana Genetics Conservation Lab (MGCL).

Only WCT genotypes were detected in 18% of the sampled sites, while another 58% of the sites had WCT allele frequencies of 0.990 or higher. High rainbow trout admixture rates were detected in sites near Whiteswan Lake, in its Outlet Creek and in the White River near where Outlet Creek meets the river. Sample collections from tributaries directly connected to Koocanusa also showed high RBT admixture (Sand Creek, Lower Elk) as did sites near Koocanusa, and low in the watershed (Wildhorse, Lower St. Mary).

Hybridization rates were compared between thirty-seven sites in the Upper Kootenay repeatedly sampled from 1986 to 2017 and are presented in this report. Temporal comparisons were made between two of the sampling years where historic (sample years between 1999 and 2007) and contemporary (sample years between 2014 and 2017) rainbow trout admixture levels were compared. Generally, since the early 2000's, hybridization rates have remained static or decreased at most sites. Five sites showed significant increased rates of hybridization (Upper Kootenay River, St. Mary River, Palliser River, Lower Sand Creek and the North Fork of the White River). Levels of hybridization significantly decreased at ten sites (Lower Bull, Lower Lodgepole, Lower Elk, Caven, Lower Gold, Lower Mather, Lower Bloom, Mid Michel, Upper Gold and Lower Morrissey). Fifteen sites showed no significant changes and six sites remained unhybridized.

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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.

WCT 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 determining, and then maintaining the native distribution and genetic diversity of populations. Population groups within the WCT core range reflect the extent to which native populations may occur. 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 Koocanusa 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 aboriginal (or native) distribution of WCT populations in this region will promote informed management decisions, provide recreational opportunities, and 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. Samples were genotyped using allozyme markers.
- Rubidge et al. in 1999 and 2000 collected 981 fish at 23 sample sites in 12 different river systems. Samples were genotyped using microsatellite markers.
- Bennett et al. from 2001 to 2006 collected 1,065 additional fish at 26 sample sites. Samples were genotyped using microsatellite markers.

More recently, to address concerns from stakeholders on limited data on Whiteswan Lake being a source of rainbow trout to downstream water, the following genetic work on WCT hybridization 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. Samples were genotyped using microsatellite markers.

Goals and Objectives

The objectives of this project are to: (1) inventory current levels of hybridization of WCT populations in the Upper Kootenay drainage; (2) compare current levels of WCT x RBT hybridization with levels 10 to 20 years ago; (3) identify pure populations to be conserved for genetic integrity; and (4) 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 WCT distribution occurs within the Upper Kootenay watershed, which encompasses drainages that ultimately flow into the Koocanusa 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 in Montana near the location of the present-day Libby dam. This project focusses on the Canadian portion of the Upper Kootenay watershed which covers approximately 20,000 km^2.

Bordering WCT populations in British Columbia also are known to occur in the Upper Columbia watershed, Flathead drainage, West Kootenay, Kettle and South Thompson. Genetic analysis will subsequently occur throughout the BC WCT range through partnership funding (FLNRORD, DFO).

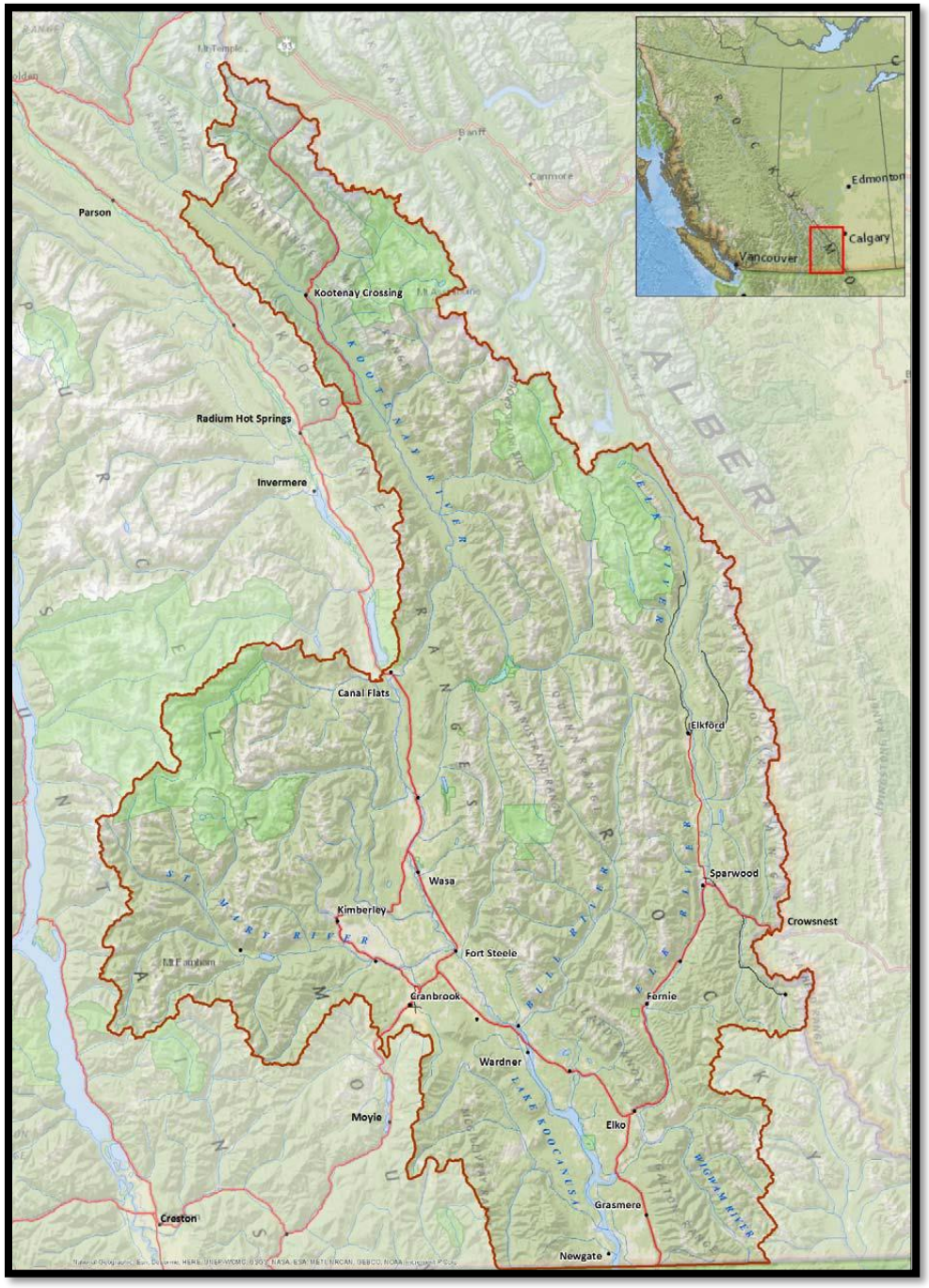

Figure 1. Westslope cutthroat trout hybridization project study area (Upper Kootenay Drainage).

Methods

WCT Hybridization Inventory

Site locations to monitor hybridization throughout the BC WCT distribution, including the Upper Kootenay drainage 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 based on a past radio telemetry study that determined distinct spawning areas upstream and downstream of St. Mary Lake (Morris and Prince 2004). In locations without data to aid in distinguishing randomly mating populations, sites were determined by distance or presence of barriers.

Because an increased number of markers were employed utilizing newer genetic analytic techniques than ten to twenty years ago, sample sizes can be lower and still provide the same power to detect RBT or YCT admixture. Consequently, 24 samples from each site were targeted for collection. Sites included those previously sampled for hybridization to monitor temporal changes (e.g. sites sampled previously by Rubidge & Bennett), sites which may have been a source of migrant rainbow trout, and sites lacking information on hybridization.

Samples were collected by angling or electrofishing. A small piece of adipose or caudal 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.

Genetic Analysis of WCT Hybridization

A species diagnostic locus has non-overlapping allele sizes or is fixed for alternate alleles in the two parental taxa. To determine WCT and RBT hybridization, an individual trout could have zero, one, or two RBT alleles at each locus. The proportion of WCT alleles (WCT frequency) in each population sample was calculated as the number of WCT alleles divided by the total number of alleles genotyped. Hybridization between WCT and Yellowstone cutthroat trout (YCT) or Coastal cutthroat trout (CCT) were similarly determined.

Samples were genotyped by the Montana Conservation Genetics Lab (MGCL) at the University of Montana Flathead Lake Biological Station. Samples collected between 2014 and 2016 were genotyped utilizing a 95 Single Nucleotide Polymorphic (SNP) chip containing both species diagnostic and WCT variable SNPs. SNP loci included 19 that differentiate RBT from WCT and YCT (RBT diagnostic markers), 20 that distinguish WCT from RBT and YCT (WCT diagnostic markers), and 20 loci that distinguish YCT from WCT and RBT (YCT diagnostic markers). Thirty-four loci that are generally variable within WCT 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.

Samples collected in 2017 were genotyped at a higher number of SNP loci using RAD-capture. Genetic samples were prepared and sequenced according to the best RAD protocol and Rapture (RAD-capture) for genotyping (Ali et al. 2016). The capture was performed targeting 3,015 regions of the genome using a MyBaits Custom Target Enrichment kit produced by Arbor

Biosciences. Baits were designed to capture RAD-tags that contain any combination of WCT polymorphic SNPs or WCT, YCT, and RBT species diagnostic SNPs (Amish et al. 2012; Hohenlohe et al. 2013; Kovach et al. 2016). SNP loci were chosen for capture based on their genotyping quality, reliability for distinguishing each (sub)species (i.e., SNP is monomorphic within a subspecies – species diagnostic), and even distribution across the assembled rainbow trout genome. RBT, YCT, and WCT diagnostic loci were separated from WCT polymorphic loci and only diagnostic markers were used in the following analyses. For each set of species diagnostic loci, loci were dropped if they were missing in more than 30% of individuals. Last, individuals were dropped from the analysis if they did not have genotypes at 25% of the loci remaining after quality score filtering.

Temporal Analysis

Sites where sampling was repeated for spatio-temporal analysis followed methodology and locations reported in previous studies (Rubidge 2003; Bennett 2007; Yau and Taylor 2013). Reported locations, methods and seasonal timing were emulated at sites as best as possible. Tissue collection and genotyping did not differ from methods described above for inventory.

Raw genotype data of individual fish at sites within the Upper Kootenay watershed were provided by Dr. Stephen Bennett. This database included locations, fish length and 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). WCT frequencies were also summarized from published literature and reports not included in this dataset.

Different genotyping methodologies have been used over time to determine hybridization in WCT as the field of genetics has quickly evolved. Table 1 summarizes the different markers that have been used over time to identify hybridization in the Upper Kootenay.

Hybridization is reported in the results as WCT allele frequency. For samples collected prior to 2012, microsatellite markers determined hybridization with RBT only and was calculated as 1- proportion of RBT alleles in a population (site). For samples analysed using the SNP chip, the WCT allele frequency utilized 1- proportion of non WCT alleles in a population (site). The SNP chip included diagnostic loci for Yellowstone cutthroat trout (YCT) (and Rad-Capture included coastal cutthroat trout CCT). Yellowstone cutthroat trout (YCT) allele frequencies were also determined for each site. First generation hybrids (F1) are offspring from a mating between a WCT and an RBT (or YCT) and are determined from having one allele of each parental type at each diagnostic loci. As SNPs use so many more diagnostic loci than microsatellite markers, the accuracy of determining F1's is much greater.

To test for significant temporal changes in hybridization, the WCT frequency in the most recent sample was subtracted from the WCT frequency from one historical sample. As sites might have been sampled more than two times temporally, the two sample years chosen were ones with large sample sizes or defaulted to years sampled by Bennett due to a higher number of markers being used. Significant changes in WCT frequency over time were tested as described by Muhlfeld et al. (2017). Briefly, at each location with repeated sampling, an exact fisher test was utilized to test for significant ($P < 0.05$) changes in WCT frequency between historical and contemporary samples. The exact Fisher test accounts for uncertainty in estimates of population

level WCT frequency due to sampling variation in the historical and contemporary samples, that is, differences in the number of diagnostic loci (alleles) and sample sizes used to calculate WCT frequency in each sample.

Table 1. Genotyping methods utilized to decipher hybridization in the Upper Kootenay Watershed.

SAMPLE YEARS	SOURCE	HYBRIDS TESTED	TECHNIQUE	MARKERS
1986	Leary, Allendorf and Knudsen	WCT, RBT, YCT	6 allozyme markers	Aat1, Ck2, Gpi3, Idh3,4, Me1, Sdh
1999, 2000	Rubidge 2003	WCT, RBT, YCT	4 microsatellite loci	Ikaros, Occ16, hsc 71, Om 13
2001 TO 2010	Bennett, 2010	WCT, RBT, YCT	7 microsatellite loci	Occ34, Occ35, Occ36, Occ37, Occ38, Occ42, Om55
2001 (CONNOR)	Taylor, Stamford and Baxter 2003	WCT, RBT, YCT	8 microsatellite loci	Omy77, Ssa85, Ssa197, Ssa456, Ots3, Ots103, One14, Oki3a
2012 (OUTLET)	McPherson and Lamson 2014	WCT, RBT, YCT	5 microsatellite loci	Omm11, Occ16, Occ34, Occ36, Occ42
2014 TO 2016	Amish, SJ	WCT, RBT, YCT	SNP chip	94 diagnostic SNPs
2017	Amish, SJ	WCT, RBT, YCT	Rad Capture	647 diagnostic RB SNPs, 275 diagnostic YCT SNPS, 291 diagnostic WCT SNPs

Results

Over 2,000 trout were sampled throughout the Upper Kootenay watershed from 2014 to 2019 and genotypes have been successfully generated for 1,711 of these trout. Table 2Table 2 presents a summary of the hybridization results from populations sampled in the Upper Kootenay Watershed. WCT allele frequencies are shown in bold and indicate the level of WCT purity of the population (site). A WCT frequency of 1.0 indicates that all alleles were diagnostic of WCT; no RBT, YCT or CCT alleles were detected.

In 13 of the 74 (18%) sample collections, only WCT alleles were detected, while another 43 (58%) collections had WCT allele frequencies of 0.990 or higher. High RBT admixture rates were detected in sample collections from sites near Whiteswan Lake, in its Outlet Creek and in the White River near where Outlet Creek meets the river. Sample collections from tributaries directly connected to Koocanusa also showed high RBT admixture (Sand Creek, Lower Elk) as did collections from sites near Koocanusa, and low in the watershed (Wildhorse, Lower St. Mary).

First generation hybrids (F1), indicative of ongoing hybridization, were detected in three sites sampled in the Upper Kootenay: the mainstem of the White River below Whiteswan Lake, the mainstem of the White River upstream of Whiteswan Lake and Lower Sand Creek.

Alleles diagnostic of Yellowstone Cutthroat Trout were detected in low frequencies in sample collections at five sites. These included Dewar Creek, Lower Wigwam River, Upper Wildhorse River, Lower Mather Creek and Upper Wigwam River.

The percent individuals in a sample collection with YCT or RBT admixture can illuminate whether this is due to a few individuals with a high non-WCT allele frequency (e.g. a high number of non-WCT alleles), or due to many individuals with a low non-WCT allele frequency. If the sample collection is a representative sample from the population, individual levels of hybridization and how evenly admixture is distributed among individuals allows inference about the extent and time since the admixture event. For example, if only two individuals with high RBT allele frequencies are detected, this suggests a recent hybridization event (or evidence for migrants from an RBT source population). In contrast, if many individuals with a very low RBT allele frequency are detected, this suggests a hybridization event occurred many generations in the past. The West Fork and White Creeks of the Upper St Mary drainage, and Upper Wildhorse River all have low levels of RBT admixture detected in a large percentage of the sample.

Table 2. Genotype results from populations sampled 2014 to 2017 in the Upper Kootenay Watershed.

SITE	DRAINAGE	SAMPLE YEAR	N	GENOTYPING METHOD	WCT ALLELE FREQUENCY	YCT ALLELE FREQUENCY	F1	PERCENT INDIVIDUALS WITH RBT OR YCT ADMIXTURE	COMMENTS
MICHEL, LOWER	Upper Elk	2012	6	SNP-Chip	**1.0000**	0.0000	0	0%	
MICHEL, MID	Upper Elk	2012	4	SNP-Chip	**1.0000**	0.0000	0	0%	
ST. MARY, UPPER	Upper Kootenay	2014	35	SNP-Chip	**1.0000**	0.0000	0	0%	
GREENHILLS	Upper Kootenay	2015	30	SNP-Chip	**1.0000**	0.0000	0	0%	
LODGEPOLE, UPPER	Upper Kootenay	2015	24	SNP-Chip	**1.0000**	0.0000	0	0%	
UPPER FORDING	Upper Kootenay	2015	29	SNP-Chip	**1.0000**	0.0000	0	0%	
CONNOR LAKE	Upper Elk	2016	24	SNP-Chip	**1.0000**	0.0000	0	0%	
ELK, MID	Upper Elk	2016	17	SNP-Chip	**1.0000**	0.0000	0	0%	
ELK, UPPER	Upper Elk	2016	25	SNP-Chip	**1.0000**	0.0000	0	0%	
FORSYTH, LOWER	Upper Elk	2016	20	SNP-Chip	**1.0000**	0.0000	0	0%	
GOLD, UPPER	Upper Kootenay	2016	23	SNP-Chip	**1.0000**	0.0000	0	0%	
GRAVE, MID AND UPPER	Upper Elk	2016	34	SNP-Chip	**1.0000**	0.0000	0	0%	
HARMER	Upper Elk	2016	15	SNP-Chip	**1.0000**	0.0000	0	0%	
MORRISSEY, LOWER	Upper Elk	2016	24	SNP-Chip	**1.0000**	0.0000	0	0%	
WEARY	Upper Elk	2016	24	SNP-Chip	**1.0000**	0.0000	0	0%	
ANGUS	Upper Kootenay	2017	24	RAD Capture	**0.9997**	0.0000	0	21%	
HELLROARING	Upper Kootenay	2017	24	RAD Capture	**0.9995**	0.0000	0	21%	
ELK, MID	Upper Elk	2017	20	RAD Capture	**0.9993**	0.0000	0	35%	
BLOOM	Upper Kootenay	2015	20	SNP-Chip	**0.9993**	0.0000	0	5%	
WHITE, ALL TRIBS AND UPPER MAINSTEM	Upper Kootenay	2015	67	SNP-Chip	**0.9990**	0.0000	0	7%	
GRAVE, LOWER	Upper Elk	2016	20	SNP-Chip	**0.9990**	0.0000	0	5%	
WILDHORSE, UPPER	Upper Kootenay	2017	14	RAD Capture	**0.9988**	**0.0066**	0	50%	
BULL, UPPER	Upper Kootenay	2015	20	SNP-Chip	**0.9987**	0.0000	0	5%	
MICHEL, UPPER	Upper Elk	2016	17	SNP-Chip	**0.9985**	0.0000	0	6%	
REDDING	Upper Kootenay	2017	20	RAD Capture	**0.9984**	0.0002	0	25%	
LIZARD	Upper Elk	2016	24	SNP-Chip	**0.9984**	0.0000	0	25%	

SITE	DRAINAGE	SAMPLE YEAR	N	GENOTYPING METHOD	WCT ALLELE FREQUENCY	YCT ALLELE FREQUENCY	F1	PERCENT INDIVIDUALS WITH RBT OR YCT ADMIXTURE	COMMENTS
ELK, MID	Upper Elk	2014	15	SNP-Chip	**0.9983**	0.0000	0	7%	
DEWAR	Upper Kootenay	2017	8	RAD Capture	**0.9982**	**0.0002**	0	13%	
GALBRAITH CREEK	Upper Kootenay	2017	23	RAD Capture	**0.9980**	0.0000	0	30%	
ST. MARY, WEST FORK	Upper Kootenay	2017	16	RAD Capture	**0.9980**	0.0002	0	50%	
TEEPEE, LOWER	Upper Kootenay	2015	24	SNP-Chip	**0.9980**	0.0000	0	13%	
HOSMER	Upper Elk	2016	24	SNP-Chip	**0.9979**	0.0000	0	17%	
MICHEL, MID	Upper Elk	2016	20	SNP-Chip	**0.9976**	0.0000	0	19%	
LEACH	Upper Elk	2017	24	RAD Capture	**0.9975**	0.0000	0	50%	
WHITE CREEK, ST. MARY	Upper Kootenay	2017	17	RAD Capture	**0.9971**	0.0000	0	65%	
CAVEN	Upper Kootenay	2015	22	SNP-Chip	**0.9964**	0.0000	0	9%	
WIGWAM, UPPER	Lower Elk	2016	22	SNP-Chip	**0.9964**	**0.0036**	0	5%	
ALEXANDER, LOWER	Upper Elk	2016	18	SNP-Chip	**0.9964**	0.0000	0	22%	Adults
MEACHEN, UPPER	Upper Kootenay	2017	24	RAD Capture	**0.9960**	0.0000	0	8%	
MICHEL, LOWER	Upper Elk	2017	19	RAD Capture	**0.9959**	0.0000	0	74%	
COAL	Upper Elk	2016	24	SNP-Chip	**0.9957**	0.0000	0	17%	
SAND, UPPER	Upper Kootenay	2017	24	RAD Capture	**0.9952**	0.0000	0	21%	
MICHEL, LOWER	Upper Elk	2016	22	SNP-Chip	**0.9944**	0.0000	0	25%	
FORDING, LOWER	Upper Elk	2016	29	SNP-Chip	**0.9943**	0.0000	0	34%	
PUDDING BURN	Upper Kootenay	2017	17	RAD Capture	**0.9910**	0.0000	0	94%	
SANDOWN	Upper Kootenay	2017	21	RAD Capture	**0.9900**	0.0000	0	48%	
WHITE, NORTH FORK	Upper Kootenay	2014	18	SNP-Chip	**0.9900**	0.0000	0	44%	
MATTHEW, LOWER	Upper Kootenay	2017	25	RAD Capture	**0.9867**	**0.0003**	0	68%	
GOLD	Upper Kootenay	2015	24	SNP-Chip	**0.9861**	0.0000	0	17%	
FINDLAY	Upper Kootenay	2015	28	SNP-Chip	**0.9836**	0.0000	0	7%	
SKOOKUMCHUCK, UPPER	Upper Kootenay	2015	29	SNP-Chip	**0.9830**	0.0000	0	7%	
ALEXANDER, LOWER	Upper Elk	2017	18	RAD Capture	**0.9783**	0.0001	0	83%	Juveniles
LUSSIER, UPPER	Upper Kootenay	2015	24	SNP-Chip	**0.9715**	0.0000	0	29%	
WHITE, MIDDLE FORK	Upper Kootenay	2014	32	SNP-Chip	**0.9700**	0.0000	0	53%	

SITE	DRAINAGE	SAMPLE YEAR	N	GENOTYPING METHOD	WCT ALLELE FREQUENCY	YCT ALLELE FREQUENCY	F1	PERCENT INDIVIDUALS WITH RBT OR YCT ADMIXTURE	COMMENTS
WIGWAM, MID	Lower Elk	2016	14	SNP-Chip	**0.9698**	0.0000	0	50%	
WICKMAN	Upper Kootenay	2015	22	SNP-Chip	**0.9691**	0.0000	0	18%	
PALLISER	Upper Kootenay	2015	24	SNP-Chip	**0.9676**	0.0000	0	79%	
GOLD, LOWER	Upper Kootenay	2016	24	SNP-Chip	**0.9620**	0.0000	0	21%	
FENWICK	Upper Kootenay	2015	23	SNP-Chip	**0.9598**	0.0000	0	22%	
PERRY, LOWER	Upper Kootenay	2014	26	SNP-Chip	**0.9500**	0.0000	0	58%	
PERRY	Upper Kootenay	2017	19	RAD Capture	**0.9373**	0.0000	0	95%	
WILDHORSE, LOWER	Upper Kootenay	2015	24	SNP-Chip	**0.9355**	0.0000	0	46%	
LUSSIER, LOWER	Upper Kootenay	2015	20	SNP-Chip	**0.9301**	0.0000	0	65%	
MATHER, LOWER	Upper Kootenay	2015	24	SNP-Chip	**0.9298**	0.0000	0	50%	
JOSEPH	Upper Kootenay	2017	21	RAD Capture	**0.9212**	0.0000	0	95%	
LODGEPOLE, LOWER	Upper Kootenay	2015	24	SNP-Chip	**0.9210**	0.0000	0	33%	
WHITE, MAINSTEM (ABOVE WHITESWAN)	Upper Kootenay	2014	27	SNP-Chip	**0.9000**	0.0000	**1**	48%	
BULL, LOWER	Upper Kootenay	2015	24	SNP-Chip	**0.8946**	0.0000	0	38%	
ELK, LOWER	Upper Kootenay	2016	25	SNP-Chip	**0.8907**	0.0000	0	40%	
ST. MARY, LOWER	Upper Kootenay	2014	37	SNP-Chip	**0.8800**	0.0000	0	57%	
WIGWAM, LOWER	Lower Elk	2016	22	SNP-Chip	**0.8679**	**0.0068**	0	55%	
UPPER KOOTENAY	Upper Kootenay	2014	14	SNP-Chip	**0.8500**	0.0000	0	57%	
SAND, LOWER	Upper Kootenay	2015	32	SNP-Chip	**0.6815**	0.0000	0	47%	
SAND, LOWER	Upper Kootenay	2017	23	RAD Capture	**0.6455**	**0.0004**	**2**	100%	
WHITE, MAINSTEM (BELOW WHITESWAN)	Upper Kootenay	2014	12	SNP-Chip	**0.4100**	0.0000	**1**	67%	
OUTLET	Upper Kootenay	2014	25	SNP-Chip	**0.0100**	0.0000	0	56%	

A map of WCT purity of genotyped populations in the Upper Kootenay drainage sampled from 2014 to 2017 is presented in **Error! Reference source not found.**. Allele frequency is categorized into five categories:

- 0 to 0.5 WCT allele frequency being pure rainbow to very highly admixed populations,
- 0.5001 t 0.850 WCT allele frequency being populations with high admixture,
- 0.8501 to 0.950 WCT allele frequency being moderately admixed,
- 0.9501 to .9950 WCT allele frequency with low admixture and
- 0.9951 to 1.000 WCT allele frequency being pure.

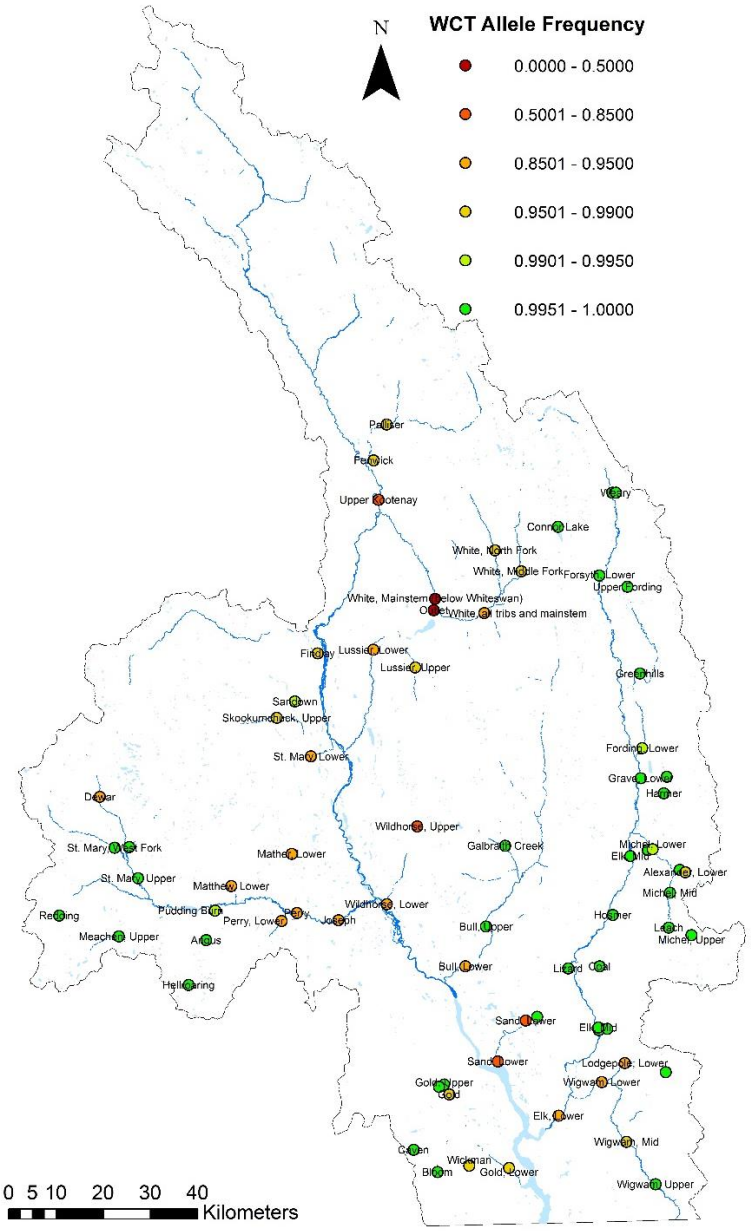

Figure 2. Westslope Cutthroat Trout Genetic purity of populations sampled 2014 to 2017.

Temporal Analysis of Hybridization in the Upper Kootenay Watershed

A total of 37 sites in the Upper Kootenay watershed had repeated hybridization testing of at least ten years apart. Table 3 summarizes changes in hybridization at sites in the Upper Kootenay and displays WCT allele frequencies. All sample years are presented but significance was tested on only two sample years (bolded in Table 3). After accounting for differences in sample size due to variation in number of molecular markers and individuals genotyped, rainbow trout hybridization significantly decreased at 10 sites (mean difference = 9.5%, range: 1-30%), increased at 5 sites (mean = 6%, range: 1-14%), and did not change at 15 sites (50%); 6 sites remained non-hybridized.

Table 3. Summary of hybridization in repeatedly sampled sites in the Upper Kootenay Watershed. Sites at the top of the list show hybridization getting better (higher WCT allele frequencies at sites) where those at the bottom of the list showed increased hybridization. A temporal binomial comparison was conducted on sample years in bold and significance is depicted by a * and bolded in the change in hybridization column.

Site	Year	N	% Indiv. with Pure RB or Hybrid	WCT Allele Freq.	F1	Gen. Method	Change in Hyb	Change in % Indiv. with Hyb
Bull, Lower	2000	30	100%	0.0290	0	Microsat.	**Large Decrease***	Decrease
	2001	30	100%	0.0302	0	Microsat.		
	2007	31	13%	0.7120	0	Microsat.		
	2015	24	38%	0.8946	0	SNP		
Lodgepole, Lower	**2000**	30	67%	0.6261	2	Microsat.	**Large Decrease***	Decrease
	2015	24	33%	0.9210	0	SNP		
Gold, Lower	1986	34	0%	1.0000	0	Allozyme	**Large Decrease***	Decrease
	1999	36	31%	0.8134	0	Microsat.		
	2000	30	37%	0.6975	1	Microsat.		
	2003	31	19%	0.8986	1	Microsat.		
	2006	30	37%	0.7119	2	Microsat.		
	2016	24	21%	0.9620	0	SNP		
Elk, Lower	**2004**	28	39%	0.7015	1	Microsat.	**Large Decrease***	Same
	2016	25	40%	0.8907	0	SNP		
Bloom, Lower	2000	30	37%	0.8409	1	Microsat.	**Large Decrease***	Decrease
	2003	29	14%	0.9630	1	Microsat.		
	2015	20	5%	0.9993	0	SNP		
Caven	**2000**	19	37%	0.9145	0	Microsat.	**Decrease***	Decrease
	2015	22	9%	0.9964	0	SNP		
Mather, Lower	**2000**	30	40%	0.8843	0	Microsat.	**Decrease***	Increase
	2015	24	50%	0.9298	0	SNP		
Gold, Upper	**2000**	30	13%	0.9746	0	Microsat.	**Decrease***	Decrease
	2016	23	0%	1.0000	0	SNP		
Morrissey, Lower	**1999**	30	3%	0.9870	0	Microsat.	**Decrease***	Decrease

Site	Year	N	% Indiv. with Pure RB or Hybrid	WCT Allele Freq.	F1	Gen. Method	Change in Hyb	Change in % Indiv. with Hyb
	2016	24	0%	1.0000	0	SNP		
Michel, Mid	2001	33	6%	0.9953	0	Microsat.	**Decrease***	Variable
	2002	37	16%	0.9595	0	Microsat.		
	2003	28	11%	0.9864	1	Microsat.		
	2005	31	10%	0.9816	0	Microsat.		
	2006	30	3%	0.9929	0	Microsat.		
	2008	31	6%	0.9953		Microsat.		
	2016	16	19%	0.9976	0	SNP		
Teepee, Lower	2000	30	17%	0.9750	0	Microsat.	Decrease	Decrease
	2003	30	3%	0.9976	0	Microsat.		
	2015	24	13%	0.9980	0	SNP		
Skookumchuck, Upper	**1999**	40	3%	0.9651	0	Microsat.	Decrease	Increase
	2015	29	7%	0.9830	0	SNP		
Coal	**2000**	36	11%	0.9872	0	Microsat.	Decrease	Increase
	2016	24	17%	0.9957	0	SNP		
Grave, Lower	**2002**	13	8%	0.9890	0	Microsat.	Decrease	Variable
	2005	7	0%	1.0000	0	Microsat.		
	2016	20	5%	0.9990	0	SNP		
Wildhorse, Lower	**2000**	45	38%	0.9250	0	Microsat.	Variable	Increase
	2007	31	10%	0.9862	0	Microsat.		
	2015	24	46%	0.9355	0	SNP		
Perry, Lower	1999	31	13%	0.9556	0	Microsat.	Variable	Increase
	2000	214	20%	0.9512	0	Microsat.		
	2001	99	30%	0.9103	0	Microsat.		
	2002	48	21%	0.9599	0	Microsat.		
	2004	57	37%	0.9316	0	Microsat.		
	2006	30	50%	0.9333	0	Microsat.		
	2014	26	58%	0.9500	0	SNP		
	2017	19	79%	0.9401	0	Rapture		
Michel, Lower	**2003**	29	7%	0.9951	0	Microsat.	Variable	Increase
	2012	6	0%	1.0000	0	SNP		
	2016	16	25%	0.9944	0	SNP		
	2017	19	68%	0.9958	0	Rapture		
Connor Lake	2001		0%	1.0000	0	Microsat.	Same	Same
	2016	24	0%	1.0000	0	SNP		
Elk, Upper	1986	29	0%	1.0000	0	Allozyme	Same	Same
	1999	38	0%	1.0000	0	Microsat.		
	2002	67	0%	1.0000	0	Microsat.		

Site	Year	N	% Indiv. with Pure RB or Hybrid	WCT Allele Freq.	F1	Gen. Method	Change in Hyb	Change in % Indiv. with Hyb
	2016	25	0%	1.0000	0	SNP		
Forsyth, Lower	2005	23	0%	1.0000	0	Microsat.	Same	Same
	2016	20	0%	1.0000	0	SNP		
Lodgepole, Upper	2004	28	0%	1.0000	0	Microsat.	Same	Same
	2015	24	0%	1.0000	0	SNP		
St. Mary, Upper	1986	40	0%	1.0000	0	Allozyme	Same	Same
	1999	31	0%	1.0000	0	Microsat.		
	2000	299	0%	1.0000	0	Microsat.		
	2014	35	0%	1.0000	0	SNP		
Upper Fording	2000	34	0%	1.0000	0	Microsat.	Same	Same
	2015	29	0%	1.0000	0	SNP		
Meachen, Mid	2000	24	0%	1.0000	0	Microsat.	Slight Increase	Same
	2017	24	0%	0.9960	0	Rapture		
Alexander, Lower	2000	30	33%	0.8258	0	Microsat.	Slight Increase	Increase
	2003	20	5%	0.9893	0	Microsat.		
	2016	18	22%	0.9964	0	SNP		
	2017	18	83%	0.9783	0	Rapture		
Bull, Upper	1986	40	0%	1.0000	0	Allozyme	Slight Increase	Increase
	1999	36	0%	1.0000	0	Microsat.		
	2003	23	0%	1.0000	0	Microsat.		
	2015	20	5%	0.9987	0	SNP		
Elk, Mid	**2000**	20	0%	1.0000	0	Microsat.	Slight Increase	Increase
	2014	15	7%	0.9983	0	SNP		
	2016	17	0%	1.0000	0	SNP		
	2017	20	5%	0.9993	0	Rapture		
Michel, Upper	**2003**	25	0%	1.0000	0	Microsat.	Slight Increase	Increase
	2016	17	6%	0.9985	0	SNP		
Sand, Upper	**2004**	12	0%	1.0000	0	Microsat.	Slight Increase	Increase
	2017	24	21%	0.9951	0	Rapture		
Lussier, Lower	**2000**	30	17%	0.9322	0	Microsat.	Slight Increase	Increase
	2015	20	65%	0.9301	0	SNP		
Wigwam, Mid	1986	20	0%	1.0000	0	Allozyme	Increase	Increase
	1999	34	9%	0.9853	0	Microsat.		
	2016	14	50%	0.9698	0	SNP		
Lussier, Upper	**2004**	30	3%	0.9878	0	Microsat.	Increase	Increase
	2015	24	29%	0.9715	0	SNP		
White, North Fork	1986	16		0.9497		Allozyme	**Variable, but**	Increase
	1999	33	12%	0.9600	0	Microsat.		

Site	Year	N	% Indiv. with Pure RB or Hybrid	WCT Allele Freq.	F1	Gen. Method	Change in Hyb	Change in % Indiv. with Hyb
	2004	31	0%	1.0000	0	Microsat.	**Increase from 2004***	
	2014	18	44%	0.9900	0	SNP		
Palliser	**2000**	21	0%	1.0000	0	Microsat.	**Increase***	Increase
	2015	24	79%	0.9676	0	SNP		
Sand, Lower	**2004**	31	39%	0.6943	1	Microsat.	**Increase***	Increase
	2007	31	29%	0.4424		Microsat.		
	2008	31	52%	0.6458		Microsat.		
	2009	32	28%	0.7330		Microsat.		
	2010	31	65%	0.5234		Microsat.		
	2015	32	47%	0.6815	0	SNP		
	2017	23	83%	0.6455	2	Rapture		
St. Mary, Lower	**2000**	195	20%	0.9502	0	Microsat.	**Large Increase***	Increase
	2014	37	57%	0.8800	0	SNP		
Upper Kootenay	1999	15	20%	0.9623	0	Microsat.	**Large Increase***	Increase
	2002	7	29%	0.9070	0	Microsat.		
	2003	40	5%	0.9912	0	Microsat.		
	2014	14	57%	0.8500	0	SNP		

The percent of individuals at a site with any admixture typically follows trends in hybridization. In some cases, however, the proportion of fish with hybridization increased when overall hybridization rates stayed static or decreased. Many fish in population with low levels of admixture would indicate hybridization events that occurred a long time ago (15 years or more). Increases in the proportion of fish in the sample with admixture could also be due to an increased power to detect low-levels of RBT and YCT alleles. Both the SNP-chip and Rad Capture have more loci than the microsatellite analysis, increasing the ability to detect admixture.

Archived DNA from Dr. Stephen Bennett was sent to MCGL for reanalysis using SNPs and Rad Capture. Three sites had archived DNA rerun with SNPs and are presented in

SITE	SAMPLE YEAR	N	# OF HYB.	# RB	# PURE WCT	% RB HYB.	% RB AND RB HYB	WCT ALLELE FREQUENCY (1- PNON WCT)	F1
SKOOKUMCHUCK, LOWER	2003	25	5	2	18	20%	28%	0.871	1
	2003	25	13	1	11	52%	56%	0.865	0
UPPER KOOTENAY	2003	40	2	0	38	5%	5%	0.991	0
	2003	50	4		46	8%	8%	0.989	0

ELK, LOWER	2004	28	4	7	17	14%	39%	0.702	1
	2004	30	10	2	18	33%	40%	0.723	0

Table 4. Archived DNA from additional sites are currently being rerun utilizing Rad Capture techniques. A comprehensive spatial temporal analysis utilizing rad capture on archived historic and contemporary DNA will be conducted and was not complete for this report.

Table 4. Comparison of sites using microsatellites and reanalysed using the SNP chip.

SITE	SAMPLE YEAR	N	# OF HYB.	# RB	# PURE WCT	% RB HYB.	% RB AND RB HYB	WCT ALLELE FREQUENCY (1- PNON WCT)	F1	LAB	GENOTYPE METHOD
SKOOKUMCHUCK, LOWER	2003	25	5	2	18	20%	28%	0.871	1	Bennett	Microsatellite
	2003	25	13	1	11	52%	56%	0.865	0	Amish	SNP Chip
UPPER KOOTENAY	2003	40	2	0	38	5%	5%	0.991	0	Bennett	Microsatellite
	2003	50	4		46	8%	8%	0.989	0	Amish	SNP Chip
ELK, LOWER	2004	28	4	7	17	14%	39%	0.702	1	Bennett	Microsatellite
	2004	30	10	2	18	33%	40%	0.723	0	Amish	SNP Chip

At these sites, Lower Skookumchuck River, Upper Kootenay River and Lower Elk River, the admixture levels (WCT allele frequency) were similar. The detection of low levels of admixture was apparent with the SNP techniques as at each site, the number of hybrids increased. It should be noted that the sample sizes for the Upper Kootenay and the Lower Elk were not identical and so the actual sample was different, and the deletion of certain samples reanalysed using SNPs will change the results.

Discussion

Genotype results, to date, have enabled a better understanding of the current status of Westslope cutthroat trout in the Upper Kootenay drainage and in the British Columbia range. These results indicate that, of the 74 sites sampled and genotyped within the Upper Kootenay drainage between 2014 and 2017, 13 WCT sites (18%) had WCT allele frequencies of 1.000 as all loci examined were diagnostic of WCT. Over half of the sampled sites (58%) had WCT allele frequencies over 0.990. Non-hybridized WCT populations are of high conservation value and will aid in fisheries and land-based management decisions that affect these populations.

Genotype results also identified sites with high RBT hybridization. Outlet Creek and the White River below and above Outlet Creek have high levels of RBT admixture. Outlet Creek drains Whiteswan Lake to the White River system and has a falls that precludes upstream fish migration. Whiteswan Lake was stocked with over 1.5 million fertile rainbow trout from 1964 to 2003 and out-migrating rainbow trout serve as the primary source for hybridization to the White River drainage. In 2015, Fisheries installed a fish barrier on Outlet Creek to limit downstream movement of rainbow trout to the White River. Monitoring admixture over time to determine the efficacy of the barrier to limit the source of hybridization will be integral to the management of the fishery at Whiteswan Lake.

Koocanusa Reservoir, which spans approximately 145 km over both USA and Canada from Libby Dam in Montana to where the reservoir flows and is considered the Upper Kootenay River. BC stocked Kikomun Creek, a tributary of Koocanusa from 1986 to 1998 with just over 60,000 reproductive Gerrard rainbow trout. The State of Montana has stocked Koocanusa from 1988 to present with over 915,000 rainbow trout. Currently, the State of Montana stocks triploid Gerrard strain rainbow into Koocanusa. From 1988 to 2009 the strain was an Ennis/ Kamloops diploid rainbow trout (n=632,922) and switched to triploid Gerrard in 2010 (total stocked to date 282,975). Koocanusa is the most likely source of rainbow trout for the sites identified with high rates of hybridization in the Lower Elk River, Lower Gold Creek, Sand Creek, the Upper Kootenay River, Wildhorse River, the Lower St. Mary River, Joseph Creek, Perry Creek and the Lower Bull River.

Temporal Changes in Hybridization in the Upper Kootenay Watershed

At most sites, hybridization rates remained static at sites repeatedly sampled in the Upper Kootenay watershed over the last 15 to 20 years. No significant changes were detected in 15 of the 37 sites (41%). Ten (27%) of the sites showed significantly decreased levels of hybridization with rainbow trout, and five sites (13.5%) showed significantly increased rainbow trout hybridization. Six of the sites remained non hybridized.

The first WCT hybridization surveys in BC were conducted in 1986 (Leary 1987) and using 6 allozyme genetic markers, found admixture in one of the seven sites sampled, the North Fork of the White River. The source of rainbow trout genes to this site was Whiteswan Lake. After poisoning the lake with toxaphene to remove undesirable fish species, the lake was stocked with fertile rainbow trout from 1964 until 2009 which established a thriving population and popular sport fishery. Hybridization in the North Fork was also sampled by Rubidge in 1999,

Bennett in 2004 and in this study in 2014. Bennett did not detect hybridization in 2004, and the 2014 sample showed low levels at the site and that 44% of the fish had some admixture, indicating that a hybridization event occurred a long time ago. The North Fork of the White River is high in the Upper Kootenay watershed (1350 m elevation) with qualities of a headwater stream. The persistence of hybridization and relative proximity to a rainbow trout source (Whiteswan Lake) indicates that the propagule pressure drives hybridization and cold water does not act as a complete barrier to introgression invasion.

The Lower Bull River showed the greatest change in hybridization over time with all fish sampled in 2000 and 2001 being pure rainbow trout or hybrids. However, these fish were sampled as fry and adults were sampled in later sampling events. In 2007 13% of the fish sampled were hybrids or rainbow and in 2015, 38% of the adult sample had some degree of hybridization. The difference of sampling fry and adults makes comparison difficult. The Lower Bull River also would have a highly migratory population and sampling would break the assumption that the site is a randomly mating population with no migrants. Given that, the decrease in hybridization from 2007 to 2015 could be a result of the cessation of stocking reproductive rainbow trout in the Lower Bull and Kikomun Creek in the late 1990's and the switch to stocking only triploid RB in BC after 2003.

The Lower St. Mary River showed a significant increase in hybridization between sample years 2000 and 2014. This aligns with reports from anglers and angling guides of catching more rainbow trout there in recent years. Hybridization will likely continue to threaten WCT in the St. Mary River drainage as climate change and anthropogenic impacts continue to degrade habitat. Other sites with significant increases in hybridization included the Palliser River, and the Upper Kootenay. Further examination of hybridization in the Palliser River and sources of rainbow trout should be undertaken.

Two sites showed significant increases in the two years chosen for the binomial comparison; Lower Sand Creek (2004 and 2017) and the North Fork of the White (between years 2004 and 2014). However, in both these populations, multiple sample years showed varying levels of hybridization that were not necessarily always negatively trending. Lower Sand Creek has high levels of hybridization and has been monitored in seven years between 2004 and 2017. Lower Sand Creek is directly connected to Koocanusa and hybridization rates there emphasize that Koocanusa remains a source of rainbow trout genes.

Aboriginal and Stocked WCT Populations

In BC, WCT are currently distributed throughout the Upper Kootenay and Flathead watersheds, as well as areas of the Lower Kootenay, Upper Columbia, Kettle and Thompson. It is known that WCT were native historically (aboriginal) in the Upper Kootenay and Flathead drainages prior to stocking. Documented stocking of reproductive WCT has occurred since the 1920's in over 340 waterbodies in BC and has included areas well outside the species historical range. These past WCT stocking efforts complicate the delineation of aboriginal and stocked populations within the species' historic and present range. Since stocking was so extensive, identification of aboriginal populations with high certainty is not possible through examination of stocking history alone. Alternatively, genetic techniques are a better tool to evaluate stocked populations through a better understanding of the genetic signature of BC's WCT brood stock populations. Though several waterbodies were used as sources for brood stock, most of the stocking in BC derived

from Kiakho Lake. Connor Lake was stocked with Kiakho Lake in 1950 and has sourced the majority of WCT in BC.
Samples collected with this project have been used to conduct a preliminary analysis of whether pure BC WCT populations derived from stocking events or colonized naturally (aboriginal). It is not possible to apply this analysis to populations with WCT allele frequencies of < 1.0 so only pure WCT populations were assessed. DAPC uses allele frequencies to partition between-group and within- group variance, in order to maximize discrimination between groups.

The preliminary DAPC results produced two well-defined clusters, one centered around Connor Lake (stocked populations) and another which included samples from within the WCT range of unknown origin (aboriginal populations). Almost all populations in the Upper Kootenay watershed that have been analyzed clustered with aboriginal populations.

Although preliminary results indicate strong clustering of aboriginal and stocked populations, remaining uncertainties in the analysis suggest additional samples and additional genetic markers would provide more definitive findings.

First, more genetic markers and additional samples from Connor Lake and sites stocked from Connor Lake will increase our certainty in the clustering detected in the DAPC. Because stocked populations originate from a limited number of individuals, they contain little genetic variation. This lack of genetic variation results in fewer genetic markers driving the allele frequency differences and the subsequent clustering patterns.

Second, clustering suggests clear genetic similarity between Connor Lake and other populations, but the origin of Kiakho Lake fish is uncertain. Stocking records of Kiakho Lake suggest re-stocking often occurred from the lake itself. Re-stocking from alternative sources was relatively rare and was mostly limited to other sources in the Upper Moyie. In the DAPC analysis, WCT populations may therefore cluster with Connor Lake due to genetic similarity to the aboriginal Upper Moyie, and therefore clustering would not be a good indicator of stocked/aboriginal population status. Next steps will include determining the genetic uniqueness of the Connor and Kiakho brood sources through genotyping isolated stocked WCT lakes throughout the WCT range. This will broaden the range of allele frequencies associated with “stocked” populations, thus increasing our confidence in the clustering results. Specifically, samples from isolated lakes stocked with reproductive WCT from known brood will improve our understanding of effects genetic drift and stocking bottlenecks have had on the genetic signature associated with Connor Lake, and allow better differentiation between stocked and aboriginal populations. Additional markers will improve our power to detect genetic differentiation among the clusters and increase the number of genetic markers driving these patterns.

Recommendations

This project has provided a current inventory of hybridization in the Upper Kootenay Watershed and has identified outstanding work that remains. The following recommendations to investigate hybridization risks to WCT in BC are as follows

1. Utilize RAD Capture genetic techniques on archived DNA to conduct an in-depth spatiotemporal analysis of hybridization in the Upper Kootenay and throughout the BC distribution of WCT. This analysis will further standardize different genotyping methods that have been used since hybridization was first detected utilizing allozyme markers in 1986, microsatellite markers (1999 to 2012), SNPs (2013 to 2016) and RAD Capture (2017 onwards).
2. Determine whether WCT populations in the BC distribution both within and outside the Upper Kootenay watershed were stocked or colonized naturally.
3. Utilizing hybridization and aboriginal vs stocked data, determine priority conservation WCT populations in BC.
4. Explore management actions that could influence risk and spread of hybridization in the Lower St. Mary watershed. Improve sample sizes in Redding and Dewar Creek and inventory tributaries of the Upper St. Mary to determine hybridization risk throughout the watershed.
5. Assess the effectiveness of the Outlet Creek fish barrier at Whiteswan Lake through future hybridization sampling in the White River drainage.
6. Continue to identify sources of RBT in the Upper Kootenay watershed and work towards limiting or removing them.
7. Work with Montana on co-management of Koocanusa Reservoir pertaining to stocking of rainbow trout in Montana and the associated ongoing risks.

Collaborative Efforts

From the onset, this study has focussed on creating and maintaining partnerships with transboundary, trans-agency and non-governmental organizations. As the global WCT distribution occurs in Idaho, Montana, Alberta and BC, transboundary collaborations with agencies managing WCT in Montana and Alberta are ongoing. Partnerships have been established and continued with the Montana Conservation Genetics Laboratory (MGCL) and the US Geological Survey (USGS), the Government of Alberta and Parks Canada.

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