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Microsatellite DNA analysis of overwintering bull trout (*Salvelinus confluentus*) and its implications for harvest regulation and habitat management

Eric B. Taylor[1] | Rachel Chudnow[2] | Ray Pillipow[3] | Ian Spendlow[3] |
Brett van Poorten[2,4,5]

[1]Department of Zoology, Biodiversity Research Centre and Beaty Biodiversity Museum, University of British Columbia, Vancouver, BC, Canada

[2]Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, BC, Canada

[3]British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Prince George, BC, Canada

[4]British Columbia Ministry of Environment and Climate Change Strategy, Vancouver, BC, Canada

[5]School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC, Canada

Correspondence
Eric B. Taylor, Department of Zoology, Biodiversity Research Centre and Beaty Biodiversity Museum, University of British Columbia, 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada.
Email: etaylor@zoology.ubc.ca

Abstract

A mixed-stock fishery occurs when multiple populations of a fish species are exploited together in a common area where they aggregate outside the breeding season (e.g. for feeding or overwintering), and the aggregation is known as a mixture. Recreational fishing often exploits such mixtures, and estimating the proportional contributions of populations to fisheries promotes more sustainable resource use. Ten DNA microsatellites were assayed in a mixture of bull trout *Salvelinus confluentus* Suckley overwintering in the Nechako River, upper Fraser River, British Columbia, and in baseline population samples from 14 tributaries putatively contributing to the overwintering mixture. A DNA microsatellite-based mixed-stock fishery analysis suggested that five populations together contributed 0.80 to the mixture. Most of the errors associated with the mixture estimates were attributable to uncertainty in baseline allele frequencies. Radiotracking data confirmed that tributary populations contributing to the mixture estimated by genetic analysis also contained individuals that moved between spawning tributaries and overwintering sites. The results better resolve habitat use by potadromous bull trout in the upper Fraser River and, in combination with assessments of baseline population-specific spawning abundances and productivity, will better inform a decision of whether or not allowing some harvest within the current catch-and-release fishery is biologically sustainable.

KEYWORDS
British Columbia, char, Fraser River, habitat use, mixture analysis, recreational fishing

1 | INTRODUCTION

There is growing recognition that recreational harvest is a significant component of global capture fisheries (Arlinghaus & Cooke, 2009; Arlinghaus et al., 2016; Pitcher & Hollingsworth, 2008). Recreational fishing, including angling, holds strong socio-economic and cultural

importance globally, and in recent decades recreational anglers have become dominant users of numerous wild fish populations in many developed nations (Arlinghaus & Cooke, 2009; Pitcher & Hollingsworth, 2008). In Canada alone, indirect and direct expenditures related to angling averaged an estimated $8.8 billion per year between 1975 and 2010 (Brownscombe et al., 2014). At the

same time, conservation-related issues ranging from climate change to overexploitation necessitate ongoing regulatory oversight of recreational fishing opportunities (e.g. Arlinghuas & Cooke, 2009; Dempson et al., 2001). British Columbia (BC) has a diverse set of marine- and freshwater-based fisheries. Freshwater recreational fishing alone currently generates almost Can 0.5 billion in indirect, direct and induced economic impacts (FFSBC, 2020).

Throughout much of its geographic range, bull trout (Salmonidae: *Salvelinus confluentus* Suckley) populations are in a state of decline (US Fish and Wildlife Service, 1999; COSEWIC, 2013). In Canada, the species has been assessed under the *Species at Risk Act* as five population assemblages or "designatable units" (DUs): Pacific populations, South Coast British Columbia populations, Western Arctic populations, Saskatchewan River–Nelson River populations and Yukon River drainage populations, and in British Columbia (BC) bull trout are "blue listed" by the British Columbia Conservation Data Centre (BC Conservation Data Centre, 2019). The Nechako River is a major tributary of the upper Fraser River, flowing from the northwest

to join the Fraser River near Prince George, BC (Figure 1). Bull trout in the Nechako River are part of the Pacific populations DU, which was assessed as "Not at Risk," a status that applies to the entire assemblage of bull trout within the DU (COSEWIC, 2013). While a DU may be assessed at one level of risk, constituent populations may be at entirely different levels of risk depending on local conditions.

The Nechako River is impounded by Kenney Dam, approximately 279 km upstream of the confluence of the Fraser and Nechako rivers. Downstream of Kenney Dam, the Nechako River contains overwintering populations of bull trout (R. Pillipow, Pers. Observation), but no known spawning areas for bull trout exist in this section of the river. Occurrence of aggregations (mixtures) of overwintering bull trout is consistent with what is known of the potadromous life history of fluvial populations, which typically spawn in smaller tributaries and migrate to larger rivers for feeding and overwintering (McPhail, 2007; Pillipow & Williamson, 2004; Taylor et al., 2014). An understanding of the spawning locations of these overwintering bull trout population mixtures would, therefore, be important
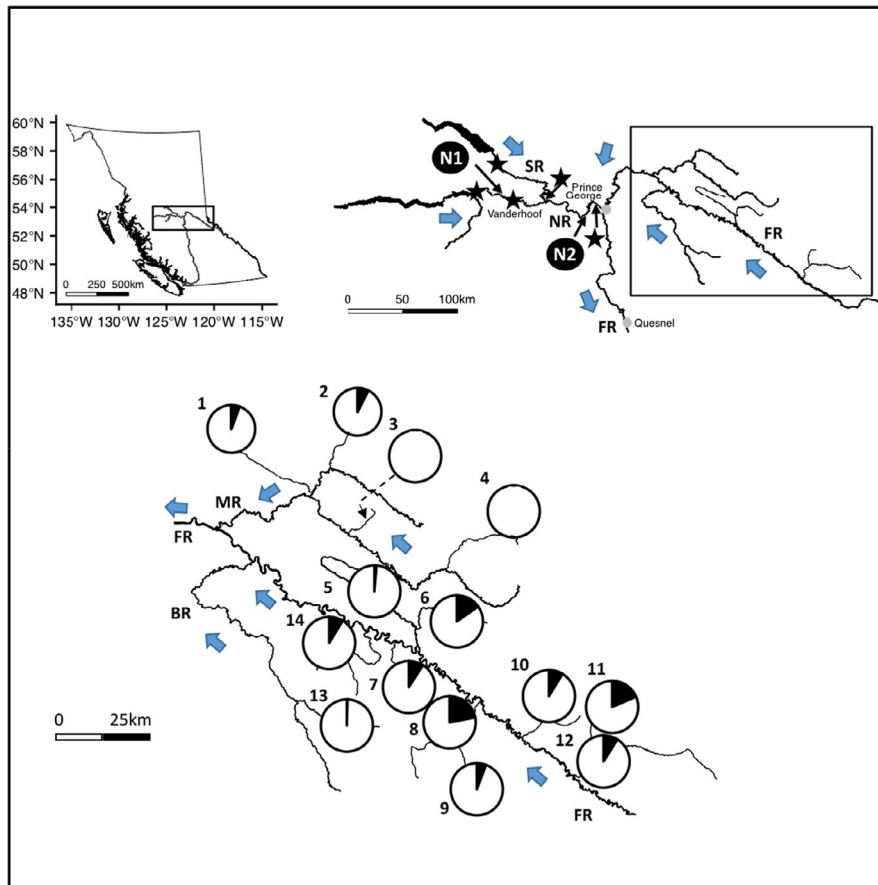
FIGURE 1 Map of sampling localities for bull trout (*Salvelinus confluentus*) in the Nechako River and tributaries of the upper Fraser River upstream of the confluence of the Nechako and Fraser rivers, British Columbia (inset). Also shown are approximate locations of fixed station radio receivers (stars) used to detect bull trout fitted with radiotags, and direction of water flow (blue arrows). BR, Bowron River; FR, Fraser River; MR, McGregor River; N1, Nechako River overwintering site 1; N2, Nechako River overwintering site 2; NR, Nechako River; SR, Stuart River, 1 = Captain Creek, 2 = Fontoniko Creek, 3 = Gleason Creek, 4 = Jarvis Creek, 5 = Torpy River, 6 = Walker Creek, 7 = Dome Creek, 8 = Goat River, 9 = Milk River, 10 = McKale River, 11 = Chalco Creek, 12 = Holmes River, 13 = Haggen Creek and 14 = Everett Creek. The pie chart adjacent to each number code indicates the mean estimated proportion of each tributary population to the Nechako overwintering mixture (see Table 2)

TABLE 1 Summary of upper Fraser River bull trout (*Salvelinus confluentus*) samples examined at 10 microsatellite DNA loci

Sample	Drainage within Fraser River	Year sampled	N	A_R	H_O	H_E
Nechako Mix 1	Nechako River	2013	85	6.3	0.54	0.68
Nechako Mix 2	Nechako River	2014	75	5.9	0.52	0.63
Nechako Mix 3	Nechako River	2015	48	6.1	0.51	0.64
Nechako Mix 4	Nechako River	2016	46	6.0	0.49	0.62
Goat River	Goat River	2011	41	5.9	0.51	0.61
Goat River	Goat River	2014	46	9.1	0.54	0.62
Milk River	Goat River	2014	59	7.5	0.69	0.73
Jarvis Creek	McGregor River	2016	42	7.2	0.51	0.67
Fontoniko Creek[a]	McGregor River	2015	16	7.7	0.59	0.61
Fontoniko Creek	McGregor River	2016	22	4.3	0.47	0.45
Gleason Creek[a]	McGregor River	2016	40	5.8	0.59	0.62
Captain Creek[a]	McGregor River	2014	32	8.1	0.59	0.74
Walker Creek	Torpy River	2011	36	4.5	0.45	0.48
Walker Creek[a]	Torpy River	2013	50	4.7	0.51	0.55
Walker Creek	Torpy River	2014	48	5.1	0.52	0.56
Torpy River	Torpy River	2016	45	7.2	0.68	0.69
McKale River[a]	McKale River	2014	48	6.6	0.62	0.67
McKale River	McKale River	2016	20	6.0	0.59	0.65
Haggen Creek[a]	Bowron River	2014	45	5.3	0.66	0.63
Haggen Creek	Bowron River	2016	31	5.8	0.67	0.67
Dome Creek	Dome Creek	2016	21	6.0	0.64	0.69
Everett Creek	Slim Creek	2016	30	6.8	0.57	0.64
Chalco Creek[a]	Holmes River	2013	65	7.1	0.69	0.74
Chalco Creek	Holmes River	2014	43	5.6	0.67	0.65
Holmes River	Holmes River	2013/14	14	6.6	0.61	0.76

Note: Values of observed heterozygosity that are underlined are significantly lower than expected.

Abbreviations: A_R, mean allele richness across 10 loci (standardised to lowest sample size of 14); H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; N, sample size.

[a]Sample consists entirely or mostly of juvenile fish less than one year of age to three years of age.

in riverscape-level management for bull trout to ensure that the full mosaic of habitats critical for the persistence of the species is protected (Taylor et al., 2014). Potadromous fishes may be particularly sensitive to habitat degradation given that they may face a so-called "triple jeopardy" of threats to spawning, overwintering and migration corridor habitats (McIntyre et al., 2016). Furthermore, bull trout is an important component of the recreational fishing catch in the Omineca Management Region, particularly because they are all wild fish (GSGislason & Associates, 2009). Fish overwintering in the Nechako River are targeted by anglers in a popular, regulated, catch-and-release recreational fishery. Anglers in this region are requesting a change in regulation to allow for harvest. It is unknown, however, which potential tributary streams may be contributing most to the fishery. If harvest is permitted in the future, a targeted fishery on the Nechako River aggregation may lead to overharvest of some low-productivity populations, a common threat in mixed-population (or "mixed-stock") fisheries (Kope, 1992).

To date, research efforts examining bull trout populations in the upper Fraser River watershed have been modest. Taylor and Clarke (2007) used microsatellite DNA and otolith microchemistry to test for population subdivision and movements of bull trout from ten localities in the upper Fraser River and reported significant genetic differentiation within (e.g. above and below migration barriers) and among localities. Both the genetic and otolith microchemistry data suggested that there was also some movement of bull trout within and among tributaries (Taylor & Clarke, 2007). These preliminary data for upper Fraser River bull trout, therefore, suggest that there is good potential for genetic techniques to assess differences between populations and to identify the source locality for individuals captured while overwintering in the Nechako River. Such mixture analyses have made important contributions to recreational fisheries in a variety of species, including bull trout in other areas (Bott et al., 2009; Brenden et al., 2015; Taylor et al., 2019; Warnock et al., 2011).

The objective of this study was to use microsatellite differentiation among bull trout sampled from different localities to

estimate the geographic origins of overwintering subadults and adults in the Nechako River. This analysis provides an initial estimate of which spawning populations contribute to the fishery at the overwintering sites. It will serve to inform an anticipated management decision with respect to possible changes to harvest regulations to protect less productive populations. It also provides further rationale for a greater focus on demographic analyses and habitat protection in overwintering and spawning/rearing environments.

2 | METHODS

2.1 | Fish samples

Fish were collected between 2011 and 2016 from 16 sites in the upper Fraser River drainage, central British Columbia (Table 1): two principal overwintering sites within the Nechako River located between 5 and 130 km upstream of the confluence between the Fraser and Nechako rivers, and 14 sites spanning tributaries of the upper Fraser River about 150 km (Captain Creek) to about 330 km (Chalco Creek) upstream of the confluence of the Nechako and upper Fraser rivers (Figure 1). These latter 14 localities represent the putative baseline populations contributing to the overwintering mixtures. The overwintering fish consisted of subadult (i.e. probably never having reproduced yet) to adult fish, all between 320 and 730 mm fork length, and fish from the baseline populations consisted both of juvenile and adult fish (Table 1). A small (5 mm x 5 mm) section of fish tissue was taken from each fish and stored in 95% non-denatured ethanol. The DNA was extracted from tissues using Qiagen Blood-Tissue DNeasy DNA Extraction Kits, and the DNA was stored at −20°C until analysis.

2.2 | Microsatellite analysis

Genetic analyses of samples using microsatellite DNA assays were conducted using the Qiagen multiplex kit and polymerase chain reactions (PCRs) in 15 μl volumes. The PCRs included fluorescently labelled primers in MJ-PTC 100 thermal cyclers, and reaction products were visualised using a Beckman–Coulter CEQ 8000 automated genotyper following the methods of Taylor et al. (2014). Bull trout were assayed at 10 loci: *Sfo*18, *Sco*102, 110, 203, 204, 212, 215, 220, *Smm*22 *and Omm*1128 (see Taylor et al., 2014 for details). The PCRs and allele visualisation for 16 fish were replicated four times each over the course of the study to verify consistency of allele and genotype determination.

2.3 | Genetic data analysis

The program MICRO-CHECKER (van Oosterhout et al., 2004) was used to check the data for evidence of scoring errors and/or null (non-amplifying) alleles. Tests for deviations from Hardy–Weinberg equilibrium were performed for each locus–population combination using

an exact test in which *p*-values were estimated using a Markov chain method using GENEPOP ver. 007 (Rousset, 2008). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were conducted using a Markov chain method with GENEPOP default values. Mean allelic richness (A_R), observed (H_O) and expected (H_E) were compiled using FSTAT (Goudet, 2005). Tests for differentiation between baseline populations were performed over all loci combined using F_{ST} estimated as θ (Weir & Cockerham, 1984) using GENETIX (Belkhir et al., 2004). Significance levels for all tests involving multiple simultaneous comparisons were adjusted following Narum (2006). For baseline populations for which there were temporally spaced samples (N = six), an analysis of molecular variance (AMOVA), implemented in ARLEQUIN (ver. 3.5, Excoffier et al., 2005), was used to partition the variation in allele frequency into that attributable to variation between populations relative to that within populations between sample years. To get an overall sense of major genetic subdivision among the baseline populations, the genetic clustering program STRUCTURE version 2.3.3 (Falusch et al., 2003) was used to estimate the number of distinct genetic clusters (K) best represented across baseline populations. STRUCTURE uses a Bayesian clustering method to assign individuals to genetic clusters based on their genotypes. An individual may be assigned to more than one cluster if its genotype indicates admixture of two or more genetic groups. A Markov chain Monte Carlo method was used to estimate posterior probability distributions for each possible number of clusters. Simulations in STRUCTURE were performed using values of K between 1 and 15, the number of baseline populations plus one. Parameters that allowed admixture and correlated allele frequencies were used, and each run consisted of a 500,000 step burn-in followed by an additional 1,000,000 steps, with five iterations run for each value of K. The value of K that most efficiently summarised the data was determined using the ad hoc statistic ΔK following Evanno et al. (2005), as implemented in STRUCTURE HARVESTER (Earl & von-Holdt, 2012).

The Bayesian approach to mixed-stock analysis as implemented in ONCOR (Anderson et al., 2008; Kalinowski et al., 2007) was applied to the data. This analysis uses the conditional maximum-likelihood algorithm to estimate mixture proportions and the Rannala and Mountain (1997) method for estimating the probability of observing a genotype in the 14 baseline populations each of which was characterised across the 10 loci. Given that interannual differences were subordinate to differences among localities (see Results) and because there were samples from several different years, all multiple year samples were pooled within localities to form the baseline sample. All mixture (overwintering) samples collected between 2013 and 2016 were also pooled given that the same fish may have overwintered in more than one year while acknowledging that some information may be lost by such pooling (e.g. variation in overwintering composition between years).

The robustness of the mixture analysis was assessed in several ways using ONCOR. First, 95% confidence intervals were generated by bootstrap resampling with replacement of both the mixture samples (across individuals) and the baseline genotypes (across alleles) during 5000 replicate analyses. Second, new mixture samples of

genotypes (N = 200 fish each) were simulated where the proportional contribution of each baseline population in turn was fixed to 1.0. Next, the mixture proportions for each baseline population contributing to these simulated mixtures ("100% mixtures") were estimated. In this case, "perfect" performance of the mixture analysis would return an estimated proportional contribution for each baseline population of 1.0. Genotype resampling mixtures were also constructed using the parametric, observed mixture proportions ("Realistic fishery simulations"). Here, the parametric (observed) mixture proportions were used to simulate mixture samples and then the proportion of each baseline sample in these more realistic overwintering mixture samples was estimated. The difference between the parametric mixture proportions and the realistic fishery simulations' average proportions is a measure of bias for each baseline population (Kalinowski et al., 2007).

For all simulations, genotype resampling was conducted using the method of Anderson et al. (2008). Three-way error decomposition analyses were conducted to examine the extent of uncertainty in mixture proportion estimation from sampling a finite number of fish in the overwintering group of bull trout ("fishery (or "mixture") error"), from genotyping a finite number of loci ("genotypic error") and from uncertainty in the estimation of baseline allele frequencies ("baseline error"). These errors are expressed as percentages of the total error in mixture estimation, and error decomposition used the method of Kalinowski et al. (Kalinowski, S.T., Taper, M.L., Manlove, K.R., Templin, W.D., and Anderson, E.C., Unpublished data).

2.4 | Tagging of bull trout

Chudnow, R., van Poorten, B., Pillipow, R., and Spendlow, I. (unpublished data) examined movements of bull trout that were fitted with a combination of radio, Floy™ and PIT (passive integrated transponder) tags within the Nechako River and several tributaries of the upper Fraser River above the confluence of these two rivers (N = 262 fish total). General methods used in applying and detecting the tags followed Pillipow and Williamson (2004). In the current study, the detection data from Chudnow et al. were examined for a subset of these fish that were genotyped and tagged in the Nechako River and in five baseline tributaries (Milk River, Holmes River, Goat River, Chalco Creek and Walker Creek). Tag detections were also searched for in two baseline tributaries (Dome Creek, Torpy River) that contained bull trout that were genotyped, but that did not receive tags (i.e. tag detections would indicate movement into these tributaries). In total, there were 214 fish both with tag detection data and genetic data. Tag detections were made with a series of five fixed receivers, and with mobile detectors on an ad hoc basis at 16 other sites in the upper Fraser River drainage (Table S1, Figure 1). In examining the tag detection data, the objective was to obtain direct evidence of movements between spawning tributaries and the Nechako river overwintering sites, not to provide a detailed description of these movements. The data were examined to determine whether fish tagged in the Nechako River overwintering sites

were detected in the different baseline tributary streams, during what times of year, and vice versa, to provide context for the genetic estimates of tributary-specific contributions to the overwintering mixture. Using these data, the degree of correspondence between the genetic assignment of individual fish to tributaries and the identification of a fish's tributary of origin determined by tagging was assessed. The program GENECLASS (Piry et al., 2004) was used to assign genetically bull trout overwintering in the Nechako River, and that were detected by tagging, to one of the tributary localities. Fish were assigned genetically to tributaries for which the likelihood of their multilocus genotypes occurring was the highest.

3 | RESULTS

3.1 | Microsatellite polymorphism within and among populations

There was no indication of null alleles or large allele dropout from the MICRO-CHECKER analysis, so all loci were retained for subsequent analysis. Across all samples, there was an average of 2.1 alleles (Sco215) to 16.8 alleles (Smm22) per locus. There were several deviations from Hardy–Weinberg equilibrium, principally in one of the Chalco Creek samples (2013, 6/10 loci), the Nechako River mixtures and the Torpy River and Walker Creek (2013) samples (4/10 loci). There were also several significant deviations from linkage equilibrium, but they were not concentrated between consistent pairs of loci, so all were considered as independent markers of genetic diversity. Expected heterozygosity averaged across all loci ranged from 0.45 (one of the Fontoniko Creek samples) to 0.76 in the Holmes River sample (Table 1).

Across all tributary samples of bull trout, θ was 0.058 and significantly greater than 0 ($p < 0.0001$). Pairwise estimates of θ ranged from 0.008 between the Milk River sample and the Goat River sample both collected in 2014 ($p = 0.03$) to 0.159 ($p < 0.001$) between the Jarvis and Everett creeks' samples, both of which were collected in 2016 (Table S2). Six tributaries had samples collected in at least two years, which allowed a comparison of inter-tributary differences relative to interannual differences within tributaries. All pairwise comparisons between years within baseline tributaries, except for the Fontoniko Creek 2015/2016 samples ($\theta = 0.014$, $p > 0.1$), were significant ($p < 0.001$) and ranged from $\theta = 0.013$ to 0.024 (between Walker Creek 2011/2013 and between McKale 2014/16, respectively; Table S2). Nevertheless, the AMOVA analysis indicated that overall θ among these six tributaries (0.027) was over twice that between years within tributaries (0.012, both $p < 0.001$). The STRUCTURE analysis indicated that the log likelihoods began to plateau between $K = 3$ and $K = 4$. The use of Evanno's ΔK indicated that a $K = 3$ was the most strongly supported model of population structure among the 14 baseline populations (Figure 2, Table S3). Eight of the 14 baseline populations had average ancestry coefficients (Q) within a single genetic group of at least 0.7. Jarvis and Gleason creeks had average Q values of ≥ 0.7 in genetic group 1, Goat River,
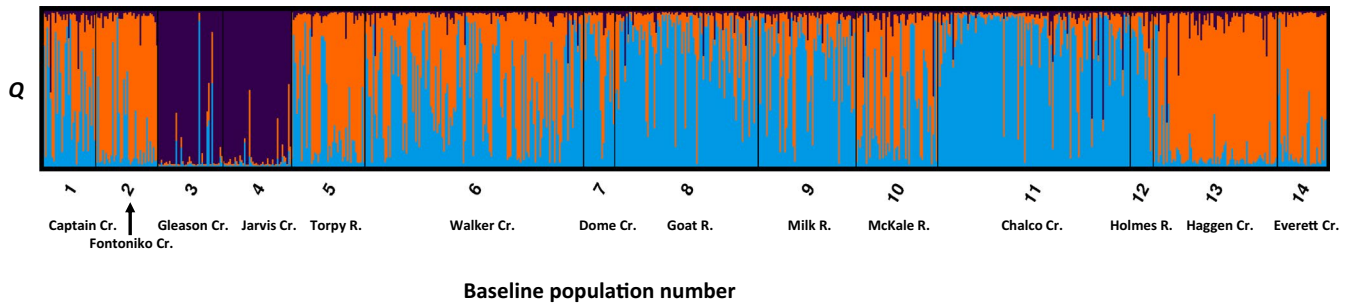
FIGURE 2 Ancestry coefficients (Q, 0–1.0) for individual bull trout (*Salvelinus confluentus*) sampled from 14 baseline populations in the upper Fraser River, BC, and assayed at ten microsatellite DNA loci. Each fish is represented by a thin vertical line where the height of each coloured bar represents the proportional contribution of one of three genetic groups to the genome of that fish. 1 = Captain Creek, 2 = Fontoniko Creek, 3 = Gleason Creek, 4 = Jarvis Creek, 5 = Torpy River, 6 = Walker Creek, 7 = Dome Creek, 8 = Goat River, 9 = Milk River, 10 = McKale River, 11 = Chalco Creek, 12 = Holmes River, 13 = Haggen Creek and 14 = Everett Creek

TABLE 2 Genetic mixture analysis of bull trout (*Salvelinus confluentus*) overwintering in the Nechako River, BC

Locality	Mixture proportion	100% mixture	Realistic fishery	Bias
Goat River	0.257 (0.160, 0.325)	0.887 (0.834, 0.950)	0.261 (0.179, 0.358)	0.004
Chalco Creek[a]	0.208 (0.153, 0.300)	0.953 (0.912, 0.986)	0.259 (0.181, 0.346)	0.051
Walker Creek	0.153 (0.101, 0.263)	0.967 (0.926, 0.993)	0.190 (0.119, 0.256)	0.037
McKale River	0.091 (0.035, 0.140)	0.885 (0.810, 0.933)	0.084 (0.047, 0.131)	−0.007
Holmes River	0.089 (0.014, 0.110)	0.198 (0.126, 0.255)	0.015 (0.000, 0.038)	−0.074
Fontoniko Creek	0.074 (0.025, 0.119)	0.883 (0.819, 0.930)	0.067 (0.029, 0.111)	−0.007
Captain Creek	0.056 (0.018, 0.094)	0.764 (0.699, 0.817)	0.034 (0.009, 0.070)	−0.002
Milk River[b]	0.046 (0.009, 0.112)	0.821 (0.736, 0.896)	0.062 (0.014, 0.102)	0.016
Torpy River	0.014 (0.000, 0.058)	0.903 (0.855, 0.955)	0.015 (0.000, 0.039)	0.001
Dome Creek	0.006 (0.000, 0.028)	0.859 (0.802, 0.909)	0.003 (0.000, 0.014)	−0.003
Haggen Creek	0.004 (0.000, 0.024)	0.982 (0.961, 0.999)	0.007 (0.000, 0.025)	0.003
Everett Creek	0.003 (0.000, 0.014)	0.961 (0.929, 0.986)	0.003 (0.000, 0.014)	0.000
Jarvis Creek	0.000 (0.000, 0.003)	0.998 (0.991, 1.000)	0.000 (0.000, 0.000)	0.000
Gleason Creek	0.000 (0.000, 0.000)	0.999 (0.991, 1.000)	0.000 (0.000, 0.000)	0.000

Note: Each value represents the mean (95% confidence interval) proportion of each baseline locality in the observed mixture (mixture proportion, N = 5000 bootstraps), in simulated mixtures each with 100% of each baseline locality (100% mixture), and in simulated mixtures based on the empirical mixture estimates (realistic fishery). The bias is the difference between the empirical mixture proportion (column 2) and the realistic fishery simulation (column 4). The 100% mixture and real fishery proportion estimates are based on 5000 simulations of N = 200 fish in each simulation.
[a]Upper tributary of the Holmes River.
[b]Upper tributary of the Goat River.

Holmes River and Chalco Creek in group 2, and Fontoniko, Haggen and Everett creeks in group 3 (Figure 2). The other six baseline populations contained more equitable proportions of all three genetic groups, but Dome Creek and the Milk River tended to be dominated by genetic group 2 (Q_2 = 0.67), and Walker and Captain creeks, and the Torpy and McKale rivers by genetic group 3 (Q_3 = 0.51–0.67, Figure 2). A second STRUCTURE analysis that included the mixture samples and tested values of K from 1 to 20 did not resolve any genetic groups unique to the mixture, i.e., there were no genetic groups resolved that were not already detected in the 14 putative source populations (Taylor, E.B., Chudnow, R., Pillipow, R., Spendlow, I. and van Poorten, B. unpublished data).

3.2 | Mixture analysis of overwintering samples

The results from the 100% mixture simulations demonstrated that the data had good power to apportion contributions from most baseline populations (average composition (SD) = 0.86 (0.20); only the 100% Holmes River simulation was poorly estimated (average = 0.198 (Table 2)). The greatest contributions to the Nechako River overwintering mixture population were estimated to be from the Goat River, Chalco Creek and Walker Creek; all other localities were estimated to contribute less than 10% to the total mixture sample and several less than 1% (Table 2). The three-way decomposition analysis indicated that baseline error was the greatest source

of mixture estimation error (59%) followed by fishery (mixture) error (28.4%); genotypic error (12.5%) was a relatively small source of error (Table S4). These sources of error varied substantially across baseline localities. The Holmes River sample with a sample size of only 14 showed, predictably, the greatest extent of baseline error (Table S4).

3.3 | Tag detections of bull trout

There were 1086 individual detections of the radio/Floy/PIT-tagged fish between 2011 and 2016. Most fish (N = 153) were tagged in the Nechako River, and most of the detections consisted of these same fish detected at least once in the Nechako River (N = 584 detections, Table S1). Eighteen fish were also tagged in Chalco Creek, and 10 were subsequently detected in the Nechako River (35 times in total, i.e. many fish had multiple detections; Figure 3). Thirty fish were tagged in the Goat River, and 9 were detected in the Nechako River (48 times in total). Eight fish were tagged in the Holmes River, and five were detected in the Nechako River (a total of 19 times). Thirteen fish were tagged in the Milk River, and six were detected in the Nechako River (a total of 15 times). Twenty-five fish were tagged in Walker Creek, and 14 were detected in the Nechako River (a total of 67 times). Other detections consisted of fish found in the same stream in which they were tagged, and fish tagged in a tributary or the Nechako River and subsequently detected in the Fraser or Stuart rivers. Fish tagged in the Nechako River were detected in tributary streams: three fish in the Goat River and in Walker Creek, two fish in each of Fontoniko Creek and the Milk River, and a single fish in Chalco Creek (Table S1, Figure 3). Two tributaries (Dome Creek and the Torpy River) that made little or no contribution to the

overwintering mixture sample had no detections of fish that were radiotagged in the Nechako River (Table 2, Table S1).

Fish were typically tagged in baseline tributaries during the spawning period (August–September) and were usually detected in the same stream during the spawning period or in the Nechako River in the winter–spring of the same or subsequent years (November–March, Table S1). Fish tagged in the Nechako River (typically in the spring-early summer) and detected in baseline tributary streams were typically detected during the spawning period (Table S1). A total of 48 fish were either tagged in the Nechako River and subsequently detected in a single baseline tributary during the spawning period, or were tagged in a baseline tributary during the spawning period and subsequently detected overwintering in the Nechako River. Of these 48 fish, 26 (54%) were genetically assigned to the same tributary in which they were tagged, or were genetically assigned to the same stream in which they were subsequently detected after being tagged in the Nechako River (p = 0.33 for departure from 50% correct assignment expected by chance).

4 | DISCUSSION

The current analysis examined microsatellite variation in potadromous bull trout, consisting of an overwintering assemblage within a large tributary of the upper Fraser River and fish from several tributaries that contribute to the overwintering mixture. Radiotracking data demonstrated that these bull trout migrate between spawning and rearing tributaries and the Nechako River overwintering area using the mainstem Fraser River as a migration corridor (see also Pillipow & Williamson, 2004). Before this
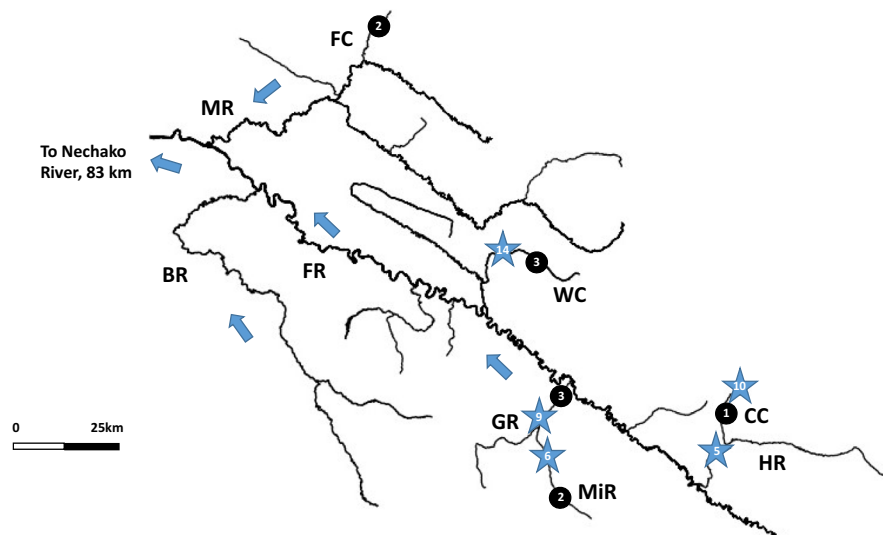
FIGURE 3 Map of localities in the upper Fraser River, British Columbia, where bull trout (*Salvelinus confluentus*) were detected after being radio/Floy/PIT-tagged in the Nechako River (blue stars) or that were tagged in tributaries and subsequently detected in the Nechako River (black circles). Numbers inside symbols indicate the numbers of fish. The direction of water flow is shown by blue arrows. BR, Bowron River; CC, Chalco Creek; FC, Fontoniko Creek; FR, Fraser River; GR, Goat River; HR, Holmes River; MiR, Milk River; MR, McGregor River; WC, Walker Creek

analysis, however, it was unknown what the relative composition of the overwintering mixture was and, consequently, how harvest of fish in the recreational fishery might impact individual spawning populations.

Seven samples showed significant inbreeding coefficients (Nechako River, Goat River, Jarvis Creek, Fontoniko Creek, Milk River, Everett Creek and Captain Creek). Several loci in each of these populations exhibited lower than expected heterozygosity values consistent with positive inbreeding coefficients. These positive inbreeding coefficients could stem from one of two causes: localised sampling of related individuals or sampling of genetically distinct breeding aggregations that were collectively analysed as a "single" population (i.e. a Wahlund Effect). Certainly, high inbreeding coefficients owing to the latter phenomenon are consistent with the Nechako River representing a mixture of bull trout from genetically distinct populations drawn from a large area of complex tributary habitats. For tributary samples that consisted of adults of variable ages, it is unlikely that sampling of relatives caused the high levels of F_{IS} because sampling of young of the year, which have the greatest chance of being closely related to each other, yielded insignificant levels of F_{IS} (i.e. upper Walker Creek 2011 and Chalco Creek 2013). It is possible that the samples from some tributaries with high F_{IS} represent mixtures of fish from migratory and non-migratory bull trout which potentially represent distinct spawning subpopulations (but see Homel et al., 2008). With the exception of the mixtures from the Nechako River and the Jarvis and Captain creeks' samples, however, the absolute differences between expected and observed heterozygosities in the other tributary samples with significant F_{IS} values were typically low (5% or lower).

Across the study area, the extent of microsatellite allele frequency variation owing to differentiation among populations (θ) was 0.058, considerably lower that reported by Costello et al. (2003, 0.39) and Taylor and Costello (2006, 0.33). These latter studies, however, sampled much broader geographic areas (e.g. between the Pine River (Peace River) and upper Kootenay River (Columbia River) watersheds, or between southwestern BC coastal areas and the BC interior), which would be expected to show greater genetic divergence among localities that are more distant from one another than in the current study. In addition, the loci used in the current study were more variable in terms of number of alleles, which would tend to increase within-population genetic variation that could contribute to lower degrees of among-population variation (Waples, 1998). The θ value in the current study is more consistent with smaller spatial scale studies, such as in the Red Deer River (θ = 0.026), Peace River (θ = 0.040) and upper Fraser River (θ = 0.068; Taylor, 2012; Taylor & Clarke, 2007; Taylor et al., 2014). The slightly higher level of divergence observed by Taylor and Clarke (2007) in the upper Fraser River may, in part, be attributable to strong divergence (θ usually >0.08) between some tributary populations that were not sampled in the current study (Hungary Creek and Small Creek). Nevertheless, population subdivision among tributaries that were well sampled (e.g. Goat River, Chalco Creek, Walker Creek) is consistent with previously documented strong differentiation among spawning

localities in bull trout (Costello et al., 2003; Leary et al., 1993; Taylor & Costello, 2006; Taylor et al., 2014).

These genetic distinctions among baseline populations could also be characterised by the existence of three major genetic groups of bull trout. The three groups had some, but not complete, geographic coherence. Group 1 contained fish from the upper reaches of the McGregor River (Jarvis and Gleason creeks), a major tributary of the upper Fraser River. Group 2 was composed of fish from the upper portions of the sample area of the Fraser River (Goat and Holmes rivers, Chalco Creek), but group 3 contained fish widely scattered across the study area (e.g. Fontoniko and Haggen creeks). Further, six baseline populations showed greater admixture of all three genetic groups. Overall, however, the significant structure resolved indicates that there is sufficient restriction in dispersal and gene flow among populations to generate detectable genetic differences among baseline spawning localities, even those within the same watershed (Bohonak, 1999), a necessary precondition for any kind of mixed-stock analysis.

The mixture analysis suggested that the greatest proportion of fish overwintering in the Nechako River originated from Goat River, although the contribution from Chalco Creek was similarly relatively high. These streams, together with Walker Creek, McKale River and Holmes River, were estimated to account for almost 0.80 of the mixture. The proportional contributions from the Holmes River must be interpreted with caution given that its baseline sample was small (N = 14) as indicated by its high estimated baseline error. Given that Chalco Creek is a tributary of the Holmes River and had the highest individual contribution to the mixture, it is perhaps not surprising that Holmes River fish also contributed a substantial proportion to the Nechako River mixture. By contrast, the Milk River (a tributary of the Goat River) had a relatively small proportional contribution to the mixture even though the Goat River mainstem was estimated to have the highest contribution. Differences in estimated mixture proportions may reflect spawning population sizes. For example, most of the spawning habitat of the best quality occurs in the Goat River and tributaries other than the Milk River, which has high, glacially generated turbidity (Pillipow & Williamson, 2004).

The McGregor River is one of the largest tributaries of the upper Fraser River whose confluence with the latter is about 100 km upstream of the Nechako River. This study examined samples from four tributaries of the McGregor River (Fontoniko, Jarvis, Gleason and Captain creeks), but together these tributaries were estimated to contribute only about 0.13 to the mixture (range: 0.0–0.074). These results, as well as the mixed results from within watersheds like the Holmes River, suggest a complex migration and seasonal distribution network within the upper Fraser River. Bull trout may overwinter in the Nechako River, in part, to await Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) and sockeye salmon *Oncorhynchus nerka* (Walbaum) smolts and feed upon these salmon when the smolts travel downstream from Stuart and Fraser lakes in the spring, as observed in other systems (Furey et al., 2015, 2016). Consequently, tributaries that vary in salmon smolt production may also vary in the extent in which bull trout use local versus distant overwintering sites

such as the Nechako River. Although there will be other contributing factors to how bull trout select habitat on a fine scale (e.g. water velocity, depth, ice cover; Jakober et al., 1998), it is likely that a primary motivation for long-distance movements is to capitalise on abundant food availability following harsh overwinter conditions (Furey et al., 2015, 2016). The estimated contributions of various populations of bull trout overwintering in the Nechako River probably reflect variation in migration distance and the relative availability of other feeding opportunities within the Fraser River watershed.

Confidence intervals bracketing the point estimates of mixture composition were relatively broad and overlapped between many populations. Consequently, although mixture point estimates for each population need to be interpreted conservatively, the current analysis suggests that tributary populations fall into three broad groups: those that contribute a substantial amount to the mixture (Goat River, Walker and Chalco creeks), those that contribute a modest fraction (Holmes River, Milk River, McKale River, Fontoniko Creek and Captain Creek) and those that appear to contribute little to no fish to the mixture (Torpy River, Jarvis, Dome, Haggen, Gleason and Everett creeks). The error decomposition analysis suggests that the most important action to increase mixture estimation accuracy would be to increase baseline sample sizes as overall baseline error was more than twice that of fishery error and almost five times that of genotypic error. Some of the baseline error may stem from the fact that these samples consisted both of juvenile and adult fish (whereas the fishery mixture consisted of subadult and adult fish), and because some baseline samples were from different years than those encompassed by the fishery mixture. Mixture estimates may also improve if sample sizes are large and equal and spread across the same year classes among tributaries. Finally, it is possible that unsampled ("ghost") baseline populations (e.g. bull trout from tributaries of the Fraser River downstream of its confluence with the Nechako River) could also be contributing to the overwintering mixture populations and thus influence the accuracy of the mixture estimates (Hansen et al., 2001; Slatkin, 2005; Smouse et al., 1990). That said, all major known spawning populations of bull trout upstream of the confluence of Nechako and Fraser rivers were included in this study. Further, many streams downstream of the confluence are considered too warm for bull trout (Parkinson et al., 2016); the nearest known spawning population downstream of the confluence is that in the Quesnel River/Lake watershed, about 140 km distant. Several aerial surveys indicated no radio-tag detections (of fish tagged in the Nechako River and upper Fraser River) in the Quesnel system, nor have stream-side surveys detected bull trout moving out of the Quesnel drainage into the Fraser River and hence potentially upstream to the Nechako River overwintering sites (R. Pillipow, personal observations). Finally, the STRUCTURE analysis found no genetic groups in the Nechako mixture that were not found in the 14 tributary populations.

By contrast to the informative mixture analysis, fish whose movements between spawning streams and the Nechako River overwintering area were identified by radiotracking were correctly assigned to those same spawning streams just over 50% of the time.

This indicates that microsatellite typing efforts would likely need to be increased substantially (e.g. a greater number of informative loci typed) if identification of individual fish to spawning tributaries, which was not the primary goal of the present study, was desired. Given that population subdivision was modest, however, genetic assignment of individual fish might be better attempted using genomic-level analyses incorporating variation across thousands of loci (e.g. Bohling et al., 2019; Gilbey et al., 2016; Small et al., 2015). Further, it is common for mixture analyses to outperform individual assignment-based methods given that the former allows for proportional contributions of individual fish to different source populations, which is more realistic approach based on the inevitable uncertainty about actual population of origin for individual fish (Manel et al., 2005).

While detailed analysis of the tagging data was beyond the scope of this study, they provided some important information. Most fundamentally, these data demonstrate that bull trout move from known spawning and rearing areas in the late summer to overwintering areas in the Nechako River (and back again). Consequently, inferences based on mixture analyses are directly corroborated by documented movements of fish from the same spawning tributaries. Conversely, tributaries with little to no estimated contribution to the overwintering population via mixture analysis (e.g. Dome Creek, Torpy River) had no documented detections of fish tagged in the Nechako River. Together, movement and genetic data highlight that distinct habitats up to hundreds of kilometres apart are important for different phases of the life cycle of potadromous bull trout (McPhail & Baxter, 1996). This is particularly notable for small tributaries such as Fontoniko Creek (McGregor River) and Chalco Creek (Holmes River) where both radiotracking and genetic data indicate bull trout move between these tributaries and the Nechako River. Travel from these small and distant tributaries to overwinter in the Nechako River might not have initially been expected given that these fish move through potential overwintering areas in larger rivers, such as the McGregor, Holmes and Fraser rivers, to get to the Nechako overwintering sites. A better understanding of the proximate drivers for seasonal distribution and migrations will be an important next step in interpreting these complex patterns. Nevertheless, such large-scale movements by potadromous fishes highlight their sensitivity to habitat degradation because potential threats must be integrated over extensive areas encompassing spawning and overwintering habitats as well as the migration corridors between them (McIntyre et al., 2016).

The current results have several implications for management of upper Fraser River bull trout. First, the current catch-and-release winter bull trout fishery on the Nechako River involves multiple spawning populations from far afield. Any potential change in regulations that permit harvest in this fishery will, therefore, have implications for sustainability of multiple populations. The impacts of a retention fishery will, however, depend on fishing effort, regulations imposed and productivity of each population. The present results are motivating managers to undertake better assessments of the abundance of baseline spawning populations and of the productivity

of these bull trout populations. A combination of mixture proportion estimates and baseline population-specific demographic analyses is required before an informed decision on potential harvest of fish in the overwintering mixture can be made. Second, if overwintering distribution of bull trout is driven primarily by the distribution of available food (primarily sockeye salmon and Chinook salmon smolts), commercial, recreational and Indigenous fishery impacts on these forage species during their adult phases may impact abundance and distribution of overwintering bull trout. Any re-distribution of bull trout driven by variation in abundance of their forage base could, therefore, impact a spatially managed bull trout fishery. Finally, the results will promote more spatially explicit evaluations of threats, of which overharvest is a component, that are integral to conservation assessments by illustrating to what relative extent component populations are exploited in mixed-stock fisheries (COSEWIC, 2013).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.