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Development and Application of Single-Nucleotide Polymorphism (SNP) Genetic Markers for Conservation Monitoring of Burbot Populations

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Abstract

The transboundary (Idaho, USA; and British Columbia, Canada) population of Burbot *Lota lota* native to the Kootenai River basin once provided a popular sport and commercial fishery and has been culturally significant to the Kootenai Tribe of Idaho for millennia. However, the population has experienced significant declines over the last 30 years, due primarily to habitat loss and alteration caused by water storage and diversion. By the late 1990s, the population was considered functionally extinct, with estimates of fewer than 50 Burbot in the wild and little to no recruitment, prompting an ongoing international recovery effort. As part of these recovery efforts, managers have been actively developing a hatchery supplementation program to rebuild the population and support future tribal subsistence harvest and recreational fisheries. Although supplementation breeding programs have the potential to rapidly rebuild depleted natural populations, careful genetic management is critical. To monitor genetic diversity and potential inbreeding in the broodstock and to provide parentage-based tagging of supplementation offspring, we developed a set ($N = 96$) of highly variable single-nucleotide polymorphism (SNP) genetic markers. The subset of 96 SNP markers was developed from a larger suite of 6,517 SNPs that were discovered by using restriction site-associated DNA sequencing. This cost-efficient technology allows for the rapid discovery of thousands of SNP markers in species that have not been extensively studied previously or for which there are little existing DNA sequence data. We demonstrated high accuracy (>99%) of our SNP set for parentage and individual identification through simulated and empirical tests. The SNP marker set provides a powerful new tool for managing broodstock and for monitoring and genetically tagging Burbot to track the growth, survival, and movement of released individuals.

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The Burbot *Lota lota* has a circumpolar distribution and is the only member of the cod family (Gadidae) that lives permanently in freshwater (McPhail and Lindsey 1970; McPhail and Paragamian 2000). Although the species is abundant throughout portions of its range and has a widespread distribution in North America, northern Europe, and Asia, many populations either have been extirpated or are in serious decline (Stapanian et al. 2010). In the United States, Stapanian et al. (2010) estimated that Burbot are vulnerable or imperiled in 12 (48%) of the 25 states in which they currently reside. Population declines have been attributed to a number of factors, including habitat loss and alteration caused by water storage and diversion; invasive species; overexploitation; and climate change (Stapanian et al. 2010 and references therein). As a result of these declines, fish managers in both North America and Europe are investigating the development of conservation breeding programs to supplement imperiled populations and reintroduce Burbot to areas where they have been extirpated (Vught et al. 2008; Paragamian and Hansen 2011; Worthington 2011; Blabolil et al. 2018).

The transboundary (Idaho, USA; and British Columbia, Canada) population of Burbot native to the Kootenai River (spelled "Kootenay River" in Canada) basin downstream of Kootenai Falls once provided a popular sport and commercial fishery and is culturally significant to the Kootenai Tribe of Idaho. The population is currently considered functionally extinct, with fewer than 50 Burbot estimated to occur in the wild and little to no recruitment (Paragamian et al. 2008), and is the subject of an ongoing international recovery effort. The collapse of the population has been primarily attributed to the construction and operation of Libby Dam, which dramatically altered the flow and temperature regimes and nutrient supply within the Kootenai River (Paragamian and Wakkinen 2008). A number of possible limiting factors associated with these alterations has been identified; all are focused on spawning and early life periods (Hardy and Paragamian 2013).

Many of the current conservation strategies for Kootenai River Burbot are directed at a broad-based habitat restoration program to address ecosystem alterations that are believed to have contributed to the population's collapse. However, with evidence that the remnant population is too small to expect recovery, even with future possible habitat improvements, managers have been actively developing a hatchery supplementation program to rebuild the population and support tribal subsistence harvest and recreational fisheries.

Since 2009, the Kootenai River has been supplemented with hatchery-reared Burbot cultured at the University of Idaho's Aquaculture Research Institute (Jensen et al. 2008a, 2008b; Neufeld et al. 2011) and, more recently, those cultured at the Kootenai Tribe of Idaho's Twin Rivers Hatchery. Broodstock collection during the first year included a small number of adults from two areas (Duncan Reservoir crossed with Moyie Lake adults; see Table 1 and Figure 1 for locations). Since then, annual broodstock collection has been only from wild adult spawners in Moyie Lake (southeastern British Columbia). The Moyie Lake stock was chosen because it resides within the Kootenai River drainage and shares the same the dominant mitochondrial DNA lineage with Burbot found in the Kootenai River (Paragamian et al. 1999; Powell et al. 2008). In addition, the Moyie Lake stock is abundant enough to provide sufficient gametes and has spawning sites that provided access to spawners (Hardy et al. 2015).

Although supplementation breeding programs have the potential to rapidly rebuild depleted natural populations, careful management is critical to maintain genetic diversity and prevent inbreeding. In addition, monitoring of supplementation fish is necessary to track the growth, survival, and movement of released individuals. Both coded-wire tags (CWTs) and PIT tags have been investigated for the marking and monitoring of Burbot in the Kootenai River (Ashton et al. 2014). These mechanical tag types showed no significant effects on survival and growth, and high tag retention (>95%) was achieved in the hatchery for a period up to 1 year posttagging. The limitation of these tagging methods is that they are labor intensive and not possible for use with juvenile fish smaller than 85 mm TL (Ashton et al. 2014). In addition, lifetime retention rates for both tags have not yet been evaluated. Application of otolith marks for the mass marking of juvenile life stages has been widely used for a variety of species (Brothers 1990; Mendoza 2006), and otolith marking with fluorescent dyes has been tested in Burbot (Stańczak et al. 2017). The limitations of otolith batch marking are (1) it precludes the tracking of families or individual fish; and (2) similar to tagging with CWTs, the recovery of the mark requires lethal sampling.

Parentage-based tagging (PBT) is a relatively new hatchery genetic management tool that uses well-validated, DNA-based parentage methodologies for genetically tagging all offspring produced at a hatchery (Anderson and Garza 2006; Steele et al. 2013). It is a cost-efficient alternative to traditional mechanical tags (e.g., CWTs) for identifying the origin and brood year of fish released from a hatchery. Furthermore, because the parents of any fish can be identified, it allows for estimation of the reproductive success of adults spawned in the hatchery.

Parentage analyses, especially involving hundreds to thousands of potential parents, requires a powerful set of genetic markers to avoid both type I (false-positive) and type II (false-negative) errors. Microsatellites have been the most common codominant genetic marker for studies of parentage and kinship in fish populations and have recently been investigated for PBT of supplementation Burbot in the Kootenai River (Ashton et al. 2016). In the Ashton et al. (2016) study, supplementation broodstock were screened

TABLE 1. Purpose of sample collection, sample locations, latitude/longitude, genetic ID, sample year, sample size (N), and the percentage polymorphic loci (%P) observed among the 6,517 single-nucleotide polymorphisms (SNPs) in each Burbot sample collection used for SNP discovery (PBT = parentage-based tagging).

Purpose	Sample location	Latitude, longitude	Genetic ID	Sample year	N	%P
SNP discovery	Duncan Lake, British Columbia	50.4030, −116.9697	LloDUNL97C	1997	9	19.7
	Columbia Lake, British Columbia	50.1813, −115.8356	LloCOLL99C	1999	8	19.4
	Kootenay Lake, British Columbia	49.6667, −116.8333	LloKOOL97C	1997	10	21.9
	Kootenai River, Idaho	48.9980, −116.5027	LloKOTN98C	1998	10	27.4
	Moyie Lake, British Columbia	49.3477, −115.8319	LloMOYL12S	2012	22	17.3
	Bull Lake, Wyoming	43.1931, −109.1077	LloBULL07C	2007	3	10.2
	Boysen Reservoir, Wyoming	43.3753, −108.1784	LloBYSR07C	2007	3	18.2
	Dinwoody Lake, Wyoming	43.3940, −109.3685	LloDWDY07C	2007	3	9.4
	Garrison Dam National Fish Hatchery, North Dakota	47.4889, −101.4127	LloGDFH07C	2007	3	11.9
	Great Slave Lake, Northwest Territories	61.6666, −114.0000	LloGSLV09C	2009	7	32.2
	Little Fox Lake, Yukon	61.3383, −135.6481	LloLFXL11C	2011	3	2.8
	Pine Lake, Yukon	60.8184, −137.4457	LloPINL12C	2012	2	9.9
	Squanga Lake, Yukon	60.4772, −133.6389	LloSQUL13C	2013	2	6.9
	Copper River, Alaska	62.6654, −143.9132	LloCOPP13C	2013	5	14.7
Parentage testing/PBT baseline	Moyie Lake, British Columbia	49.3477, −115.8319	LloMOYL11S	2011	46	N/A
			LloMOYL12S	2012	156	N/A
			LloMOYL13S	2013	74	N/A
			LloMOYL14S	2014	66	N/A
Parentage testing	Moyie Lake, British Columbia	49.3477, −115.8319	LloMOYL11B	2011	39	N/A
			LloMOYL12B	2012	274	N/A
Origin and relatedness	Kootenai River, Idaho	48.9980, −116.5027	LloKOTN13S	2013	121	N/A
			LloKOTN14S	2014	149	N/A
			LloKOTN15S	2015	600	N/A

with 14 microsatellite loci previously developed for Burbot (Sanetra and Meyer 2005). That screening, which involved multiplexes of four panels, indicated low levels of genetic diversity within supplementation broodstock and high false-assignment rates during parentage testing (19.0%). In addition, all of those microsatellite loci exhibited dinucleotide repeats, which can be difficult to score and multiplex due to the presence of "stutter" bands (Walsh et al. 1996).

Although further efforts could have tried to optimize and incorporate additional dinucleotide microsatellites developed for Burbot (Zhao et al. 2009) or to construct additional microsatellite libraries specific to Kootenai River basin Burbot, this study instead focused on employing recent advances in genomewide restriction site-associated DNA sequencing (RADseq) technology to identify single-nucleotide polymorphism (SNP) markers. This relatively new and cost-efficient technology allows the discovery of hundreds to tens of thousands of SNP markers in nonmodel organisms and is accomplished by sequencing short fragments of genomic DNA that flank the recognition site of a particular restriction enzyme (Davey et al. 2011;

Narum et al. 2013). Some of the advantages that SNPs have over microsatellite markers include their higher abundance and uniform distribution across the genome, their lower genotyping error rate, and the reduced costs and labor associated with genotyping (Fabbri et al. 2012).

The overall objectives of this study were to (1) use RADseq to identify highly variable SNP markers for parentage analysis and monitoring of genetic diversity; (2) once SNPs were identified, develop a working panel of 90–100 TaqMan assays for SNP genotyping and demonstrate the accuracy of this panel for PBT using simulated and known parent/offspring data sets; and (3) use the final panel of SNP assays to determine the origin and relatedness of Burbot sampled in the Kootenai River during 2013–2015.

METHODS

Sample Collection

For SNP discovery, we sampled 90 Burbot from 14 sample locations (Table 1). The majority of samples came
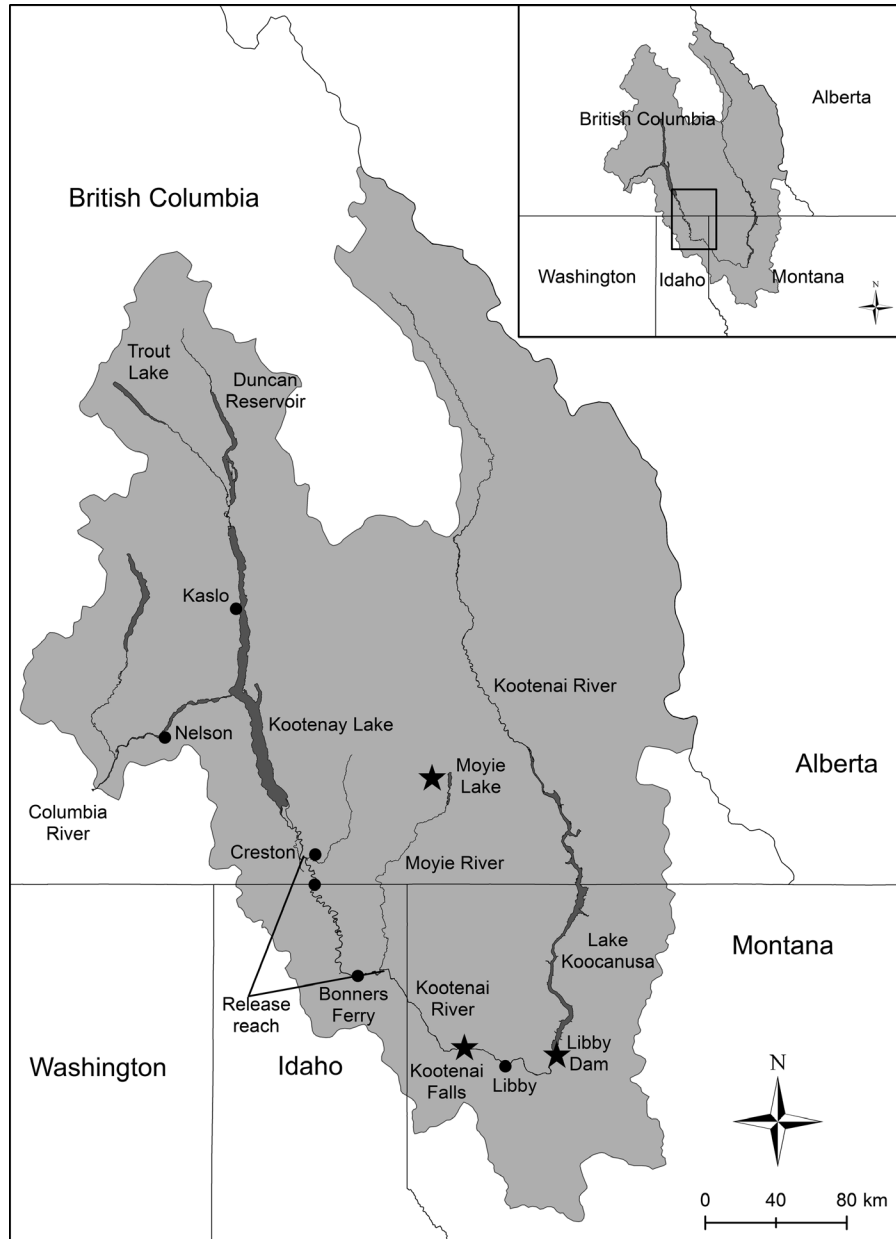
FIGURE 1. Map of the Kootenai/Kootenay River basin in Idaho, Montana, and British Columbia. Moyie Lake is the source of Burbot broodstock collection for the supplementation program. Stocking occurs in the main-stem Kootenai River from the mouth of the Moyie River, Idaho, to Creston, British Columbia.

from locations within the Kootenai River and Moyie Lake drainages in Idaho and British Columbia, Canada. However, to capture intraspecific variation, we also included samples from Wyoming; Alaska; Yukon, Canada; and Northwest Territories, Canada. To build the PBT baseline, 342 adult Burbot that were used as broodstock from Moyie Lake were sampled in 2011–2014. In 2011, sampling was incomplete, with 46 (75.4%) of the 61 broodfish sampled. For the remaining years, all adults were successfully sampled (2012: $N = 154$; 2013: $N = 75$; 2014:

$N = 66$; 2015: $N = 125$). For parentage testing, offspring of known parentage were sampled at the hatchery in 2011 ($N = 39$) and 2012 ($N = 274$). To determine the origin and relatedness of Burbot in the Kootenai River after supplementation, adults were sampled in 2013 ($N = 121$), 2014 ($N = 149$), and 2015 ($N = 600$).

DNA Extraction

Genomic DNA was extracted from fin tissue samples by using Nexttec Genomic DNA Isolation Kits from

XpressBio (Frederick, Maryland) following the manufacturer's recommended protocols. Extracted genomic DNA was quantified using Quant-iT PicoGreen dsDNA (double-stranded DNA) Assay Kits (Invitrogen, Grand Island, New York) and a Victor2 Microplate Fluorometer (Perkin Elmer, Waltham, Massachusetts).

Library Construction and Sequencing

The RADseq libraries prepared for Illumina sequencing were produced using a protocol modified from one previously published (Miller et al. 2007; Hecht et al. 2013). The 90 Burbot samples were individually quantified using Quant-iT PicoGreen dsDNA Assay Kits and a Tecan Infinite M200 Plate Reader. Using the DNA quantities, 150 ng of each sample were added to a 100-μL restriction digest using the Sbf1 restriction enzyme (New England Biolabs, Ipswich, Massachusetts). Each sample was then tagged by ligation of 1 of 90 unique barcoded adapters (P1 adapter) to the Sbf1 site. The barcoded samples were mixed together into a library of 90 individuals, and approximately 4 μg of each were sheared using a Bioruptor Plus UCD-300 Sonication System (Diagenode, Denville, New Jersey). After sonication, each library was concentrated using the Qiagen MinElute PCR Purification Kit (Qiagen, Valencia, California) prior to size selection by AMPure XP magnetic beads. Size selection was performed using the standard protocol, targeting fragments between 200 and 700 base pairs (bp).

The remainder of the library preparation followed the methods outlined by Hecht et al. (2013). Prior to sequencing, RAD libraries were quantified by quantitative PCR custom library quantification standards prepared from an RNA sequencing library with a known concentration on an ABI 7900HT Sequence Detection System (Life Technologies, Grand Island, New York). Libraries were sequenced with paired-end, 100-bp reads on an Illumina HiSeq 1500 Sequencer (Illumina, Inc., San Diego, California). Only single-end data were used for analyses in this study.

SNP Discovery, Polymorphism, and Genetic Structure

Genotyping and SNP discovery were performed using STACKS version 1.10 (Catchen et al. 2011, 2013). Quality of raw reads was evaluated using the program FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), indicating that the last 25 bases were most prone to sequencing errors. Therefore, 100-bp reads were trimmed from the 3′ end to 75 bp to remove the portion of the read that was most prone to sequencing error. Trimming, demultiplexing, and quality filtering were performed using the "process_radtags" module in STACKS. Quality filtering steps included (1) removing reads with any uncalled bases or low-quality scores and (2) rescuing barcodes and partial Sbf1 recognition sites.

Reads were sorted into stacks of similar sequences using "ustacks" in STACKS. The deleveraging (-d) and removal (-r) functions were utilized to exclude paralogous and repetitive sequences. The minimum read depth of a stack was set to 5 (-m); a maximum mismatch of 2 bases (-M) was allowed when merging primary stacks, and a maximum mismatch of 4 bases (-N) was allowed when merging secondary stacks.

A representative catalog of RAD tags was created in "cstacks," including two individuals per population and averaging 500,000–1,100,000 retained reads per individual. All individuals were aligned to the catalog using "sstacks" and subsequently genotyped via the "populations" program in STACKS. The RAD tag loci were pruned to exclude those (1) containing less than 1 SNP or more than 4 SNPs per tag, (2) with a minor allele frequency less than 0.01, (3) that were genotyped in less than 70% of individuals, and/or (4) showing significant deviations from Hardy–Weinberg equilibrium (HWE). The RAD tag locus sequences are available from the National Center for Biotechnology Information (Sequence Read Archive, Accession Number SUB5125488).

To develop a panel of SNPs for PBT, we chose 1,690 variable SNPs observed in sample collections from Moyie Lake and the Kootenai River. These SNPs were further filtered by removing ones that did not conform to HWE expectations ($P < 0.05$); the remaining SNPs were ranked from highest to lowest genetic diversity (minor allele frequency). Paired-end sequences for the top-100 ranked SNPs were aligned in Sequencher version 5.2.4 (Gene Codes Corp., Ann Arbor, Michigan; http://www.genecodes.com) and were submitted to Applied Biosystems' Assays-by-Design service for SNP TaqMan assay development. Of the 100 sequences submitted, ninety-six 5′ exonuclease assays (Holland et al. 1991) were successfully designed with 6-FAM and VIC allele-specific probes for genotyping (Table 1). Primer and probe sequences for all assays are publicly available from the FishGen database (www.fishgen.net; after registering, the user can go to the Marker Sets tab and find and export the Marker Panel file, "Lota Lota SNP").

Genotyping of these 96 TaqMan SNP assays was subsequently performed on study samples by using Fluidigm 96.96 Dynamic Array Integrated Fluidic Circuits (IFCs). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and the 96 TaqMan SNP assays were hand-pipetted onto the 96.96 IFCs. Sample and SNP assay cocktail recipes and PCR protocols are available by request from the corresponding author (M. R. Campbell). The IFCs were imaged on a Fluidigm EP1 system and were analyzed and scored using Fluidigm SNP Genotyping Analysis version 3.1.1. Genotypes were imported and organized in a Progeny database (Progeny Software, South Bend, Indiana).

Following the discovery of polymorphic SNPs, we estimated the percentage of polymorphic loci observed in each sample collection by using the program GenAlEx version 6.1 (Peakall and Smouse 2006). We estimated Nei's genetic distance (Nei 1972) between collections using GENDIST in PHYLIP version 3.5 (Felsenstein 1993). To help visualize genetic relationships, a neighbor-joining dendrogram was generated from chord distances with the program NEIGHBOR in PHYLIP by using a bootstrapping algorithm. Bootstrap replicates of 1,000 iterations were attained with SEQBOOT, and a consensus tree was formed with CONSENSE in PHYLIP. The dendrogram was edited and visualized using TreeGraph 2 (Stöver and Müller 2010).

Testing the Resolving Power of the SNP Panel for Parentage-Based Tagging Using Simulated and Empirical Testing

Simulated parentage testing.— We calculated nonexclusion probabilities (probability of not excluding a nonparent) using the parentage software Cervus version 3.0.3 (Kalinowski et al. 2007) based on allele frequencies observed in broodstock collected from Moyie Lake in 2011–2014 ($N = 342$). We also completed a parentage simulation in Cervus to estimate the critical values of LOD (i.e., the natural logarithm of the overall likelihood ratio) and Delta (the difference between LOD scores of the two most likely candidate parents) associated with a strict (95%) level of statistical confidence in assignment (Kalinowski et al. 2007). The following parameters were used in the parentage simulation: the analysis type was "parent sexes unknown"; the number of offspring was 1,000; the number of candidate parents was 344; the proportion of parents sampled was 0.95; the proportion of loci typed was 0.90; the proportion of loci mistyped was 0.01; and the error rate in likelihood calculations was 0.01.

Empirical parentage testing.— For empirical testing of type I and type II errors, we tested offspring of known parentage sampled at the hatchery in 2011 ($N = 39$) and 2012 ($N = 274$) against all adult broodstock ($N = 342$) in Cervus. For type II error (failing to assign a true parent pair), we used the complete adult broodstock baseline. For type I error (assigning an untrue parent pair), we removed all known parents ($N = 54$) and re-ran the parentage analyses. All parentage analyses were conducted in Cervus.

RESULTS

SNP Discovery, Polymorphism, and Genetic Structure

After quality filtering, 6,517 SNPs were discovered and genotyped successfully (the genotypes for all 6,517 SNPs are available as a variant call format [VCF] file from the authors upon request). The percentage of polymorphic loci among sample collections used for SNP discovery (Table 1) averaged 15.9%, ranging from a low of 2.78% in samples from Little Fox Lake (LloLFXL11C) to a high of 32.2% in samples from Great Slave Lake (LloGSLV09C). To depict the genetic relationships among the sample locations used for SNP discovery, we constructed a dendrogram based on pairwise genetic distances observed using the 6,517 SNPs (Figure 2). We observed geographic clustering of sample collections, with 100% bootstrap support for grouping collections from the upper Columbia River basin, 99.8% support for grouping sample collections from the Missouri River basin, and 100% support for grouping sample locations from Alaska and northern Canada. Within the upper Columbia River basin, sample collections from the main-stem Kootenai River and Moyie Lake clustered together with 90.1% bootstrap support.

Testing of Simulated and Empirical Data Sets

Simulated parentage testing.— Average expected heterozygosity over all loci was 0.438, and the mean polymorphism information content value was 0.340. Combined nonexclusion probabilities for the first and second candidate parents were both greater than 99.9%. The probability of two independent samples or two full siblings having the same identical genotype were both less than 0.01%. Parentage simulations resulted in 93% assignment at the 95% confidence level. The critical LOD value at 95% confidence was 6.00.

Empirical parentage testing.— When all known parents ($N = 54$) were included in the full adult broodstock baseline ($N = 342$), all 314 juveniles assigned to the correct parent pair (100% accuracy). The highest number of trio locus mismatches for an assigned offspring was 1. The total number of observed trio locus mismatches for all offspring was 123 out of 29,015 loci (i.e., 0.42%). When all known parents were removed from the adult broodstock baseline, no parent pairs assigned to any offspring at 95% confidence. The average number of trio locus mismatches observed for all juveniles was 9 (range = 4–15).

Origin and relatedness of Burbot sampled in the Kootenai River in 2013–2015.— Of the 870 adult Burbot collected from the Kootenai River between 2013 and 2015, 660 assigned to parents in the PBT baseline (Table 2). The percentage of adults assigned to the PBT baseline increased each year from 36.4% in 2013 to 83.3% in 2015. In 2015, 500 of the 600 samples examined assigned to the parents in the PBT baseline. Of the 100 that failed to assign, all were of an age (estimated based on PIT tags or length) indicating that they came from stocking events that pre-dated the PBT program (Idaho Department of Fish and Game [IDFG], unpublished data). The 660 PBT-assigned individuals were produced from 40 families and 68 individual parents. However, 445 individuals (67.4%) were produced from only five families
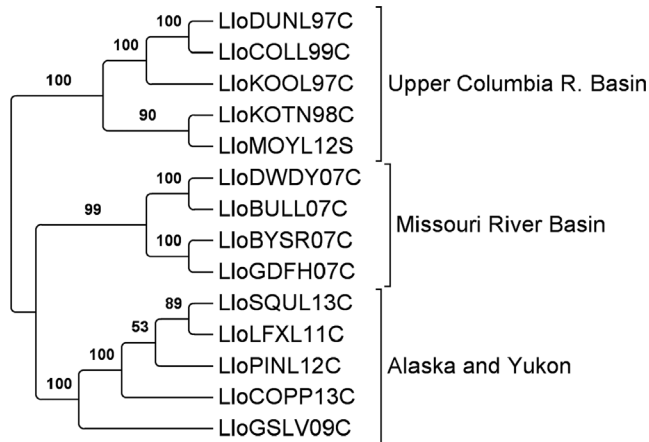
FIGURE 2. Dendrogram showing genetic relationships among Burbot sample collections (neighbor-joining tree based on pairwise Nei's genetic distances). All bootstrap values exceeded 50%. Collection codes are defined in Table 1.

(Figure 3)—all belonging to brood year 2011—consisting of nine parents.

DISCUSSION

Parentage-based tagging resolves many of the limitations of traditional tagging methods. It provides the ability to individually tag offspring and is a noninvasive approach because the parents, not the offspring, are genetically sampled at spawning. The parentage "tag" is permanent and can be recovered from offspring at any life stage via a nonlethal fin clip. It is also especially efficient, particularly for highly fecund species like Burbot (up to 3.4 million eggs; Roach and Evenson 1993), as the genotyping of a parent pair effectively tags all of their offspring.

In this study, we developed a highly powerful SNP genetic marker panel for PBT of Burbot in the Kootenai River basin. Accuracy of this panel for parentage was demonstrated through simulation testing and the empirical screening of juveniles of known parentage. These results indicate that managers can have high confidence in parentage and individual identification results in the future. This is important because although stocking efforts have been initially successful in rebuilding the abundance of Burbot within the Kootenai River, substantial research is still needed in areas related to assessing survival, growth, and reproductive success of stocked fish. For example, hatchery rearing is expensive, and the ability to release larval Burbot would significantly reduce costs and threats from domestication selection by reducing rearing in the hatchery environment. Since Burbot smaller than 85 mm cannot be mechanically tagged, PBT provides the first opportunity to evaluate the survival of this early life history stage.

Monitoring the survival of released larval Burbot may contribute to decisions related to rearing and release strategies, but perhaps more importantly, it may help managers pinpoint the life stage at which recruitment failure is occurring within the Kootenai River. It is hypothesized that changes in Kootenai River flow patterns after the construction of Libby Dam disrupted or delayed adult spawning migration behavior (Paragamian and Wakkinen 2008) and food availability for larval Burbot (Hardy and Paragamian 2013). In addition, it is known that Burbot have very specific temperature requirements (0–6°C) for inducing spawning behavior (.Zarski et al. 2010), successful egg incubation (McPhail and Paragamian 2000), hatching success, and posthatch embryo development (Vught et al. 2008). Survival and growth documentation of released larval Burbot would help to confirm that the limiting factor occurs at the spawning or egg incubation period.

The PBT program will also greatly benefit the monitoring of older juveniles, since the program has only been able to PIT tag approximately 10% of the juveniles released each year due to the high costs and labor requirements (Ross et al. 2018). Since 2012, PBT rates have averaged over 99% for the Moyie Lake broodstock, which will significantly improve the precision of survival and growth estimation. This will greatly benefit managers as they develop harvest models for Burbot in the Kootenai River. Additionally, because virtually all Burbot released from the hatchery are now genetically tagged, the sampling of juvenile Burbot that fail to assign to Moyie Lake broodstock would indicate successful reproduction of released hatchery fish. This information is important, since managers' long-term goal is to restore a viable, self-sustaining population of Burbot to the Kootenai River.

Until natural production is documented and is deemed sufficient to meet harvest and conservation goals, hatchery supplementation will have to continue. Although the program has relied on broodstock from Moyie Lake, a management goal is to transition to collecting eggs solely from Burbot that have matured in the Kootenai River. This will significantly reduce the costs and logistical demands associated with spawning operations at Moyie Lake and the international transport of fertilized eggs. However, managers will have to carefully consider broodstock sourcing given the large variance in family size observed among Burbot collected from the Kootenai River in 2013–2015. Large variance in family size is one of the most important factors in reducing the effective size of a population and increasing the rate of inbreeding and genetic drift (Crow and Kimura 1970). If managers can briefly hold potential broodstock prior to spawning, thus allowing time for sampling and genotyping, pedigree information could then be used to reduce variance in family size among spawners

TABLE 2. Collection year and sample size (N) of Burbot adults sampled from the main-stem Kootenai River and the number assigned or not assigned to the Moyie Lake parentage-based tagging (PBT) broodstock baseline. For fish assigned to the PBT baseline, the brood year assigned (BY2011, BY2012, or BY2013) is shown along with the percentage successfully assigned out of the total.

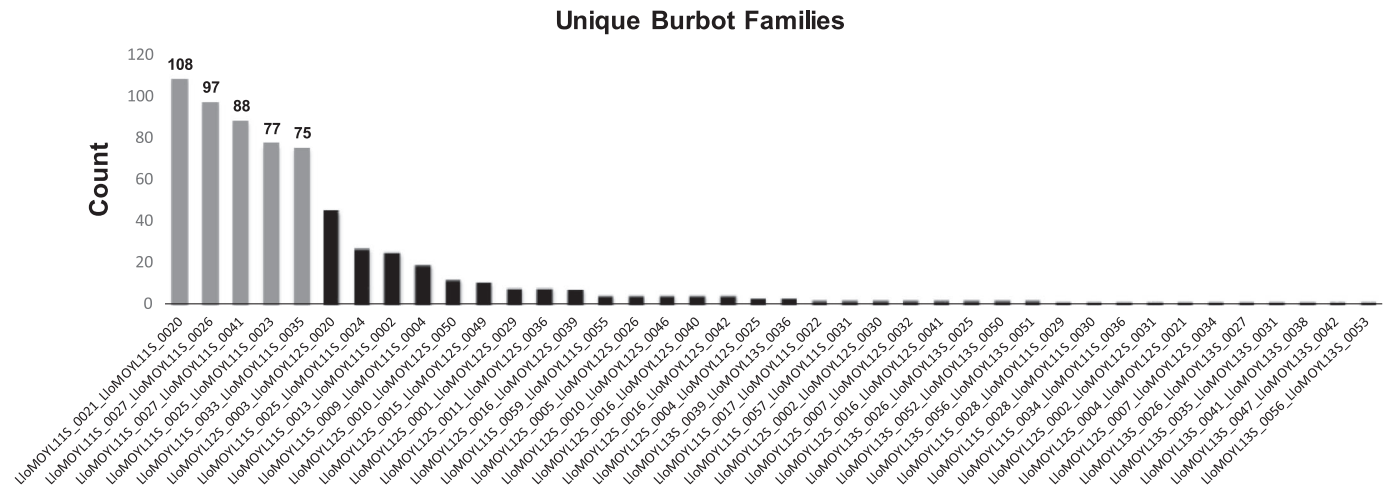| Collection year | N | Not assigned | Assigned | Brood year assigned | | | Percent assigned |
				BY2011	BY2012	BY2013	
LloKOTN13S	121	77	44	44			36.36
LloKOTN14S	149	33	116	103	13		77.85
LloKOTN15S	600	100	500	380	106	14	83.33
Total	870	210	660	527	119	14	

FIGURE 3. Number of Burbot belonging to each of 40 families identified among the 660 individuals that were collected from the main-stem Kootenai River and assigned to a brood year (2011, 2012, or 2013) by use of parentage-based tagging. Five families (gray bars), all from brood year 2011, produced 445 (67.4%) of the 660 individuals. Collection codes (x-axis) are defined in Table 1.

and to avoid inbreeding. This is a common strategy used by conservation breeding programs to maximize effective population size and reduce domestication selection (O'Reilly and Kozfkay 2014; Fisch et al. 2015).

The final SNP panel developed in this study was specifically designed using markers that were found to be variable within Moyie Lake and the Kootenai River. However, this panel may also be variable in Burbot populations outside of these areas and could be used for similar PBT and genetic monitoring projects. To determine this will require the screening of a larger number of samples than was used for discovery purposes in this study. Alternatively, the ascertainment bias we introduced during the selection of our 96-SNP panel may limit its transferability to Burbot populations outside of the upper Columbia River basin. However, the percentage of polymorphic loci observed within the 6,517 SNPs that were discovered and genotyped successfully among sample collections suggests that alternative panels of SNPs could be developed from our RAD sequences for PBT and genetic monitoring of

populations outside of the Kootenai River basin. For example, study samples from Little Fox, Squanga, and Pine lakes were from the Yukon province of Canada. These populations are geographically distant from the upper Columbia River basin and exhibited a smaller percentage of polymorphic loci (2.8, 6.9, and 9.9%, respectively). Nevertheless, there would still be between 182 and 645 SNPs available for assay design. The Little Fox, Squanga, and Pine Lake Burbot populations have supported popular recreational fisheries in the past. However, recent assessments of each have found them depleted (Barker 2013; Barker et al. 2014a, 2014b); therefore, managers are interested in monitoring these populations for changes in genetic diversity and effective population size (Oliver Barker, Senior Fisheries Biologist, Government of Yukon, personal communication).

In conclusion, we identified a powerful panel of 96 SNP markers with which PBT and individual identification of supplemented Burbot can be monitored in the Kootenai River. The creation of PBT baselines provides a

foundation for addressing numerous management and research questions in the future. Although the application of this specific 96-SNP panel for PBT monitoring outside of the Kootenai River basin may be limited, our study provides another example of the robustness of RADseq for the rapid and cost-effective detection of SNPs in non-model organisms. In addition, the DNA sequence data generated in this study may be of use for conservation and management applications across the species' range.

ACKNOWLEDGMENTS

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