American Fisheries Society Symposium 59:3–27, 2008
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Mitochondrial Variation in Western North American Burbot with Special Reference to the Kootenai River in Idaho and Montana

MADISON POWELL*
Hagerman Fish Culture Experiment Station, University of Idaho
3059F National Fish Hatchery Road, Hagerman, Idaho 83332, USA

VAUGHN L. PARAGAMIAN
Idaho Department of Fish and Game
2885 West Kathleen Avenue, Coeur d'Alene, Idaho 83815, USA

JAMES DUNNIGAN
Montana Department of Fish Wildlife and Parks, Libby Field Station
Libby, Montana, 59923, USA

Abstract.— Burbot *Lota lota* are the only freshwater members of the cod family Gadidae and possess a circumpolar distribution. Studies of phylogenetic relationships between palearctic and nearctic burbot have demonstrated the presence of distinct groups between North American and European populations. Higher resolution separation within western North American populations has thus far not been undertaken. In this study, we examined 372 burbot collected from 28 sample locations across its range in western North America. A 572 base pair portion of the mitochondrial cytochrome b gene was used to estimate diversity and divergence among populations and further characterize matrilineal lines. Three distinct haplogroups of burbot were observed that correspond to past allopatric distributions within Pleistocene refugia, namely the Pacific, Mississippi, and Missouri. Within the Columbia/Kootenai River basins west of the Continental Divide, admixtures of Pacific and Mississippi matrilines are indicative of both postglacial expansion and sorting as well as clinal variation resulting from vicariant events as seen in the Kootenai River population below Kootenai Falls. The study was also designed to help determine which northwest population would be most suitable in genetic terms as a donor stock to rehabilitate the Kootenai River, Idaho population. Of eight haplotypes found in the Kootenai River, Idaho and Columbia Lake, British Columbia, six are shared.

Introduction

Burbot *Lota lota* are the sole freshwater members of the cod family Gadidae and possess a circumpolar distribution (Scott and Crossman 1998; Berra 2001). The oldest fossil of these benthic carnivores, found in Austria, dates from the lower Pliocene (Cavender 1986). It is not surprising then that significant variation exists among burbot across its extensive range, and this, in turn, has led to numerous reexaminations from its original classification using various morphological characters (Hubbs and Shultz 1941; Lawler 1963; McPhail and Lindsey 1970; Pivnicka 1970; Snyder 1979).

* Corresponding author: mpowell@uidaho.edu

More recently, the burbot's widespread distribution and persistence has been the subject of phylogeographic studies comparing palearctic and nearctic postglacial dispersal (Van Houdt et al. 2003, 2005). In these studies, two phylogenetically distinct forms of burbot in North America were confirmed: *L. l. maculosa* (Hubbs and Shultz 1941) south of the Great Slave Lake in Canada and *L. l. lota* (Hubbs and Shultz 1941) comprising the remainder of the Nearctic region and all of Eurasia. Additionally, *L. l. maculosa* was shown to consist of three mitochondrial clades ostensibly arising from allopatric separation in different Wisconsonian glacial refugia (Van Houdt et al. 2003, 2005).

Two of these North American clades were observed east of the Continental Divide (Mississippi and Missouri clades) and had distinct distributions within the southern portions of their refuge zones. However, these clades were integrated in more northern, previously glaciated areas. The third clade (Pacific) was exclusively observed west of the Continental Divide and confirmed previous assertions by McPhail (1997) of burbot surviving in Pacific refugia during the last glacial advance (Van Houdt et al. 2005).

On a much smaller scale, genetic divergence among burbot has also been demonstrated at the watershed level. Paragamian et al. (1999) described significant clinal variation and distinct populations within the Kootenai River in Montana, Idaho, and British Columbia using restriction fragment length polymorphism analysis of mitochondrial DNA. Interest in burbot within the Kootenai River has grown as studies of its abundance in Idaho have shown significant declines over recent years (Paragamian et al. 2000, 2008, this volume). Burbot in Idaho are now considered a species of special concern and were petitioned for listing as a distinct population segment under the U.S. Endangered Species Act (1978; U.S. Office of the Federal Register 2003). However, the U.S. Fish and Wildlife Service ruled that although this population was declining in abundance, it was not a distinct population segment and did not warrant listing (U.S. Office of the Federal Register 2003).

In this study, we examined burbot samples from locations within western North America and compared them to mitochondrial cytochrome b sequences from previous studies (Miya et al. 2003; Van Houdt et al. 2003). The objectives were to (1) provide a more regional analysis and greater resolution of genetic divergence among burbot populations in relation to those within the Kootenai River, (2) provide a more complete examination of burbot dispersal adjacent to the Continental Divide in the southern portion of the Pacific refugia, and (3) provide a guideline to management decisions regarding donor stock of burbot for rehabilitation of the Kootenai River stock.

Methods

Sample Collection

Burbot were sampled from 28 locations within an area encompassed by 42°–52° north latitude and 108°–115° west longitude, which included populations both east and west of the Continental Divide in western North America. Sample locations are diagramed in Figure 1. Tissue samples collected were preserved in ethanol or lysis buffer (50 mM tris HCl, pH 8.0; 200 mM NaCl; 50 mM EDTA; 1% sodium dodecyl sulfate; 0.2% dithiothreitol) until DNA was isolated in the laboratory.

Mitochondrial DNA Analysis

Total genomic DNA was extracted from samples using a common, salt-chloroform protocol modified from Sambrook et al. (1989) and Hillis et al. (1996). Nucleotide primers L14299Gm (5'-AATTGATAT-

GAAAAACCACCGT-3') and H14942Gm (5'-ATTTGAGCCTGTTTCATGGA-3') were used along with the polymerase chain reaction amplification protocol from Van Houdt et al. (2003) to amplify and sequence an approximate 600 base pair (bp) fragment from the 5' end of the cytochrome b gene using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, California). The Phred-Phrap base-calling program (Ewing et al. 1998) was used to assess quality in base calls from sequencing. All fragments were initially aligned to a complete *L. lota* cytochrome b reference sequence (Genbank Accession NC_004379, National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov) from Miya et al. (2003) using Sequencher (Gene Codes Corp., Ann Arbor, Michigan). Aligned fragments were trimmed to 572 bp to correspond with Genbank accessions deposited from Van Houdt el al. (2003) and then translated and checked for internal stop codons, frame shifts, and other changes.

Trimmed cytochrome b fragments from individual samples were used as input for CLUSTAL W (Thompson et al. 1994) for multiple sequence alignment to the Van Houdt et al. (2003) Genbank accessions for phylogeographic comparison. Nested clade analysis (Templeton et al. 1987; Templeton 1998) was used to construct simple haplotype networks based upon nucleotide changes. Haplotypes were subdivided into (j + 1…) nested clades using the most common haplotype(s) as interior haplotypes (j = 0, initially). Estimates of pairwise genetic distance between haplotypes were generated using the Kimura 2-parameter distance model (Kimura 1980) in DNADIST (PHYLIP version 3.5c; Felsenstein 1989). Nucleotide frequencies and transition/transversion ratios were set for the Kimura model using empirical observations from all aligned sequences. The resulting evolutionary distance matrix was used to infer separate unrooted phylogenetic networks for each clade using DRAWTREE (PHYLIP version 3.5c; Felsenstein 1989). An urooted cladogram with haplotypes combined from all three clades was generated using CLUSTALTREE (CLUSTAL W; Thompson et al. 1994). TREEVIEW (Page 1996) was used to visualize the cladogram and add the percentage of occurrence to each node from 1,000 boot strap replications.

ARLEQUIN version 2.0 (Schneider et al. 2000) was used to examine hierarchical molecular variation using analysis of molecular variance (Excoffier et al. 1992) as well as to calculate estimates of within population haplotype diversity (h), nucleotide diversity (π), and the mean number of pairwise differences in each sample population. ARLEQUIN was also used to test differentiation between sample populations using Markov-Chain exact tests with 10,000 bootstrap iterations.

Results

Sequence Analysis

Of the 372 DNA samples amplified and examined, 24 samples (6.45%) were ineffectively sequenced or contained numerous nucleotide ambiguities. These samples were removed from further analysis leaving a total of 348 samples comprising 53 unique haplotypes (Table 1). When aligned with the Miya et al. (2003) sequence, a total of 22 variable sites were observed over the 572 bp region with a transition/transversion ratio of 1.25. Of these, three nonsynonymous transitions were observed at base positions #14384 (1st base substitution, Asp to Asn), #14418 (2nd base substitution, Ile to Thr), #14423 (1st base substitution, Ile to Val), and one nonsynonymous transversion at base position #14396 (1st base substitution, Asp to Tyr). The remaining variable sites comprised 12 synonymous transitions and 11 synonymous

TABLE 1. Sequence variation at 22 polymorphic sites within a 572 bp sequence of cytochrome B from 348 samples of burbot. Numbers above the sequence indicate the position of the polymorphism as aligned to the reference haplotype: NC_004379 (Miya et al. 2003). A "." indicates the same nucleotide.

	Polymorphic sites by base number																						
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	3	3	3	3	3	4	4	4	4	4	4	5	5	5	5	6	7	7	7	8	8	8	
	5	0	4	5	6	7	8	3	0	3	8	2	0	1	7	2	2	7	5	6	3	6	
Haplotype	A	T	G	A	G	C	T	G	T	T	C	C	A	T	C	A	T	A	A	A	T	T	
PAC-01	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	(Pac)*
PAC-02	.	A	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-03	.	A	A	.	T	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-04	.	A	A	.	.	.	.	.	.	.	T	T	.	.	.	.	C	T	.	.	.	.	
PAC-05	C	A	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-06	.	.	A	.	.	.	.	.	.	.	T	T	.	.	.	.	C	T	.	.	.	.	
PAC-07	C	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	G	.	.	
PAC-08	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	G	C	T	.	.	.	.	
PAC-09	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	G	C	T	.	.	A	.	
PAC-10	C	.	A	.	.	.	.	.	.	.	T	.	.	.	.	G	C	T	.	.	.	.	
PAC-11	G	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-12	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	A	.	
PAC-13	.	.	A	T	T	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-14	.	.	A	.	T	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-15	.	.	A	.	.	G	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	
PAC-16	.	A	A	.	.	.	.	.	.	.	T	.	.	.	.	G	C	T	.	.	.	.	
MOR-01	.	.	A	.	.	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	(Ms 1)*
MOR-02	.	A	A	.	.	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-03	G	A	A	.	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-04	.	.	A	.	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-05	.	.	A	T	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-06	G	A	A	T	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-07	.	A	A	T	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-08	.	A	A	.	T	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-09	.	.	A	.	T	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-10	.	.	A	.	.	.	.	.	.	.	T	.	G	G	.	.	C	T	.	.	.	.	
MOR-11	.	A	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	T	.	.	.	.	
MOR-12	.	.	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	T	.	.	.	.	
MOR-13	.	.	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	.	.	(Ms 2)*
MOR-14	.	.	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	C	.	
MOR-15	G	A	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-16	.	.	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	.	G	
MOR-17	.	A	A	.	.	.	.	A	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-18	.	.	A	.	.	G	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	.	
MOR-19	.	.	A	.	.	.	.	.	.	.	T	.	G	G	.	.	C	.	.	.	.	C	
MOR-20	.	.	A	.	.	.	.	.	C	.	T	.	G	G	.	.	C	.	.	.	.	.	
MSR-01	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.	(Mp 1)*
MSR-02	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	A	.	
MSR-03	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	G	C	.	.	.	A	.	

TABLE 1. Continued.

	Polymorphic sites by base number																					
Haplotype	14365A	14307T	14344G	14355A	14366G	14447C	14448T	14443G	14440T	14443T	14448C	14452C	14450A	14451T	14457C	14462A	14472T	14477A	14475A	14486A	14483T	14486T
MSR-04	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	C	.
MSR-05	.	.	A	.	.	.	C	.	C	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-06	.	.	A	.	.	.	C	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-07	G	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-08	G	.	A	.	T	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-09	.	.	A	.	T	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-10	.	A	A	.	T	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-11	.	A	A	T	T	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-12	.	A	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	A	.
MSR-13	.	A	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	.	.
MSR-14	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	C	C	.	G	.	G	.
MSR-15	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	G	C	.	G	.	.	.
MSR-16	.	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	C	.	.	.	G	.
MSR-17	.	.	A	.	T	.	.	.	.	G	T	.	.	.	T	.	C	T	.	.	.	.

* Designation from Van Houdt et al. (2003).

transversion mutations. Multiple, synonymous base substitutions were observed at base positions #14365, #14662, #14863, and #14896 (Table 1).

Phylogeographic Analysis

Three nucleotide changes in the anterior portion of the cytochrome b gene at base positions #14384, #14488, and #14702 were found common to all burbot samples in this study as well as North American burbot sequences from Van Houdt et al. (2003). However, all European and Beringian haplotypes from Van Houdt et al. (2003) and Miya et al. (2003) differ at these three nucleotide positions and thus provide fixed differences between Eurpean/Beringian and North American matrilines. The 53 burbot haplotypes observed were broadly divided into three major North American phylogroups based upon shared similarities. Naming of each phylogroup (Pacific, Missouri, and Mississippi) generally followed Van Houdt et al. (2003). However, each haplotype within each phylogroup was uniquely numbered for this sample set. Burbot haplotypes from this study homologous to the Van Houdt et al. (2003) Genbank accessions are indicated in Table 1 for comparison.

The greatest diversity was observed within the Missouri phylogroup (MOR) with 20 haplotypes followed by the Mississippi (MSR) and Pacific (PAC) phylogroups with 17 and 16 haplotypes, respectively. All MOR haplotypes are united by two unique, synonymous mutations at base positions #14530 and #14551. Likewise, MSR haplotypes are united by two unique synonymous mutations at base positions #14443 and #14587. A single transversion at base position #14767 unites PAC haplotypes, but this polymorphism was also observed in three Missouri haplotypes (MOR-10, MOR-11, and MOR-12) and one Mississipi haplotype (MSR-17). Percent

sequence divergence (p) between all pairs of burbot haplotypes is shown in Appendix A. Overall, sequence divergence within phylogroups ranged from 0.18% to 0.71% in PAC, 0.18–1.06% in MSR, and 0.18–1.06% in MOR. As expected, sequence divergence was higher between phylogroups with p greatest between MOR and MSR haplotypes ranging from 0.70 to 1.78% and similar between MSR–PAC and MOR–PAC haplotypes ranging from 0.53 to 1.42%.

The distribution and percentage of each burbot phylogroup is shown among all sample locations in Figure 1. Mississippi haplotypes were observed on either side of the Continental Divide, whereas Missouri and Pacific haplotypes were confined to east and west of the Continental Divide, respectively. Thus, no samples locations were observed to contain admixtures of more than two phylogroups, and no sample locations contained both PAC and MOR phylogroups.

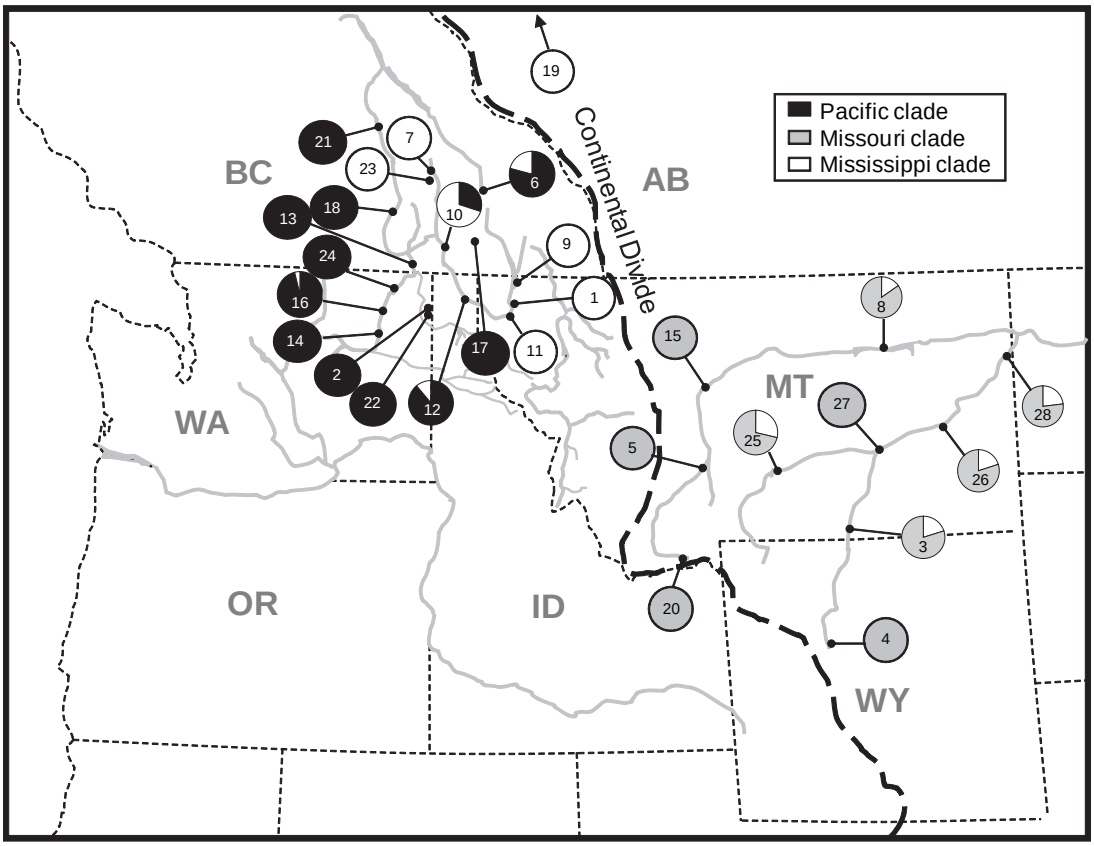

FIGURE 1. Sample locations surveyed for burbot and the relative frequency of each phylogroup at that location. Numbers designating each sample location correspond to those in Table 2. (1 = Libby Dam Afterbay, 2 = Bead Lake, 3 = Big Horn River, 4 = Boysen Reservoir, 5 = Canyon Ferry Reservoir, 6 = Columbia Lake, 7 = Duncan Reservoir, 8 = Fort Peck Reservoir, 9 = Lake Koocanusa, 10 = Kootenay Lake, 11 = Kootenai River, Montana, 12 = Kootenai River, Idaho, 13 = Columbia River at Keenlyside, 14 = Lower Lake Roosevelt, 15 = Missouri River, 16 = Mid Lake Roosevelt, 17 = Moyie Lake, 18 = Arrow Lake at Nakusp, 19 = Peace River, 20 = Red Rock Lakes, 21 = Columbia River at Revelstoke, B.C., 22 = Sullivan Lake, 23 = Trout Lake, 24 = Upper Lake Roosevelt, 25 = Yellowstone River at Big Timber, 26 = Lower Yellowstone River, 27 = Yellowstone River at Huntley, 28 = Yellowstone River at Sidney).

Figure 2 shows that burbot haplotypes generally cluster in accordance with their phylogroup. The exceptions are haplotypes PAC-03, PAC-13, and PAC-14, which cluster with the MSR phylogroup. These haplotypes were observed in sample sets from Columbia Lake, Kootenay Lake, and Kootenai River, Idaho, all of which contain admixtures of both MSR and PAC phylogroup haplotypes. In Figure 2, PAC haplotypes appear to diverge basally to the MOR and MSR phylogroups when compared with European and Beringian haplotypes from Van Houdt et al. (2003). Comparison of haplotype clusters in Figure 2 with haplotype distributions in Appendix B indicate that the pattern of haplotype clustering generally follows geographic distributions with the most common haplotypes (PAC-1, PAC-2, MOR-1, MOR-13, MSR-1, and MSR-13) widely distributed and haplotypes that cluster closely together found in the same or geographically proximate populations. Unrooted phylogenetic networks graphically show the geographic content of the cluster analysis and also provide informative geographic patterns to haplotype distributions. In general, each phylogroup was characterized by a typical, predominant, widespread haplotype (PAC-1, MOR-1, MSR-1) occurring in most populations along with presumptively derived haplotypes containing one to three mutational changes from the common haplotypes. Exceptions were observed in populations with small sample numbers such as Moyie Lake ($n = 4$), which was fixed for PAC-2, and Big Horn River ($n = 5$), which was admixed with MOR haplotypes and MSR 13. However, the Mid Lake Roosevelt sample location ($n = 28$) also contained one individual with a MSR13 haplotype.

Population Analyses

The distribution of burbot haplotypes over all 28 sample locations is shown in Appendix B. Within populations, haplotypes from the MSR phylogroup are the most widespread, being observed in 15 of 28 sample locations. Haplotype diversity, nucleotide diversity, and pair wise differences between haplotypes within sample locations are compared in Table 2. Overall, populations of admixed MOR and MSR haplotypes within the Yellowstone and Big Horn River drainages showed the greatest nucleotide diversity with pair wise differences among haplotypes ranging from 3.23 in the Big Timber sample location on the Yellowstone River to 0.00 in the Moyie Lake sample. Likewise, haplotype diversity ranged from 0.00 in the Moyie Lake population, which was monomorphic for PAC-2, to 1.00 in the Upper Lake Roosevelt and Yellowstone River at Huntley populations, which contained equal frequencies of haplotypes.

Analysis of molecular variance (Table 3) partitions the greatest amount of genetic variation among the three burbot phylogroups (67%), with a modest amount of variation within populations (28%) and minor variation among populations within phylogroups (5%). Exact tests for population differentiation shown in Appendix C indicate significant differences (+) between disjunct populations such as Columbia Lake, British Columbia and the lower Yellowstone River, as well as proximate populations without shared haplotypes, Boysen Reservoir and the Big Horn River.

The Kootenai River Basin

The Kootenai River drainage contained a total of 17 haplotypes, six PAC and 11 MSR (Appendix B). The PAC haplotypes were all found only below Kootenai Falls, Montana. The Kootenai River, Idaho and Kootenay Lake, British Columbia had a mix of PAC and MSR haplotypes, Moyie Lake, British Columbia was PAC only (a single haplotype), and Trout Lake and Duncan Reservoir, British Columbia consisted of only MSR.

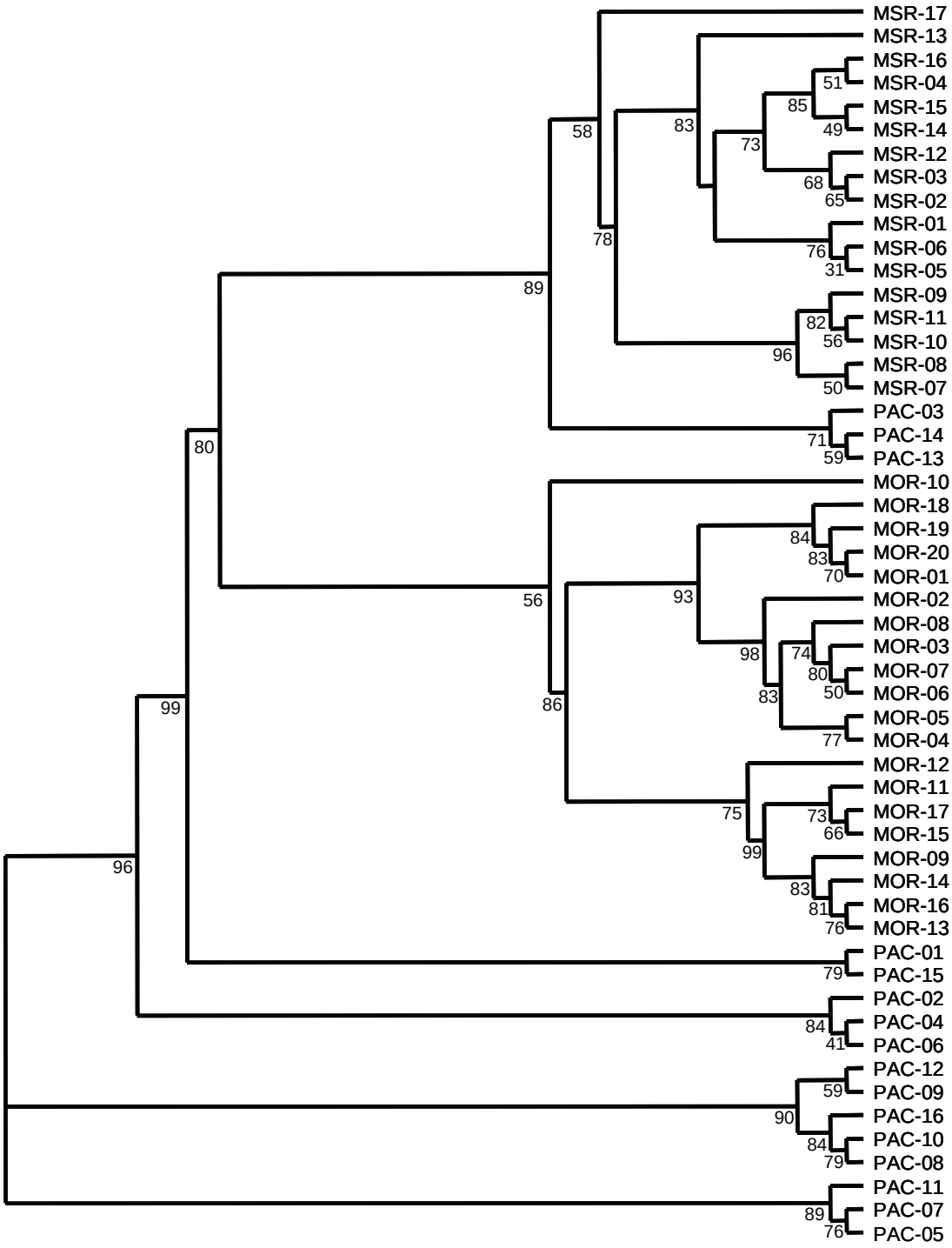

FIGURE 2. Neighbor-joining tree of burbot haplotypes constructed in PHYLIP from percent sequence divergence estimates in Appendix A.

TABLE 2. Estimates of haplotype diversity (h), mean pairwise differences and nucleotide diversity (π) for 28 burbot populations.

Population	Haplotype diversity	Mean number of pairwise differences	Nucleotide diversity
Libby Dam Afterbay	0.2355 +/– 0.1093	0.242754 +/– 0.286853	0.000424 +/– 0.000559
Bead Lake	0.4545 +/– 0.1701	0.909091 +/– 0.676029	0.001589 +/– 0.001331
Big Horn River	0.9000 +/– 0.1610	3.000000 +/– 1.874085	0.005245 +/– 0.003830
Boysen Reservoir	0.4762 +/– 0.1545	0.800000 +/– 0.611010	0.001399 +/– 0.001198
Canyon Ferry	0.8000 +/– 0.0826	1.580952 +/– 0.995148	0.002764 +/– 0.001951
Columbia Lake	0.7609 +/– 0.0596	1.797101 +/– 1.074610	0.003142 +/– 0.002094
Duncan Reservoir	0.7500 +/– 0.1391	1.571429 +/– 1.041814	0.002747 +/– 0.002075
Fort Peck	0.7821 +/– 0.1045	2.461539 +/– 1.421746	0.004303 +/– 0.002794
Lake Koocanusa	0.1947 +/– 0.1145	0.389474 +/– 0.380941	0.000681 +/– 0.000744
Kootenay Lake	0.8444 +/– 0.1029	2.311111 +/– 1.378890	0.004040 +/– 0.002726
Kootenai River, MT	0.8000 +/– 0.1138	1.309091 +/– 0.882414	0.002289 +/– 0.001740
Kootenai River, ID	0.7386 +/– 0.0989	1.562091 +/– 0.976851	0.002731 +/– 0.001910
Columbia, Keenlyside	0.3778 +/– 0.1813	0.800000 +/– 0.628045	0.001399 +/– 0.001242
Lower Lake Roosevelt	0.2333 +/– 0.1256	0.233333 +/– 0.285145	0.000408 +/– 0.000558
Missouri River	0.1667 +/– 0.1343	0.166667 +/– 0.240121	0.000291 +/– 0.000473
Mid Lake Roosevelt	0.2300 +/– 0.1095	0.553333 +/– 0.469605	0.000967 +/– 0.000915
Moyie Lake	0.0000 +/– 0.0000	0.000000 +/– 0.000000	0.000000 +/– 0.000000
Arrow at Nakusp	0.5263 +/– 0.0887	0.561404 +/– 0.478851	0.000981 +/– 0.000936
Peace River	0.7048 +/– 0.1139	1.000000 +/– 0.712444	0.001748 +/– 0.001397
Red Rock Lakes	0.5111 +/– 0.1643	0.555556 +/– 0.494259	0.000971 +/– 0.000977
Revelstoke	0.6667 +/– 0.3143	0.666667 +/– 0.666667	0.001166 +/– 0.001454
Sullivan Lake	0.6571 +/– 0.1384	0.971429 +/– 0.698138	0.001698 +/– 0.001368
Trout Lake	0.7949 +/– 0.1091	1.307692 +/– 0.871289	0.002286 +/– 0.001712
Upper Lake Roosevelt	1.0000 +/– 0.5000	1.000000 +/– 1.000000	0.001748 +/– 0.002472
Yellowstone, Big Timber	0.8571 +/– 0.1371	3.238095 +/– 1.895060	0.005661 +/– 0.003795
Yellowstone, lower	0.9000 +/– 0.1610	2.600000 +/– 1.662792	0.004545 +/– 0.003398
Yellowstone Huntley	1.0000 +/– 0.1768	1.333333 +/– 1.024942	0.002331 +/– 0.002140
Yellowstone, Sidney	0.9103 +/– 0.0559	2.820513 +/– 1.589753	0.004931 +/– 0.003124

Discussion

Variations in Western North American Burbot

Burbot mitochondrial haplotypes within this study can be chiefly characterized by three fixed differences when compared to Beringian and European haplotypes. Moreover, intraspecific divergence within Western North American matrilines also showed pronounced differences delineating three major lineages or phylogroups (PAC, MOR, and MSR). Two of these phylogroups, PAC and MOR, are allopatric in their contemporary distributions with well-defined geographic separation. The third lineage, MSR, is broadly codistributed over the study area among both PAC and MOR assemblages. On one hand, this type of phylogeographic distribution could have arisen within North American burbot if high gene flow and a very large effective population sizes (N_e) were initially present. Thus, contemporary distributions would then be a reflection of lineage sorting and balancing selection over time among dissimilar geographic areas east and west of the Continental Divide. Far more likely, however, is

TABLE 3. Analysis of molecular variance at different hierarchical levels of population structure (AMOVA). Probabilities (*P*) were estimated via permutation tests (Excoffier et al. 1992; Weir 1996).

Source of variation	d.f.	Variance components	Percentage of variation	Fixation indices
Among groups	2	1.34962 Va	66.72	F_{SC} : 0.15325*
Among populations within groups	25	0.10317 Vb	5.10	F_{ST} : 0.71818*
Within populations	320	0.57008 Vc	28.18	F_{CT} : 0.66718*
Total	347	2.02288	100.0	

* $P < 0.001$

the supposition that PAC, MOR, and MSR phylogroups evolved in allopatry and current distributions are the result of secondary admixture of MSR into historical MOR and PAC distributions following glacial retreat (Avise 1989, 2000). Supporting evidence for an allopatric origin comes from additional work on burbot from Van Houdt et al. (2005 and 2006) as well as comparative systematic evidence from lake whitefish *Coregonus clupeaformis* mitochondrial DNA, which also shows similar distributional patterns (Bernatchez and Dodson 1991). However, a partial explanation for the introgression within the Columbia Lake and upper Kootenai River drainage is the access to either drainage through a man-made canal at Canal Flats, British Columbia. At Canal Flats, the two drainages are in close proximity. The canal was constructed in 1888 and would have allowed gene flow of PAC or MSR in either direction. However, if this was to have occurred, we have evidence only for the gene flow to have gone from the Kootenai River to Columbia Lake and not the reverse.

Within this study, haplotype MSR-1 was the most widely distributed being observed in 46% (13/28) of the sample locations. The second most prevalent Mississippi haplotype, MSR-13, is shared among samples from the Peace River and Kootenai and Columbia River drainages but not observed in the Missouri and Yellowstone drainages (though MSR-1 is). Designating MSR-1 as an interior haplotype ($j = 0$) between watersheds east and west of the Continental Divide (using nested clade analysis) results in haplotypes with single mutational changes from MSR-1 (i.e., MSR-9 and MSR-13) distributed among east and west populations. In contrast, tip haplotypes ([j + 1]+1…) (i.e., haplotypes with two or more mutational changes) are not distributed among populations on both sides of the Continental Divide. This pattern of haplotype distribution suggests that a time frame for separation and subsequent expansion of the MSR lineage to either side of the Continental Divide may be calculated based upon the accumulation of observed nucleotide changes. Caution should be exercised here, however, in any estimation of divergence time for several reasons, even if one may wish to assume the requisite, randomly stochastic, steady-state of mutational change required for a molecular clock. First, sample sizes within some locations are small and haplotypes more than one mutational change away from the MSR-1 are rare (<5.0% among MSR). Chance alone may preclude our observance of these haplotypes between east and west populations in this data set. Second, the observed distribution of lineages is this

study differ somewhat from distributions observed in more northern locations in previous studies (Van Houdt et al. 2005), suggesting that the postglacial expansion of MSR may be more complex than assumed. Third, there are reticulations present within the sequence data giving rise to equally parsimonious pathways between several haplotypes such as MSR-1, MSR-2, MSR-12, and MSR-13.

The PAC-1 haplotype was the most frequently observed haplotype (27.8%), but its slightly higher frequency over MSR-1 (22.4%) is likely influenced by the choice of locations sampled and the emphasis on examining the Kootenai River and proximate populations. All populations with PAC haplotypes were observed with PAC-1 with the exception of the Moyie Lake, which only consisted of PAC-2 haplotypes.

The distribution of Missouri haplotypes is less complex and reflects the widespread appearance of the most common haplotype MOR-1 in all populations within the upper Missouri River drainage. Interestingly, the Big Horn River and Boysen Reservoir do not share haplotypes, and MOR-1 was not observed in these populations either. Sample size from the Big Horn River location was small ($n = 5$), which may explain this difference. Boysen Reservoir does, however, contain haplotypes broadly distributed elsewhere (MOR-13) in the Yellowstone River. Overall, populations within the Missouri/Yellowstone River drainages had the highest nucleotide diversity and pairwise differences.

The Kootenai River

Comparison of the ratio of sample size to observed haplotype number generally indicate that sample sizes included within the Kootenai populations are sufficient to describe the majority of haplotype variation (the majority of haplotypes observed among sample populations were rare, e.g., <5% in frequency) with all common haplotypes (PAC-1, PAC-2, and MSR-1) accounted for. Thus, a concern of small sample size bias raised by Hammond and Anders (2003) to the Paragamian et al. (1999) study appears to be circumvented. Suffice to say, further sampling within the Kootenai/y system would not significantly change the distributions or numbers of haplotypes observed unless there were significant stocks or populations left unsampled. The one exception here is the sample size from Moyie Lake, $n = 4$. Nonetheless, burbot colonization of the Kootenai River is more problematic than distributions observed in other drainages in this study and is likely shaped by the complex physiographic history of the watershed. Lake Koocanusa, the Libby Dam afterbay and the Kootenai River above Kootenai Falls in Montana were not observed to contain PAC haplotypes, whereas the Kootenai River in Idaho is predominately PAC haplotypes admixed with MSR-1 and MSR-9 haplotypes. However, farther down river, the haplotype distributions and frequencies are predominately MSR haplotypes as observed in Kootenay Lake, Trout River, and Duncan Reservoir.

One possible explanation for the presence of PAC haplotypes in the Kootenai River and Kootenay Lake is the invasion of burbot from the Pacific Refuge through waterways that may have existed during glacial times. Geologists believe that a large river flowed 50 to 70 million years ago south from the region of Kootenay Lake through the Purcell trench into an even larger stream now in the vicinity of the Pend Oreille River (Alt and Hyndman 1991). Others believed that ice dams formed at the west arm outlet of Kootenai Lake, causing the Kootenai River to flow south through the Purcell trench during the last glacial periods. We speculate that this allowed Pacific Ridge burbot to pass to the north into the Kootenai River and Kootenai Lake.

To add further to this complexity, Paragamian and Wakkinen (2008, this volume) found that the Kootenai River, Idaho popu-

lation was composed of several different life history strategies with multiple spawning locations. This life history variation could be the result of local adaptation and/or due to post glacial differences originating in stocks colonizing this reach of river. These life history variants require further examination and could be important to population rehabilitation efforts (Paragamian and Wakkinen 2008).

The Kootenai River burbot population downstream of Kootenai Falls and upstream of Kootenay Lake has a high probability of demographic extinction (Goodman 1996) with perhaps fewer than 40 adults remaining (Paragamian et al. 2008). The population is below what conservation biologists and geneticists infer as a typical minimum effective breeding population (N_e) of at least 50–500 individuals thought necessary to sustain a viable population over time (Soule 1980; Lande and Barrowclough 1996). Although immediate habitat changes (winter dam discharge and temperature management, nutrient restoration, etc.) are most important to burbot rehabilitation (Paragamian and Wakkinen 2008), the concept of donor stocks and or conservation aquaculture may also be considered as a logical measure in addition to habitat amelioration to reduce the risk of extinction. Results of this investigation suggest the best donor stock may be that of burbot from Columbia Lake, British Columbia since five of eight haplotypes are shared between populations and three of these haplotypes (PAC-3, PAC-13, and and Pac-14) are rare (<5% occurrence throughout the study). Within basin populations such as Moyie Lake may also serve as a source population for restoration efforts. Unfortunately, the sample size from Moyie Lake in this study is too small ($n = 4$) and the haplotypes observed were not shared in the Kootenai River, Idaho population. Additional sampling of the Moyie system is warranted. If Columbia Lake burbot are to be considered as a broodstock source, other important factors must also be taken into account such as disease transfers out of basin and availability of broodstock (M. Neufeld, British Columbia Ministry of Environment, personal communication) and environmental compatibility.

This investigation has provided higher resolution to separation within western North American populations of burbot. Three distinct haplogroups of burbot were observed that correspond to past allopatric distributions within Pleistocene refugia, namely the Pacific, Mississippi, and Missouri. Within the Columbia/Kootenai River basins west of the Continental Divide, admixtures of Pacific and Mississippi matrilines are indicative of both postglatial expansion and sorting as well as clinal variation resulting from vicariant events as seen in the Kootenai River population below Kootenai Falls. This more detailed strategy of matrilines may prove helpful to a better understanding of parental lineage of other burbot populations throughout there range and further enhance the restoration efforts of burbot stocks globally.

Acknowledgments

We acknowledge the dedicated time and effort of the many individuals and agencies that collected burbot tissue samples for this investigation: Matt Neufeld and Colin Spence of the British Columbia Ministry of Environment; Kenton Andreashuk of the Canadian Department of Fisheries and Oceans; Al Scholz and Derrick Raider of Eastern Washington University; Jason McLellan of the Washington Department of Fish and Wildlife; Travis Horton of the Montana Fish Wildlife and Parks; Scott Millar with Main Stream Aquatics of Alberta, Canada; Joe Deromedi of the Wyoming Game and Fish Department and Kootenai River collections by Vint Whitman (formerly of the Idaho Department of Fish and Game [IDFG]); and the many bio-aides that helped in this study. Special thanks to Joyce Faler for laboratory work. We would also like

to thank IDFG and especially to David Bennett for technical review of our manuscript. The Bonneville Power Administration and the Montana Department of Fish Wildlife and Parks provided the funding for this study.

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APPENDIX A. Persent sequence divergence in cytochrome b among observed burbot haplotypes generated using a Kimura 2-parameter model (Kimura 1980).

	PAC-01	PAC-02	PAC-03	PAC-04	PAC-05	PAC-06	PAC-07	PAC-08	PAC-09	PAC-10	PAC-11	PAC-12	PAC-13	PAC-14
PAC-02	0.0018													
PAC-03	0.0035	0.0018												
PAC-04	0.0035	0.0018	0.0035											
PAC-05	0.0035	0.0018	0.0035	0.0035										
PAC-06	0.0018	0.0035	0.0053	0.0018	0.0053									
PAC-07	0.0035	0.0053	0.0070	0.0070	0.0035	0.0053								
PAC-08	0.0018	0.0035	0.0053	0.0053	0.0053	0.0035	0.0053							
PAC-09	0.0035	0.0053	0.0070	0.0070	0.0070	0.0053	0.0070	0.0018						
PAC-10	0.0035	0.0053	0.0070	0.0070	0.0035	0.0053	0.0035	0.0018	0.0035					
PAC-11	0.0018	0.0035	0.0053	0.0053	0.0035	0.0035	0.0035	0.0035	0.0053	0.0035				
PAC-12	0.0018	0.0035	0.0053	0.0053	0.0053	0.0035	0.0053	0.0035	0.0018	0.0053	0.0035			
PAC-13	0.0035	0.0053	0.0035	0.0070	0.0071	0.0053	0.0070	0.0053	0.0070	0.0070	0.0053	0.0053		
PAC-14	0.0018	0.0035	0.0018	0.0053	0.0053	0.0035	0.0053	0.0035	0.0053	0.0053	0.0035	0.0035	0.0018	
PAC-15	0.0018	0.0035	0.0053	0.0053	0.0053	0.0035	0.0053	0.0035	0.0053	0.0053	0.0035	0.0035	0.0053	0.0035
PAC-16	0.0035	0.0018	0.0035	0.0035	0.0035	0.0053	0.0070	0.0018	0.0035	0.0035	0.0053	0.0053	0.0070	0.0053
MSR-01	0.0053	0.0070	0.0088	0.0088	0.0088	0.0070	0.0088	0.0070	0.0088	0.0088	0.0070	0.0070	0.0088	0.0070
MSR-02	0.0070	0.0088	0.0106	0.0106	0.0106	0.0088	0.0106	0.0088	0.0070	0.0106	0.0088	0.0053	0.0106	0.0088
MSR-03	0.0088	0.0106	0.0124	0.0124	0.0124	0.0106	0.0124	0.0070	0.0053	0.0088	0.0106	0.0070	0.0124	0.0106
MSR-04	0.0070	0.0088	0.0106	0.0106	0.0106	0.0088	0.0106	0.0088	0.0088	0.0106	0.0088	0.0070	0.0106	0.0088
MSR-05	0.0088	0.0106	0.0124	0.0124	0.0124	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0124	0.0106
MSR-06	0.0070	0.0088	0.0106	0.0106	0.0106	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0106	0.0088
MSR-07	0.0070	0.0088	0.0106	0.0106	0.0088	0.0088	0.0088	0.0088	0.0106	0.0088	0.0053	0.0088	0.0106	0.0088
MSR-08	0.0088	0.0106	0.0088	0.0124	0.0106	0.0106	0.0106	0.0106	0.0124	0.0106	0.0070	0.0106	0.0088	0.0070
MSR-09	0.0070	0.0088	0.0070	0.0106	0.0106	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0070	0.0053
MSR-10	0.0088	0.0070	0.0053	0.0088	0.0088	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0088	0.0070
MSR-11	0.0106	0.0088	0.0070	0.0106	0.0106	0.0124	0.0142	0.0124	0.0142	0.0142	0.0124	0.0124	0.0070	0.0088
MSR-12	0.0088	0.0070	0.0088	0.0088	0.0088	0.0106	0.0124	0.0106	0.0088	0.0124	0.0106	0.0070	0.0124	0.0106
MSR-13	0.0070	0.0053	0.0070	0.0070	0.0070	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0106	0.0088
MSR-14	0.0106	0.0124	0.0142	0.0142	0.0142	0.0124	0.0142	0.0106	0.0106	0.0124	0.0124	0.0106	0.0142	0.0124
MSR-15	0.0088	0.0106	0.0124	0.0124	0.0124	0.0106	0.0124	0.0070	0.0088	0.0088	0.0106	0.0106	0.0124	0.0106
MSR-16	0.0070	0.0088	0.0106	0.0106	0.0106	0.0088	0.0106	0.0088	0.0088	0.0106	0.0088	0.0070	0.0106	0.0088
MSR-17	0.0053	0.0070	0.0053	0.0088	0.0088	0.0070	0.0088	0.0070	0.0088	0.0088	0.0070	0.0070	0.0053	0.0035
MOR-01	0.0053	0.0070	0.0088	0.0088	0.0088	0.0070	0.0088	0.0070	0.0088	0.0088	0.0070	0.0070	0.0088	0.0070
MOR-02	0.0070	0.0053	0.0070	0.0070	0.0070	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0106	0.0088
MOR-03	0.0106	0.0088	0.0070	0.0106	0.0088	0.0124	0.0124	0.0124	0.0142	0.0124	0.0088	0.0124	0.0106	0.0088
MOR-04	0.0070	0.0088	0.0070	0.0106	0.0106	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0070	0.0053
MOR-05	0.0088	0.0106	0.0088	0.0124	0.0124	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0053	0.0070
MOR-06	0.0124	0.0106	0.0088	0.0124	0.0106	0.0142	0.0142	0.0142	0.0160	0.0142	0.0106	0.0142	0.0088	0.0106
MOR-07	0.0106	0.0088	0.0070	0.0106	0.0106	0.0124	0.0142	0.0124	0.0142	0.0142	0.0124	0.0124	0.0070	0.0088
MOR-08	0.0088	0.0070	0.0053	0.0088	0.0088	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0088	0.0070
MOR-09	0.0088	0.0106	0.0088	0.0124	0.0124	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0088	0.0070
MOR-10	0.0035	0.0053	0.0070	0.0070	0.0070	0.0053	0.0070	0.0053	0.0070	0.0070	0.0053	0.0053	0.0070	0.0053
MOR-11	0.0070	0.0053	0.0070	0.0070	0.0070	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0106	0.0088
MOR-12	0.0053	0.0070	0.0088	0.0088	0.0088	0.0070	0.0088	0.0070	0.0088	0.0088	0.0070	0.0070	0.0088	0.0070
MOR-13	0.0070	0.0088	0.0106	0.0106	0.0106	0.0088	0.0106	0.0088	0.0106	0.0106	0.0088	0.0088	0.0106	0.0088
MOR-14	0.0088	0.0106	0.0124	0.0124	0.0124	0.0106	0.0124	0.0106	0.0106	0.0124	0.0106	0.0088	0.0124	0.0106
MOR-15	0.0106	0.0088	0.0106	0.0106	0.0088	0.0124	0.0124	0.0124	0.0142	0.0124	0.0088	0.0124	0.0142	0.0124
MOR-16	0.0088	0.0106	0.0124	0.0124	0.0124	0.0106	0.0124	0.0106	0.0124	0.0124	0.0106	0.0106	0.0124	0.0106

APPENDIX A. Continued.

	PAC-15	PAC-16	MSR-01	MSR-02	MSR-03	MSR-04	MSR-05	MSR-06	MSR-07	MSR-08	MSR-09	MSR-10	MSR-11	MSR-12
PAC-02														
PAC-03														
PAC-04														
PAC-05														
PAC-06														
PAC-07														
PAC-08														
PAC-09														
PAC-10														
PAC-11														
PAC-12														
PAC-13														
PAC-14														
PAC-15														
PAC-16	0.0053													
MSR-01	0.0070	0.0088												
MSR-02	0.0088	0.0106	0.0018											
MSR-03	0.0106	0.0088	0.0035	0.0018										
MSR-04	0.0088	0.0106	0.0018	0.0018	0.0035									
MSR-05	0.0106	0.0124	0.0035	0.0053	0.0070	0.0053								
MSR-06	0.0088	0.0106	0.0018	0.0035	0.0053	0.0035	0.0018							
MSR-07	0.0088	0.0106	0.0018	0.0035	0.0053	0.0035	0.0053	0.0035						
MSR-08	0.0106	0.0124	0.0035	0.0053	0.0070	0.0053	0.0070	0.0053	0.0018					
MSR-09	0.0088	0.0106	0.0018	0.0035	0.0053	0.0035	0.0053	0.0035	0.0035	0.0018				
MSR-10	0.0106	0.0088	0.0035	0.0053	0.0070	0.0053	0.0070	0.0053	0.0053	0.0035	0.0018			
MSR-11	0.0124	0.0106	0.0053	0.0071	0.0088	0.0070	0.0088	0.0070	0.0070	0.0053	0.0035	0.0018		
MSR-12	0.0106	0.0088	0.0035	0.0018	0.0035	0.0035	0.0070	0.0053	0.0053	0.0070	0.0053	0.0035	0.0053	
MSR-13	0.0088	0.0070	0.0018	0.0035	0.0053	0.0035	0.0053	0.0035	0.0035	0.0053	0.0035	0.0018	0.0035	0.0018
MSR-14	0.0124	0.0124	0.0053	0.0053	0.0053	0.0053	0.0088	0.0070	0.0070	0.0088	0.0070	0.0088	0.0106	0.0070
MSR-15	0.0106	0.0088	0.0035	0.0053	0.0035	0.0053	0.0070	0.0053	0.0053	0.0070	0.0053	0.0070	0.0088	0.0070
MSR-16	0.0088	0.0106	0.0018	0.0018	0.0035	0.0018	0.0053	0.0035	0.0035	0.0053	0.0035	0.0053	0.0071	0.0035
MSR-17	0.0070	0.0088	0.0035	0.0053	0.0070	0.0053	0.0070	0.0053	0.0053	0.0035	0.0018	0.0035	0.0053	0.0071
MOR-01	0.0070	0.0088	0.0070	0.0088	0.0106	0.0088	0.0106	0.0088	0.0088	0.0106	0.0088	0.0106	0.0124	0.0106
MOR-02	0.0088	0.0070	0.0088	0.0106	0.0124	0.0106	0.0124	0.0106	0.0106	0.0124	0.0106	0.0088	0.0106	0.0088
MOR-03	0.0124	0.0106	0.0124	0.0142	0.0160	0.0142	0.0159	0.0142	0.0106	0.0088	0.0106	0.0088	0.0106	0.0124
MOR-04	0.0088	0.0106	0.0088	0.0106	0.0124	0.0106	0.0124	0.0106	0.0106	0.0088	0.0070	0.0088	0.0106	0.0124
MOR-05	0.0106	0.0124	0.0106	0.0124	0.0142	0.0124	0.0142	0.0124	0.0124	0.0106	0.0088	0.0106	0.0088	0.0142
MOR-06	0.0142	0.0124	0.0142	0.0160	0.0178	0.0160	0.0177	0.0160	0.0124	0.0106	0.0124	0.0106	0.0088	0.0142
MOR-07	0.0124	0.0106	0.0124	0.0142	0.0160	0.0142	0.0160	0.0142	0.0142	0.0124	0.0106	0.0088	0.0070	0.0124
MOR-08	0.0106	0.0088	0.0106	0.0124	0.0142	0.0124	0.0142	0.0124	0.0124	0.0106	0.0088	0.0070	0.0088	0.0106
MOR-09	0.0106	0.0124	0.0106	0.0124	0.0142	0.0124	0.0141	0.0124	0.0124	0.0106	0.0088	0.0106	0.0124	0.0142
MOR-10	0.0053	0.0070	0.0088	0.0106	0.0124	0.0106	0.0124	0.0106	0.0106	0.0124	0.0106	0.0124	0.0142	0.0124
MOR-11	0.0088	0.0070	0.0124	0.0142	0.0160	0.0142	0.0159	0.0142	0.0142	0.0160	0.0142	0.0124	0.0142	0.0124
MOR-12	0.0070	0.0088	0.0106	0.0124	0.0142	0.0124	0.0141	0.0124	0.0124	0.0142	0.0124	0.0142	0.0160	0.0142
MOR-13	0.0088	0.0106	0.0088	0.0106	0.0124	0.0106	0.0123	0.0106	0.0106	0.0124	0.0106	0.0124	0.0142	0.0124
MOR-14	0.0106	0.0124	0.0106	0.0106	0.0124	0.0088	0.0141	0.0123	0.0123	0.0141	0.0124	0.0142	0.0160	0.0124
MOR-15	0.0124	0.0106	0.0124	0.0142	0.0159	0.0141	0.0159	0.0141	0.0106	0.0124	0.0142	0.0124	0.0142	0.0124
MOR-16	0.0106	0.0124	0.0106	0.0124	0.0142	0.0124	0.0141	0.0124	0.0124	0.0142	0.0124	0.0142	0.0160	0.0142
MOR-17	0.0106	0.0088	0.0106	0.0124	0.0142	0.0124	0.0141	0.0124	0.0124	0.0142	0.0124	0.0106	0.0124	0.0106

APPENDIX A. Continued.

	MSR-13	MSR-14	MSR-15	MSR-16	MSR-17	MOR-01	MOR-02	MOR-03	MOR-04	MOR-05	MOR-06	MOR-07	MOR-08	MOR-09
PAC-02														
PAC-03														
PAC-04														
PAC-05														
PAC-06														
PAC-07														
PAC-08														
PAC-09														
PAC-10														
PAC-11														
PAC-12														
PAC-13														
PAC-14														
PAC-15														
PAC-16														
MSR-01														
MSR-02														
MSR-03														
MSR-04														
MSR-05														
MSR-06														
MSR-07														
MSR-08														
MSR-09														
MSR-10														
MSR-11														
MSR-12														
MSR-13														
MSR-14	0.0070													
MSR-15	0.0053	0.0035												
MSR-16	0.0035	0.0035	0.0053											
MSR-17	0.0053	0.0088	0.0070	0.0053										
MOR-01	0.0088	0.0124	0.0106	0.0088	0.0106									
MOR-02	0.0070	0.0142	0.0124	0.0106	0.0124	0.0018								
MOR-03	0.0106	0.0178	0.0159	0.0142	0.0124	0.0053	0.0035							
MOR-04	0.0106	0.0142	0.0124	0.0106	0.0088	0.0018	0.0035	0.0035						
MOR-05	0.0124	0.0160	0.0142	0.0124	0.0106	0.0035	0.0053	0.0053	0.0018					
MOR-06	0.0124	0.0196	0.0177	0.0160	0.0142	0.0070	0.0053	0.0018	0.0053	0.0035				
MOR-07	0.0106	0.0178	0.0160	0.0142	0.0124	0.0053	0.0035	0.0035	0.0035	0.0018	0.0018			
MOR-08	0.0088	0.0160	0.0142	0.0124	0.0106	0.0035	0.0018	0.0018	0.0018	0.0035	0.0035	0.0018		
MOR-09	0.0124	0.0160	0.0141	0.0124	0.0106	0.0035	0.0053	0.0053	0.0018	0.0035	0.0070	0.0053	0.0035	
MOR-10	0.0106	0.0142	0.0124	0.0106	0.0088	0.0018	0.0035	0.0070	0.0035	0.0053	0.0088	0.0071	0.0053	0.0053
MOR-11	0.0106	0.0178	0.0159	0.0142	0.0124	0.0053	0.0035	0.0070	0.0070	0.0088	0.0088	0.0070	0.0053	0.0053
MOR-12	0.0124	0.0160	0.0141	0.0124	0.0106	0.0035	0.0053	0.0088	0.0053	0.0070	0.0106	0.0088	0.0070	0.0035
MOR-13	0.0106	0.0142	0.0123	0.0106	0.0124	0.0018	0.0035	0.0070	0.0035	0.0053	0.0088	0.0070	0.0053	0.0018
MOR-14	0.0124	0.0142	0.0141	0.0106	0.0142	0.0035	0.0053	0.0088	0.0053	0.0070	0.0106	0.0088	0.0070	0.0035
MOR-15	0.0106	0.0177	0.0159	0.0142	0.0160	0.0053	0.0035	0.0035	0.0070	0.0088	0.0053	0.0070	0.0053	0.0053
MOR-16	0.0124	0.0160	0.0141	0.0124	0.0142	0.0035	0.0053	0.0088	0.0053	0.0070	0.0106	0.0088	0.0070	0.0035
MOR-17	0.0088	0.0160	0.0141	0.0124	0.0142	0.0035	0.0018	0.0053	0.0053	0.0070	0.0070	0.0053	0.0035	0.0035

APPENDIX A. Continued.

	MOR-10	MOR-11	MOR-12	MOR-13	MOR-14	MOR-15	MOR-16
PAC-02							
PAC-03							
PAC-04							
PAC-05							
PAC-06							
PAC-07							
PAC-08							
PAC-09							
PAC-10							
PAC-11							
PAC-12							
PAC-13							
PAC-14							
PAC-15							
PAC-16							
MSR-01							
MSR-02							
MSR-03							
MSR-04							
MSR-05							
MSR-06							
MSR-07							
MSR-08							
MSR-09							
MSR-10							
MSR-11							
MSR-12							
MSR-13							
MSR-14							
MSR-15							
MSR-16							
MSR-17							
MOR-01							
MOR-02							
MOR-03							
MOR-04							
MOR-05							
MOR-06							
MOR-07							
MOR-08							
MOR-09							
MOR-10							
MOR-11	0.0035						
MOR-12	0.0018	0.0018					
MOR-13	0.0035	0.0035	0.0018				
MOR-14	0.0053	0.0053	0.0035	0.0018			
MOR-15	0.0070	0.0035	0.0053	0.0035	0.0053		
MOR-16	0.0053	0.0053	0.0035	0.0018	0.0035	0.0053	

APPENDIX B. Distribution and overall frequencies of 53 cytochrome B haplotypes observed in 28 populations of burbot.

Population #	1	2	3	4	5	6	7	8	9
Haplotype	Libby Dam Afterbay	Bead Lake	Big Horn R.	Boysen Res.	Canyon Ferry	Columbia Lake	Duncan Res.	Fort Peck	Lake Koocanusa
PAC-01		9				10			
PAC-02						5			
PAC-03						1			
PAC-04									
PAC-05		1							
PAC-06									
PAC-07		1							
PAC-08									
PAC-09									
PAC-10		1							
PAC-11									
PAC-12									
PAC-13						1			
PAC-14						2			
PAC-15									
PAC-16									
MOR-01					6			6	
MOR-02					1				
MOR-03					1				
MOR-04								2	
MOR-05								1	
MOR-06								1	
MOR-07					1				
MOR-08					1				
MOR-09									
MOR-10			2						
MOR-11			1						
MOR-12			1						
MOR-13				11					
MOR-14				1					
MOR-15				1					
MOR-16				1					
MOR-17								1	
MOR-18					4				
MOR-19									
MOR-20				1	1				
MSR-01	21					5	4	2	18
MSR-02									
MSR-03									
MSR-04									
MSR-05									
MSR-06									
MSR-07									
MSR-08									
MSR-09	1								
MSR-10									
MSR-11									1
MSR-12									
MSR-13	2								1

Appendix B. Continued.

Population # Haplotype	1 Libby Dam Afterbay	2 Bead Lake	3 Big Horn R.	4 Boysen Res.	5 Canyon Ferry	6 Columbia Lake	7 Duncan Res.	8 Fort Peck	9 Lake Koocanusa
MSR-14							1		
MSR-15							2		
MSR-16							1		
MSR-17									
n	24	12	5	15	15	24	8	13	20

Population # Haplotype	10 Kootenay Lake	11 Kootenai R. MT	12 Kootenai R. ID	13 Columbia Keenlyside	14 Lower Lake Roosevelt	15 Missouri R.	16 Mid Lake Roosevelt	17 Moyie Lake
PAC-01	2		9	8	14		22	
PAC-02					2		1	4
PAC-03			1					
PAC-04				1				
PAC-05								
PAC-06								
PAC-07								
PAC-08								
PAC-09								
PAC-10				1			1	
PAC-11								
PAC-12								
PAC-13			2					
PAC-14	1		3					
PAC-15			1					
PAC-16								
MOR-01						11		
MOR-02								
MOR-03								
MOR-04								
MOR-05								
MOR-06								
MOR-07								
MOR-08								
MOR-09								
MOR-10								
MOR-11								
MOR-12								
MOR-13								
MOR-14								
MOR-15								
MOR-16								
MOR-17								
MOR-18								
MOR-19						1		
MOR-20								
MSR-01	4	5	1					
MSR-02								
MSR-03								

APPENDIX B. Continued.

Population #	10	11	12	13	14	15	16	17
Haplotype	Kootenay Lake	Kootenai R. MT	Kootenai R. ID	Columbia Keenlyside	Lower Lake Roosevelt	Missouri R.	Mid Lake Roosevelt	Moyie Lake
MSR-04								
MSR-05	1	1						
MSR-06	1							
MSR-07		1						
MSR-08								
MSR-09	1	1	1					
MSR-10	1							
MSR-11								
MSR-12								
MSR-13		2					1	
MSR-14								
MSR-15								
MSR-16								
MSR-17								
n	10	11	18	10	16	12	25	4

Population #	18	19	20	21	22	23	24	25
Haplotype	Arrow at Nakusp	Peace River	Red Rock L.	Revelstoke BC	Sullivan Lake	Trout River	Upper Lake Roosevelt	Yellowstone Big Timber
PAC-01	12			1	9		1	
PAC-02	6			2	1		1	
PAC-03								
PAC-04								
PAC-05								
PAC-06	1							
PAC-07								
PAC-08					1			
PAC-09					1			
PAC-10								
PAC-11					1			
PAC-12					1			
PAC-13								
PAC-14								
PAC-15								
PAC-16					1			
MOR-01			7					1
MOR-02			2					
MOR-03								
MOR-04								
MOR-05								
MOR-06								
MOR-07								
MOR-08								
MOR-09								1
MOR-10								
MOR-11								
MOR-12								
MOR-13								3
MOR-14								

Appendix B. Continued.

Population #	18	19	20	21	22	23	24	25
Haplotype	Arrow at Nakusp	Peace River	Red Rock L.	Revelstoke BC	Sullivan Lake	Trout River	Upper Lake Roosevelt	Yellowstone Big Timber
MOR-15								
MOR-16								
MOR-17								
MOR-18			1					
MOR-19								
MOR-20								
MSR-01		8				6		1
MSR-02		1						
MSR-03		1						
MSR-04		1						
MSR-05								
MSR-06`						1		
MSR-07								
MSR-08								1
MSR-09						1		
MSR-10						1		
MSR-11						1		
MSR-12		1						
MSR-13		3				2		
MSR-14								
MSR-15								
MSR-16						1		
MSR-17								
n	25	4	19	15	10	3	15	13

Population #	26	27	28		
Haplotype	Yellowstone lower	Yellowstone Huntley	Yellowstone Sidney	Haplotype *n*	Frequency
PAC-01				97	27.87
PAC-02				22	6.32
PAC-03				2	0.57
PAC-04				1	0.29
PAC-05				1	0.29
PAC-06				1	0.29
PAC-07				1	0.29
PAC-08				1	0.29
PAC-09				1	0.29
PAC-10				3	0.86
PAC-11				1	0.29
PAC-12				1	0.29
PAC-13				3	0.86
PAC-14				6	1.72
PAC-15				1	0.29
PAC-16				1	0.29
MOR-01	1	1	3	36	10.34
MOR-02			1	4	1.15
MOR-03				1	0.29
MOR-04		1	1	4	1.15
MOR-05			1	2	0.57

APPENDIX C. Exact tests for population differentiation using a Markov-Chain with 10,000 iterations (Rayomond and Rousset 1995).

	1 Libby Dam Afterbay	2 Bead lake	3 Big Horn R.	4 Boysen Res.	5 Canyon Ferry	6 Columbia Lake	7 Duncan Res.	8 Fort Peck	9 Lake Koocanusa	10 Kootenay Lake	11 Kootenai R. MT	12 Kootenai R. ID
Libby Dam Afterbay		+	+	+	+	+	+	+	–	+	+	+
Bead lake	+		+	+	+	+	+	+	+	+	+	–
Big Horn R.	+	+		+	+	+	+	+	+	+	+	
Boysen Res.	+	+	+		+	+	+	+	+	+	+	+
Canyon Ferry	+	+	+	+		+	+	+	+	+	+	+
Columbia Lake	+	+	+	+	+		+	+	+	–	+	–
Duncan Res.	+	+	+	+	+	+		+	+	–	–	+
Fort Peck	+	+	+	+	+	+	+		+	+	+	+
Lake Koocanusa	–	+	+	+	+	+	+	+		+	+	+
Kootenay Lake	+	+	+	+	+	–	–	+	+		–	–
Kootenai R., MT	+	+	+	+	+	+	–	+	+	–		+
Kootenai R., ID	+	–	+	+	+	–	+	+	+	–	+	
Columbia, Keenlyside	+	–	+	+	+	–	+	+	+	+	+	–
Lower Lake Roosevelt	+	–	+	+	+	+	+	+	+	+	+	+
Missouri R.	+	+	+	+	+	+	+	+	+	+	+	+
Mid Lake Roosevelt	+	–	+	+	+	+	+	+	+	+	+	+
Moyie Lake	+	+	+	+	+	–	+	+	+	+	+	+
Arrow at Nakusp	+	+	+	+	+	–	+	+	+	+	+	+
Peace River	+	+	+	+	+	+	–	+	+	–	–	+
Red Rock L.	+	+	+	+	–	+	+	–	+	+	+	+
Revelstoke	+	–	–	+	+	–	+	+	+	–	+	–
Sullivan Lake	+	–	+	+	+	–	+	+	+	+	+	–
Trout River	+	+	+	+	+	+	–	+	+	–	–	+
Upper Lake Roosevelt	+	–	–	–	–	–	–	–	+	–	–	–
Yellowstone, Big Timber	+	+	–	–	+	+	+	–	+	–	+	+
Yellowstone, lower	+	+	–	–	–	+	–	–	+	–	–	+
Yellowstone Huntley	+	+	–	–	–	+	–	–	+	–	–	+
Yellowstone, Sidney	+	+	+	+	–	+	+	–	+	–	–	+
Yellowstone, Sidney	–	–		–								

APPENDIX C. Continued.

	13 Columbia Keenlyside	14 Lower Lake Roosevelt	15 Missouri River	16 Mid Lake Roosevelt	17 Moyie Lake	18 Arrow at Nakusp	19 Peace River	20 Red Rock L.	21 Revelstoke BC	22 Sullivan Lake	23 Trout River	24 Upper Lake Roosevelt
Libby Dam Afterbay	+	+	+	+	+	+	+	+	+	+	+	+
Bead lake	–	–	+	–	+	+	+	+	–	–	+	–
Big Horn R.	+	+	+	+	+	+	+	+	–	+	+	–
Boysen Res.	+	+	+	+	+	+	+	+	+	+	+	–
Canyon Ferry	+	+	+	+	+	+	+	–	+	+	+	–
Columbia Lake	–	+	+	+	–	–	+	+	–	–	+	–
Duncan Res.	+	+	+	+	+	+	–	+	+	+	–	–
Fort Peck	+	+	+	+	+	+	+	–	+	+	+	–
Lake Koocanusa	+	+	+	+	+	+	+	+	+	+	+	+
Kootenay Lake	+	+	+	+	+	+	–	+	–	+	–	–
Kootenai R., MT	+	+	+	+	+	+	–	+	+	+	–	–
Kootenai R., ID	–	+	+	+	+	+	+	+	–	–	+	–
Columbia, Keenlyside		–	+	–	+	+	+	+	–	–	+	–
Lower Lake Roosevelt	–		+	–	+	–	+	+	–	–	+	–
Missouri R.	+	+		+	+	+	+	–	+	+	+	+
Mid Lake Roosevelt	–	–	+		+	+	+	+	–	+	+	–
Moyie Lake	+	+	+	+		+	+	+	–	+	+	–
Arrow at Nakusp	+	–	+	+	+		+	+	–	–	+	–
Peace River	+	+	+	+	+	+		+	+	+	–	–
Red Rock L.	+	+	–	+	+	+	+		+	+	+	–
Revelstoke	–	–	+	–	–	–	+	+		–	–	–
Sullivan Lake	–	–	+	+	+	–	+	+	–		+	–
Trout River	+	+	+	+	+	+	–	+	–	+		–
Upper Lake Roosevelt	–	–	+	–	–	–	–	–	+	–	–	
Yellowstone, Big Timber	+	+	+	+	+	+	+	+	–	+	+	–
Yellowstone, lower	+	+	+	+	+	+	+	+	–	+	–	_
Yellowstone Huntley	+	+	+	+	+	+	+	–	–	–	–	–
Yellowstone, Sidney	+	+	+	+	+	+	+	–	–	+	+	_

APPENDIX C. Continued.

	25 Yellowstone Big Timber	26 Yellowstone lower	27 Yellowstone Huntley	28 Yellowstone Sidney
Libby Dam Afterbay	+	+	+	+
Bead lake	+	+	+	+
Big Horn R.	–	–	–	+
Boysen Res.	–	–	–	+
Canyon Ferry	+	–	–	–
Columbia Lake	+	+	+	+
Duncan Res.	+	–	–	+
Fort Peck	–	–	–	–
Lake Koocanusa	+	+	+	+
Kootenay Lake	–	–	–	–
Kootenai R., MT	+	–	–	–
Kootenai R., ID	+	+	+	+
Columbia, Keenlyside	+	+	+	+
Lower Lake Roosevelt	+	+	+	–
Missouri R.	+	+	+	+
Mid Lake Roosevelt	+	+	+	+
Moyie Lake	+	+	+	+
Arrow at Nakusp	+	+	+	+
Peace River	+	+	+	+
Red Rock L.	+	+	–	–
Revelstoke	–	–	–	–
Sullivan Lake	+	+	–	+
Trout River	+	–	–	+
Upper Lake Roosevelt	–	–	–	–
Yellowstone, Big Timber		–	–	–