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Phylogenetic and Ecological Relationship Between "Giant" Pygmy Whitefish (*Prosopium* spp.) and Pygmy Whitefish (*Prosopium coulteri*) in North-Central British Columbia

by

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Abstract

Populations of pygmy whitefish (*Prosopium coulteri*) from McLeese Lake and Tyhee Lake display unusually fast growth and are identified as "giant" pygmy whitefish. Their phylogenetic status is unresolved yet the "giant" pygmy whitefish have been red listed as a potential sub-species by the province of British Columbia. The main objective of this thesis is to determine if the "giant" pygmy whitefish of Tyhee and McLeese lakes should be classed as a sub-species based on mitochondrial DNA sequence, nuclear intron sequence and RAPD (Random Amplified Polymorphic DNA) data. The other objectives included 1) comparing growth data to confirm the "giant" pygmy whitefish status, 2) finding microsatellite primers to work on pygmy whitefish and 3) to review and compare existing ecological data for pygmy whitefish and data taken for this study to identify possible ecological factors that may produce "giant" pygmy whitefish. The Tyhee Lake pygmy whitefish can be considered "giant" pygmy whitefish based on their size at age; the McLeese Lake pygmy whitefish were large, but were within the normal range of size at age as defined by six other pygmy whitefish populations. The sequence data from cytochrome b, the control region and the intron D of type-2 growth hormone did not distinguish the "giant" pygmy whitefish population as being genetically differentiated from other BC populations, while the Lake Superior populations was differentiated from the BC populations. The RAPD analysis determined that all BC pygmy whitefish populations are differentiated from one another, however the "giant" pygmy whitefish were no more differentiated than any other population of pygmy whitefish. It was concluded that the "giant" pygmy whitefish are not genetically differentiated from other pygmy whitefish and should not be considered a sub-species. The search for

microsatellites yielded a few promising results which may be used in future research. Although some patterns emerged from the ecological data, more needs to be collected in order to determine ecological factors that may be contributing to the unusually large size of the Tyhee Lake "giant" pygmy whitefish. Several factors are noted which may contribute to the growth of "giant" pygmy whitefish, such as the lack of potential competitors (ie. other whitefish and kokanee), the lack of aggressive piscivorous fish (ie. bull and lake trout) and the lake's fairly shallow, eutrophic environment. Although not phylogenetically distinct, the "giant" pygmy whitefish are still a unique form not known to exist in any other BC lake. In light of this it is recommended that changes to the environment of Tyhee lake should be kept to a minimum in order to conserve this rare form.

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General Introduction

Pygmy whitefish, *Prosopium coulteri,* are small fish belonging to the family Salmonidae sub-family Corigoninae (Cannings & Ptolemy 1998; Behnke 1972). Pygmy whitefish were first described by Eigenmann & Eigenmann in 1892 and are sometimes referred to as the brown back whitefish (Scott & Crossman 1973). It was not until the 1950's, however, that ecological studies on pygmy whitefish were conducted. Carl et al. (1959) were the first to document the existence of "giant" pygmy whitefish in Maclure (Tyhee) Lake, British Columbia. McCart (1963) described four populations of pygmy whitefish in British Columbia, two of which were described as "giant" pygmy whitefish (McLeese and Maclure (Tyhee) lakes). These two populations have since been red listed as a threatened species in British Columbia due to the rare occurrence of the "giant" pygmy whitefish and the eutrophication of both lakes. The Fisheries Branch (BC) has indicated that these two populations are probably a separate species or sub-species of pygmy whitefish and have classified them simply as *Prospium* spp. (Cannings & Ptolemy 1998).

When a species is considered to be threatened or endangered it may become the focus of conservation efforts. It is becoming increasingly common to seek answers to conservation problems through the use of molecular genetic techniques, to determine genetic differentiation and the best possible way to proceed with conservation efforts (Vrijenhoek et al. 1985; Swart & Ferguson 1997; Milligan et al. 1994). Molecular genetic data such as mitochondrial DNA (mtDNA) and nuclear DNA sequence can be used to identify divergent populations and Evolutionarily Significant Units (ESU). As taxonomic units are not always clear, genetically divergent populations are being given greater consideration as worthy of conservation in their own right (Moritz 1994). Another useful application of mtDNA is at the

population level to define Management Units (MU), specific populations within a geographic region (Moritz 1994).

Given that "giant" pygmy whitefish are red listed as a threatened species (potentially a sub-species), they constitute a serious conservation concern. The main objective of this thesis is to determine whether "giant" pygmy whitefish are genetically distinct from normal pygmy whitefish and whether they should be classed as a sub-species. Chapter 1 first compares the sizes of the two "giant" pygmy whitefish populations to normal pygmy whitefish to determine if the Tyhee and McLeese populations are "giant" pygmy whitefish. In order to test genetic differentiation among "giant" pygmy whitefish and normal pygmy whitefish, phylogenetic analysis was done using sequence data from the cytochrome b gene, the control region, and an intron from the type-2 growth hormone gene. Phylogenetic analysis was also done using RAPD (Random Amplified Polymorphic DNA) data. Chapter 2 describes another class of molecular genetic marker that is useful in determining genetic differentiation, microsatellites. Although microsatellite data were not obtained, the chapter contains useful information for future pygmy whitefish research. The final chapter contains ecological data collected while sampling for pygmy whitefish. The information contained in chapter 3 is used to identify potential ecological factors that might also contribute to the unusual size of the "giant" pygmy whitefish.

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Chapter 1

Genetic differentiation among pygmy whitefish (*Prosopium coulteri)* populations, of varying growth rates, using RAPDs, and mitochondrial and nuclear intron DNA sequence

1.1 Introduction:

Evolutionary relationships among fish species have been inferred, in some cases, using life history variation, not genetic variation. However, life history traits such as growth, fecundity, age at maturity and generation time have been documented to vary widely within freshwater fishes of northern temperate waters. Specific examples include growth rate in lake whitefish, *Coregonus clupeaformis* (Bernatchez et al. 1996; Pigeon et al. 1997), cisco, *Coregonus artedii* (Shields et al. 1990; Shields & Underhill 1993), rainbow smelt, *Osmerus mordax* (Taylor & Bentzen 1993), and yellow perch, *Perca flavescens* (Heath & Roff 1996), growth and age at maturity in pygmy whitefish (Bird & Roberson 1979) and spawning and fecundity in the American shad, *Alosa sapidissima* (Bentzen et al.1989). Morphological variation (such as colour) has been used to define sub-species classifications in whales (Hoelzel 1992). There is debate over whether such distinctions should be made for arctic char (*Salvelinus alpinus*) which display high levels of variation in life history and morphological traits (Hindar et al. 1986). Variation in some species can be so striking that different life history and morphological variants may be classed as sub-species, without any evidence of genetic differentiation. Fish such as coregonids (Behnke 1972), Arctic char (Hindar et al. 1986) and brown trout, *Salmo trutta* L.,(Apostolidis et al. 1997) display much variation and it is not clear whether this variation is a result of phenotypic plasticity or whether it is under genetic control. Shields and Underhill (1993) demonstrated that dwarf cisco were plastic enough to change body shape and size once transplanted to other

environments. Later, molecular genetic data demonstrated that the dwarf cisco were not genetically differentiated from normal cisco (Shields et al. 1990). Although such variation is interesting, it is not taxonomically defensible to designate each variant as a different species or sub-species, particularly when the variants can change with the environment. Often it is not clear which life history traits can be reliably used to infer taxonomic differences, especially since variation in morphology and life history may have a genetic or environmental basis, or both. However, molecular genetic data can be used to objectively test whether life history variants are truly genetically differentiated populations. For example the morphological, ecological and behavioural variations in brown trout have made classification of this species difficult. Differentiation of brown trout populations was demonstrated using mitochondrial DNA sequence, but was insufficient to warrant the present sub-species classification (Apostolidis et al. 1997). Several molecular genetic studies on sympatric dwarf versus normal-sized morphotypes in a variety of species have been reported (Bernatchez et al. 1996; Pigeon et al. 1997; Taylor & Bentzen 1993; and Shields et al. 1990). In most cases the dwarf morphotypes did not cluster together as separate from the normal-sized morphs. The strongest factor influencing divergence appeared to be geographic proximity; ie. dwarfs and normal fish from their respective lakes clustered together (Bernatchez et al. 1996; Pigeon et al. 1997; Taylor & Bentzen 1993; and Shields et al. 1990).

In the last five years, use of DNA-based analyses has become increasingly common in population studies (Mitton 1994). Two very common types of such analysis include random amplified polymorphic DNA (RAPD) and mitochondrial DNA sequence analysis. RAPD (Williams et al. 1990) uses short random sequence primers, approximately 10 base pairs (bp) in length. Each primer is generally capable of amplifying several DNA fragments

of various lengths from coding and non-coding regions dispersed throughout the genome using the polymerase chain reaction (PCR). This makes it possible to detect both inter- and intra- population variation (Macaranas et al. 1995). RAPDs have been used to differentiate populations in a wide range of species, including redclaw crayfish, *Cherax quadricarinatus*, (Macaranas et al. 1995) the American cranberry, *Vaccinium macrocarpon* (Stewart & Excoffier 1996), copepod sea lice , *Lepeophtheirus salmonis* (Todd et al. 1997) and the northern leopard frog, *Rana pipiens* (Kimberling et al. 1996). RAPDs have also been used effectively to differentiate closely related species of barramudi and Nile perch, *Lates* sp. (Partis & Wells 1996). One region of the genome that has been particularly useful in population genetics is mitochondrial DNA. Two mitochondrial regions, the cytochrome b gene and the control region (also known as the displacement loop (d-loop) or heavy strand replication region, Alavadro Bremer 1995,1996) have been used to demonstrate population differentiation in many species including fishes (Baker et al. 1995; Cecconi et al. 1995). Mitochondrial DNA is maternally inherited, which reduces the effective population size and makes genetic drift more detectable. The occurrence of nucleotide substitutions are also more frequent in mtDNA than nuclear DNA with a typical sequence divergence of 1-4% within a species (Mitton 1994). Mismatch in mtDNA replication is thought to be responsible for the higher mutation rate (MacKay et al. 1996). This makes mtDNA excellent for detecting relatively close genetic relationships (Moritz 1994).

The cytochrome b gene is a well defined mtDNA region with readily available PCR primers and is thus a popular choice in many systematic and population studies (Wiley & Hagen 1997; Kitamura et al. 1996; Apostolidis et al. 1997; Árnason & Pálsson 1996). The control region evolves at a faster rate as there are limited selective pressures on this non-

coding region. The control region is comprised of three sections each evolving at different rates (Alvarado Bremer et al. 1995). The central control region is fairly conserved and is therefore appropriate for higher taxonomic-level studies. The left and right regions evolve faster, making them good choices for population level-studies (Faber & Stepien 1997; Kitamura et al. 1996). There is little homoplasy in the control region at the population level making it easier to analyze.

One other type of molecular genetic data that is useful in population-level studies are nuclear intron sequences. Moran et al. (1997) showed nuclear intron sequence from several regions of the genome to be useful in detecting intraspecific variation in Pacific salmon. Devlin (1993) found that intron D of the type-2 growth hormone (GH2) gene differed in size among five salmon species. The introns of type-2 growth hormone show twice as much variation as the exons (MacKay et al. 1996). However, in that study, there was more genetic variation in mtDNA than the GH2 introns (MacKay et al. 1996). A variety of data sources including RAPDs, mtDNA and nuclear intron sequence, which utilize different areas of the genome, are recommended for population studies, since one type of data may not be conclusive to infer population differentiation (Degnan 1993).

The main objective of this study is to test for consistent genetic differentiation between the giant and normal-growth life history forms of pygmy whitefish populations in North-Central BC. The ultimate goal is to determine whether the "giant" pygmy whitefish are differentiated enough to be classed as a separate sub-species, or at least an ESU. Genetic differences will be determined using both nuclear (RAPD and GH2 intron sequence) and mtDNA sequence analyses (cytochrome b and control region). However, I start by demonstrating the size at age differences between the "giant" pygmy whitefish populations

and the other pygmy whitefish populations are great enough to warrant the expectation of genetic differentiation.

1.2 Materials and Methods:

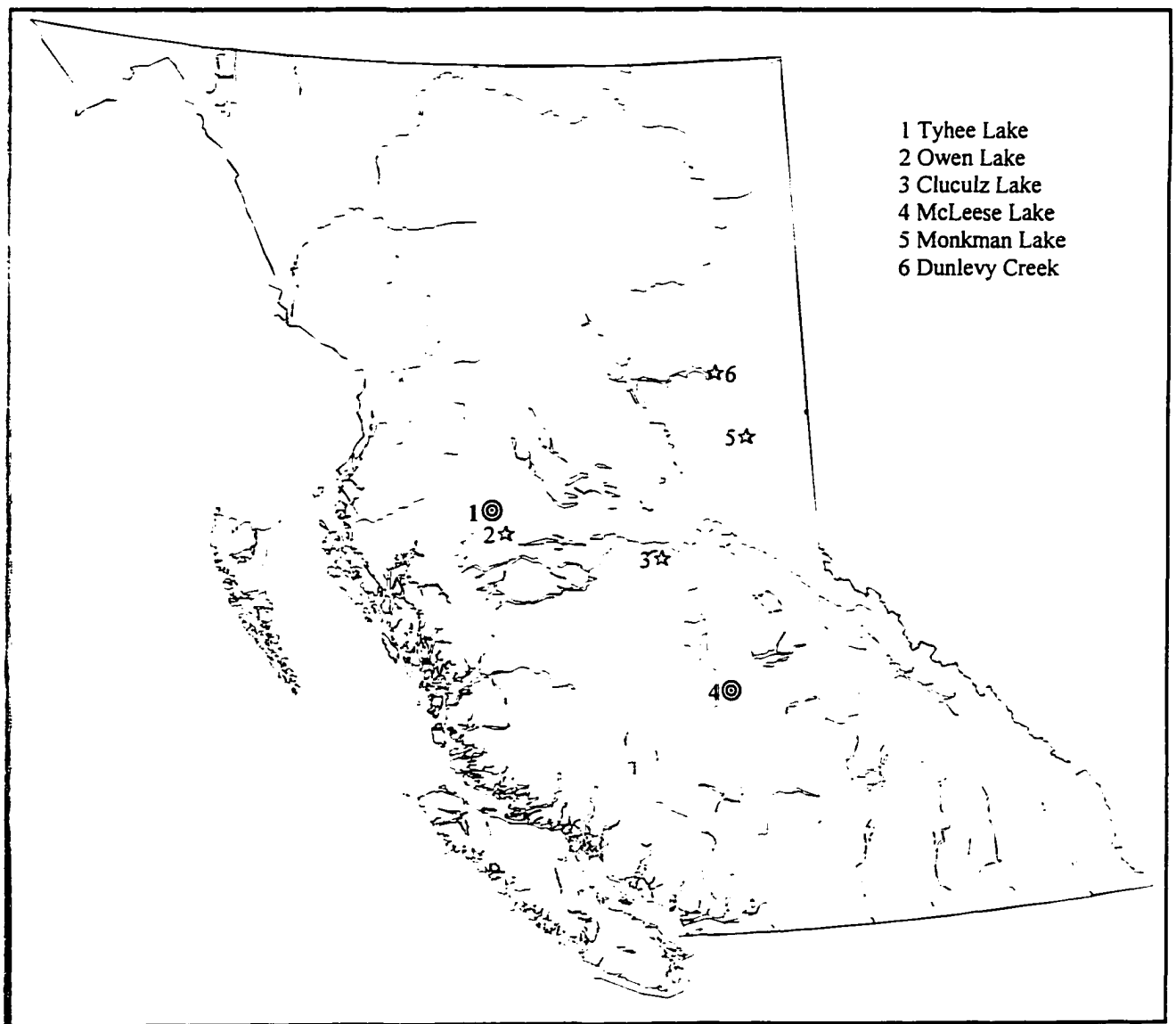
Sampling

Three lakes from each of three river drainages (Skeena, Peace and Fraser) were sampled for pygmy whitefish using gillnets. The lakes were chosen based on accessibility and presence of pygmy whitefish, and included; Tyhee, Chapman and Owen lakes in the Skeena drainage, Cluculz, McLeese and Jack of Clubs lakes in the Fraser drainage, and Monkman Lake, Tacheeda Lake and Dunlevy Creek on Williston Reservoir in the Peace drainage (Figure 1.01). Two of the lakes were reported to be populated with "giant" pygmy whitefish while the remaining seven were reported to harbour populations of normal sized pygmy whitefish. Detailed lake sampling protocols are given in Chapter 3. Briefly, gillnets were set for approximately 10 hours during the day and then re-set overnight and collected in the morning. Nets were set at depths ranging from 4.5m to 38m. Sampling was done between June 11 and September 22, 1996. Blood samples were taken from each pygmy whitefish and stored on ice. Pygmy whitefish from Lake Superior were also obtained courtesy of Dr. R.A. Bodaly.

Age data

Scale and otolith samples were taken for age determination from all pygmy whitefish caught from 8 of the BC populations. Scales were taken from the left side beneath the dorsal

Figure 1.01 Map of British Columbia indicating where the six populations of pygmy whitefish are located. The fish from these populations were used in the RAPD and sequencing analyses. Location of the "giant" pygmy whitefish populations are indicated by "◎"



fin above the lateral line (McCart 1963). In a few cases the scales had been stripped due to handling, and in such cases the scales were taken closer to the caudal peduncle on the left side. Both otoliths were also removed from all fish. The scales and otoliths were sent to Birkenhead Scale Analysis (D'Arcy, BC) to be aged using standard protocols. Briefly, an 8 1/2" x 11" photograph of the best scale(s) was made using a microfiche reader/printer at a magnification of 50X, (or 35.5X for larger scales). Fish were aged as 0+, 1+, 2+, etc. based on the number of annuli present. An annulus is identified by the relative distance between circuli, with wider spacing in spring and summer, and narrower spacing in fall and winter. Other criteria include 'crossing or cutting' of circuli at the annulus, and finally the distance and the number of circuli between annuli.

The otoliths from each fish were immersed in water and viewed against a black background using reflected light at a magnification of 100X and/or 250X for optimal annuli contrast. The age of the best otolith was taken. The ages determined by scales and otoliths were compared for each fish, and both were double-checked when the age did not correspond.

Size at Age Analysis

Only the fish for which the aging agreed between scales and otoliths were used to estimate mean standard length for each age class of the 8 pygmy whitefish populations. Standard length was thought to be a better measure of size than weight, as some of the female fish were gravid and therefore proportionately heavier.

DNA extraction and verification

Five microlitres of blood was incubated at 36°C overnight in 475 µL of digestion buffer (50 mM Tris-HCL, 1,0% SDS and 25 mM EDTA), with 25µL of 10 mg/mL proteinase K. DNA was extracted using phenol chloroform isoamyl alcohol and precipitated with 0.1 volumes 3 M NaOAc added to 0.6 volumes of isopropanol (Devlin et al. 1991). The precipitate was washed with 70% ethanol, dried and reconstituted in 100 µL of TE (0.01 M Tris-HCL and 0.005 M EDTA). All DNA was quantified using Gene Quant (spectrophotometer, Pharmacia) and diluted to $10\mu g \cdot \mu L^{-1}$.

Pygmy whitefish from Lake Superior had been stored in DMSO, thus a modification of the DNA extraction protocol was necessary. A small sample of muscle tissue was soaked in distilled water overnight to remove excess DMSO and salts. The tissue was then pressed to remove all liquid before being placed in 475µL of digestion buffer along with 25 µL of 10 mg/mL proteinase K. The same DNA extraction protocol that was used for the blood was then used for the tissue (see above). All DNA was visualized on 1.8% agarose gels stained with ethidium bromide to determine approximate quality and quantity.

RAPD Amplification

Two hundred RAPD primers were screened for potential use in this study. Twenty primers which gave clear bands with some variation were further screened with five individuals from each of three populations, (Cluculz, Monkman and Tyhee). All RAPD data were tested for reproducibility by running replicates of two individuals from each population for each primer. If the RAPD bands did not replicate the primer was not used in the study. Nine primers were chosen based on variability, clarity and reproducibility of bands (Table

1.01). For the nine primers chosen, half the samples were replicated. RAPDs were run using DNA from ten individuals from the following 6 populations; Tyhee, Chapman, Cluculz, McLeese, Monkman lakes and Dunlevy Creek. Specimens from Lake Superior were not run. Two rocky mountain whitefish (*Prosopium williamsoni*) samples were chosen as an outgroup. Final RAPD PCR conditions were as follows; 2.5μL of 1X buffer (50 mM KCL, 10 mM Tris HCL and 0.1% triton X-100), 2.5 mM $MgCl_2$, 200 μM of each dNTP , 1 unit *Taq* DNA polymerase, 0.1 μg RAPD primer, 10 ng genomic DNA and distilled water to make up a 25 μL reaction. Each of the 35 cycles were made up of the following: 94° for 50 s, 36° for 50s and then 72° for 1 min and 50 s. Amplified products were electrophoresed on 2.1% "high resolution blend" (AMRESCO) agarose gel stained with 0.04 mg of ethidium bromide. The gel was run at $2.22V \cdot cm^{-1}$ for four hours and then viewed under UV transillumination. The images were digitized for later analysis.

RAPD Analysis

The 60 pygmy whitefish samples and two rocky mountain whitefish were run on a total of four gels for each primer. Only five individuals from each population underwent PCR at one time and were always run on a gel with PCR's from 2 other populations (also five individuals each). This ensured that observed variation was not due to gel bias or PCR condition bias. RAPDs have been reported to be unreliable by some researchers due to scoring error and non-reproducibility. Skroch and Nienhuis (1995) did an extensive study to test these criticisms, and found that the dependability of RAPD data increased significantly when all PCR conditions were held constant, and when primers were initially chosen with care. Skroch and Nienhuis (1995) found that since errors were random they did not affect the

Table 1.01 RAPD primers used in the analysis of six populations of pygmy whitefish in North-Central BC. The number of bands scored between 200 and 1000bp are indicated on the right. These bands were scored as present or absent for each individual.

RAPD primer	Sequence 5'→3'	#of bands scored between 200-1000bp
UBC 14	CCT GGG TTT C	6
UBC 40	TTA CCT GGG C	7
UBC 48	TTA ACG GGG A	13
UBC 55	TCC CTC GTG C	10
UBC 59	TTC CGG GTG C	7
UBC 130	GGT TAT CCT C	11
UBC 131	GAA ACA GCG T	6*
UBC 134	AAC ACA CGA G	10
UBC 142	ATC TGT TCG G	10

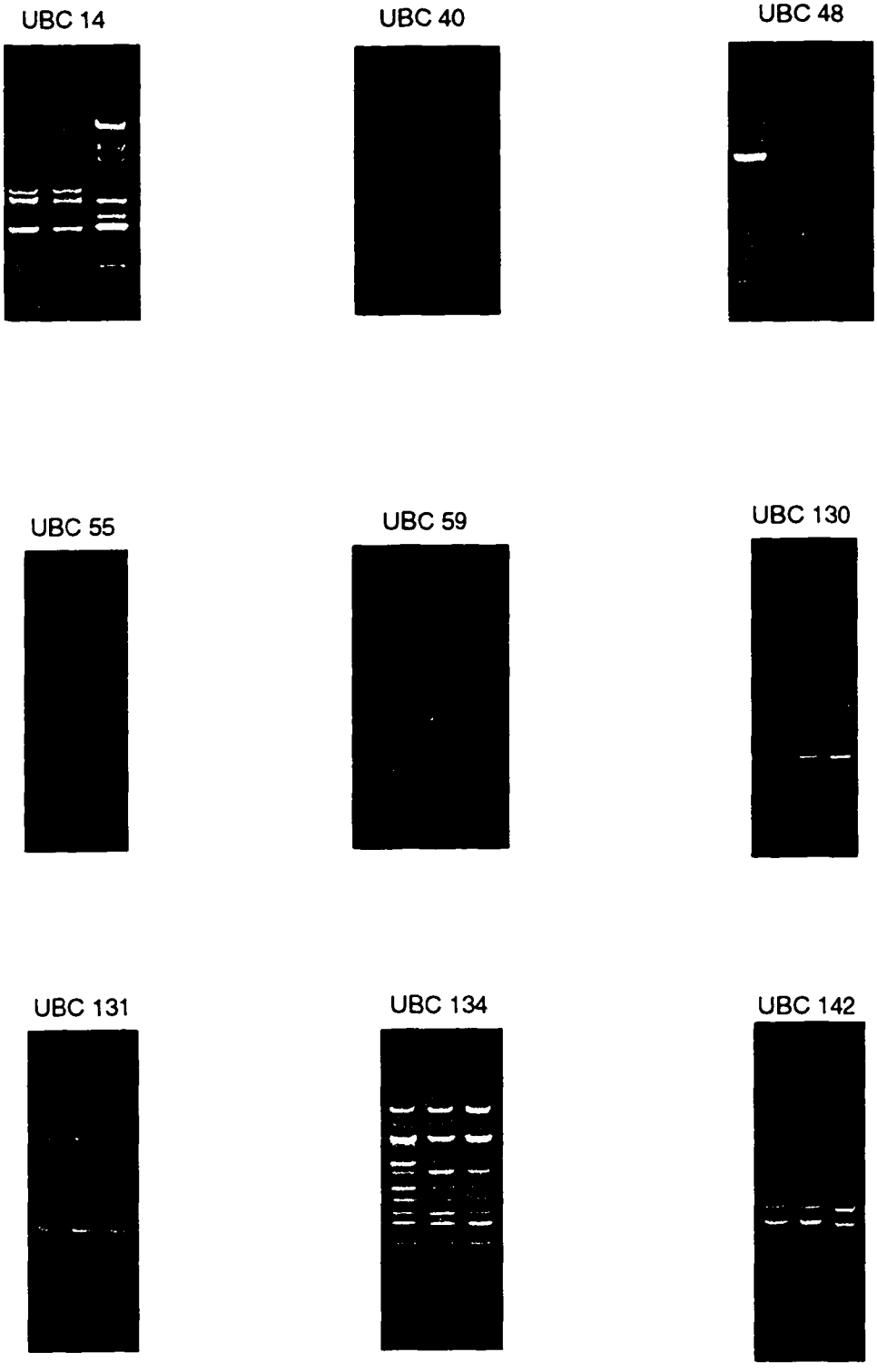
* Only bands below 500bp were counted for primer 131. Bands above 500bp were difficult to score across gels and were not counted.

calculated genetic distance. This study was carried out to keep all conditions constant to minimize any possible errors.

Only bands below 1000 bp and above 200 bp were scored. Bands above 1000 bp were generally crowded and difficult to separate and bands below 200 bp were often too faint to score reliably. Bands that were shared between rocky mountain whitefish and pygmy whitefish were not scored. If there was any doubt as to whether or not a band was being affected by a particular gel run, the band was ruled out. As there was replicated data for much of the data set, many of the questionable bands could be checked against the replicate. The same scoring procedure was used for all nine primers. The RAPDs were scored as present or absent and imported into TFPGA (Miller 1997) (see Appendix A for data). Banding patterns for each primer are shown in Figure 1.02.

The Taylor Expansion for estimating allele frequencies for a dominant marker was chosen in TFPGA (Lynch & Milligan 1994). To test the genetic differentiation among the 6 populations, two genetic distances were used, Wright's modification of Roger's distance (Wright 1978), and Nei's unbiased distance (Nei 1978). Once the distance matrices were calculated, the matrix was imported into MEGA (Kumar et al. 1993) to construct a Neighbour Joining tree. An exact test (Raymond & Rousset 1995), which tests if there are significant differences in marker frequencies among populations, was performed in TFPGA with all populations run simultaneously. Isolation by distance was tested for to determine if there is a relationship between the genetic distance and geographic distance. It is assumed that populations geographically further away from one another will be more genetically distant as well. Isolation by distance was tested for by plotting Wright's modification of

Figure 1.02 Photos of transilluminated agarose gels for each of the nine RAPD primers used in the analysis of six BC pygmy whitefish populations. Three individuals are shown for each primer to provide an indication of the bands amplified for each primer.



Rogers's distance against the geographic distance (straight line) for all possible combinations of pairs of populations.

Mitochondrial DNA Amplification and Sequencing

The populations used for RAPD analysis were also used for sequence analysis of the cyt b and control region fragments. Three individuals from each of the 6 BC populations, along with two individuals from Lake Superior and a rocky mountain whitefish sample were used. A fragment of the cyt b gene was amplified using "universal" primers ("IRS" and "III"; Kocher et al. 1989) with the PCR protocol already noted for RAPD's; however the annealing temperature was changed to 47°C. The resulting approximately 500 bp fragment was sequenced in both directions at the University of British Columbia (UBC) on an ABI 370 automated sequencer (Applied Biosystems). Primers specific for pygmy whitefish were designed using the resulting aligned sequence (Table 1.02). The specific primers amplified a 255 bp fragment at an annealing temperature of 53°C, other PCR conditions remaining constant. Amplified fragments from 3 fish from each lake were sequenced in both directions. A similar protocol was used to generate primers for pygmy whitefish control region (Table 1.02). Primers developed to amplify the left region of the control region of swordfish, *Xiphias gladius* (Alvarado Bremer et al. 1995) were used on pygmy whitefish. Those primers amplified an approximately 500bp fragment from pygmy whitefish. The fragment was sequenced and specific primers developed for pygmy whitefish (Table 1.02). These primers amplified a 266bp fragment at an annealing temperature of 54°C. To amplify the rocky mountain whitefish a combination of L15998 and Pco-cr2 was used (see Table 1.02)

Table 1.02 Primers used to obtain fragments for sequencing mitochondrial and nuclear intron DNA. Pygmy whitefish primers refer to primers developed specifically for this study.

Primer	Sequence 5'➡3'	Target Region
IRS	ATC GGA ATT CTG ACT TGA ARA ACC AYC GTT G	Universal cytochrome b
III	ATC GGA ATT CCC TCA GAA TGA TAT TTG TCC TCA	
Pco-cytb1	GCC ATA GTA AAG ACC TCG GG	Pygmy whitefish cytochrome b
Pco-cytb2	CCA CCC CCT CCT GAA AAT TG	
L15998	TAC CCC AAA CTC CCA AAG CTA	Universal control region
CSBDH	TGA ATT AGG AAC CAG ATG CCA G	
Pco-cr1	GCC ACA TAA GGC ATG TAA TA	Pygmy whitefish cytochrome b
Pco-cr2	TGG GAT CGT TGG TCG GTT CT	
GH2-Ex4	CAG CCT AAT GGT CAG AAA CT	Type-2 growth hormone intron D, Pacific salmon
GH2-Ex5a	CGT AGT TCC TCC TGA CGT TG	
Pco-gh2d1	AGA AGC TCA GCG ACC TCA AA	Pygmy whitefish, type-2 growth hormone intron D
Pco-gh2d2	CCA CAT CAG GCC TGC AAG TA	

with an annealing temperature of 52 °C. The same 21 fish used in the cyt b analysis were used for the control region analysis. All amplified fragments were sequenced in both directions.

Nuclear DNA Amplification and Sequencing

To amplify the selected nuclear region, 3 fish from each of the same 6 BC populations mentioned above, 2 Lake Superior fish and a rocky mountain whitefish were used as template. The selected region was a portion of intron D of the type-2 growth hormone. Primers originally designed for use in Pacific salmon (Park et al. 1995) were used to amplify a fragment in pygmy whitefish (Table 1.02) using an annealing temperature of 52°C. This fragment was sequenced and new primers were developed specific to pygmy whitefish (Table 1.02). The M13 universal primer was added to the 5' end of these primers so that they could be used in the Visible Genetics automated sequencer. The PCR annealing temperature for these elongated primers ranged from 56°C to 68°C, depending on individual fish. Once a fragment was obtained, it was used as a template in a cycle sequencing reaction with an annealing temperature of 52°C (following Visible Genetics protocols). The Lake Superior individuals amplified very weakly during the initial PCR amplification at 60°C. Once a weak fragment was obtained, the PCR product was diluted to 1:100 and used as a template in another round of PCR. This re-amplification was run at 67°C to produce a strong band to be used in the sequencing reaction.

Sequence Analysis

The sequences for cyt b, control region and GH2 intron were aligned using the clustal based program in OMIGA 1.1 (Oxford Molecular, England). The gap penalty and gap cost were set at ten and five respectively. The sequences for each region were aligned separately.

The GH2 intron amplified fragment was just under 500bp. For analysis however, only the consensus sequence from the forward and reverse sequence was used. Some consensus sequences were longer than others. For analysis all sequences had to begin and end at the same location within the fragment, therefore all sequences were brought down to a size of 350 bp. It was this edited sequence that was used in the analysis.

Neighbour Joining was done using Tamura-Nei, Jukes-Cantor and Kimura-2 parameter distance models. Kimura-2 parameter and Jukes-Cantor distance models were available on the programs DAMBE (Xia 1998) and MEGA, while the Tamura-Nei distance model was only available on MEGA. The Kimura-2 parameter and Tamura-Nei distance models are both relatively new, and both correct for assumption violations encountered in other models (Stepien & Kocher 1997).

The assumptions for the Jukes Cantor and Kimura-2 parameter models are different from one another. The Jukes Cantor model assumes that transversions are as likely as transitions. As there were two different regions in the combined sequence, there were different constraints acting, and the Jukes-Cantor would ignore transversion bias. The Kimura-2 parameter model calculates distances using proportions of transversion-type differences and transition-type differences (Swofford et al. 1996). The Kimura-2 parameter model assumes a higher rate of transitions but also assumes all four nucleotides are in equal frequencies, however this assumption is violated by organisms showing nucleotide bias

(Kocher & Carleton 1997). All three models were used to make comparisons among the different distance estimates. In addition, a bootstrap of 1000 replicates was done using the Tamura-Nei and Kimura-2 parameter distance models in MEGA and a consensus Neighbour Joining tree was constructed. When constructing a tree, deletions were treated as complete deletions in MEGA and both transitions and transversions were used. The two mitochondrial regions were initially analyzed separately, then they were combined and re-analyzed using the same models as above.

1.3 Results

Age Data/Size Data

The Tyhee population has much larger fish at all ages than any of the other sampled populations (Figure 1.03). Furthermore Tyhee Lake pygmy whitefish grow to much larger sizes. Although a range of size at age curves are seen for the various lakes, none are drastically different from one another except the Tyhee population (Figure 1.03). These data clearly show that the McLeese population are not "giant" pygmy whitefish based on the size at age data.

RAPD Analysis

Figure 1.04 illustrates the type of variation that RAPDs can detect. Primer UBC 55 was the only primer to detect a population-specific band. The band highlighted in Figure 1.04 did not appear in any other population other than McLeese Lake. The RAPDs also showed species-specific bands. Depending on which primer was used, there were at least 1 to 4 bands that differentiated rocky mountain whitefish from the pygmy whitefish.

Figure 1.03 Size at age curve of 8 populations of pygmy whitefish. The two "giant" pygmy whitefish populations are indicated by open symbols, Tyhee and McLeese lakes. Size was determined by the standard length in mm and the age was calculated from consensus readings from both scales and otoliths.

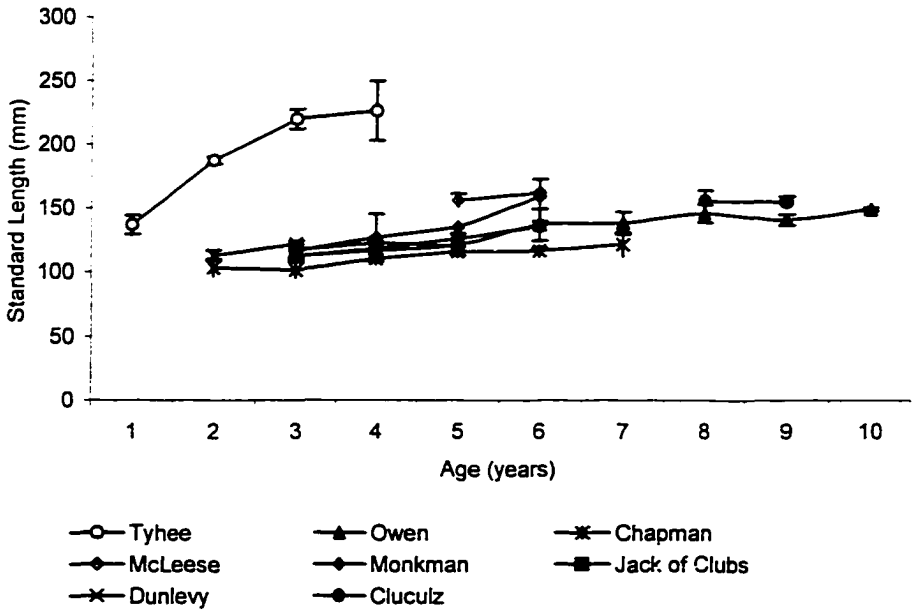
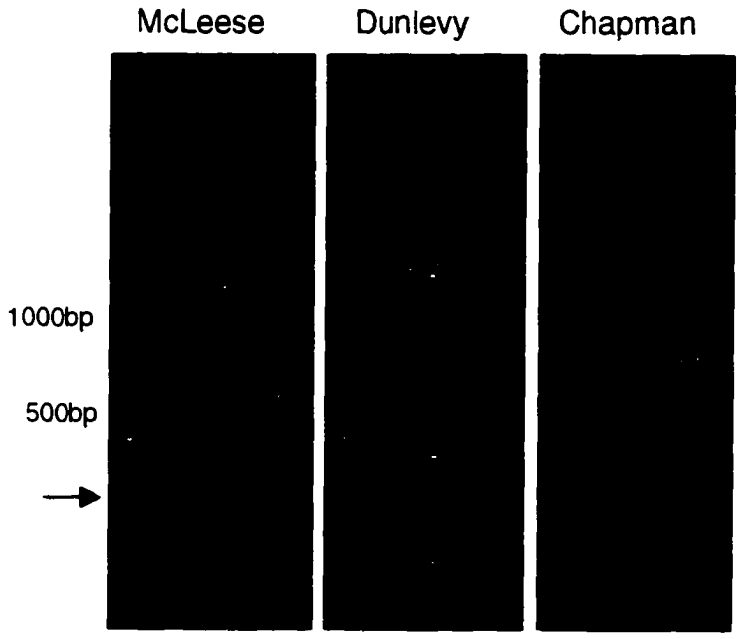


Figure 1.04 Image of transilluminated agarose gel with RAPD (primer UBC 55) generated bands using pygmy whitefish DNA from three populations. The arrow indicates a band seen only in the McLeese Lake population.



These species-specific bands confirmed that the questionable juvenile rocky mountain whitefish, first labeled as pygmy whitefish, were rocky mountain whitefish. Sixty-one percent of the bands that were scored as at least 95% polymorphic, showing the utility of RAPDs for scoring differentaition among individuals and populations (Table 1.03). The Neighbour Joining tree of Wright's distance based on RAPD data can be seen in Figure 1.05. The topologies for Wright's modification of Roger's distance and Nei's distance were identical, so only the results from Wright's modification of Roger's distance are shown. The trees constructed using the RAPD data gave ties, so the branches are not necessarily in the correct order. The distance estimates were also very similar. The Tyhee Lake population is not uniquely separated from the rest of the populations. However, there was significant differentiation among all populations ($P< 0.00001$; Chi-square=314; df=160) using the exact test. The isolation by distance plot did not show any relationship between genetic distance and geographic distance. Populations that are geographically closer to one another are not necessarily genetically more similar to one another than a population at a greater geographic distance, indicating that the populations probably all diverged from one another at the same time and have had little contact since.

Sequence Data

The cytochrome b primers amplified a 255 bp fragment. There were 2 site changes among pygmy whitefish in BC, and one difference between the Lake Superior and BC pygmy whitefish, and 33 changes between pygmy whitefish and rocky mountain whitefish. Of these 33 differences, all but 4 were transitions (Figure 1.06). The control region amplified a 266 bp fragment in all the BC pygmy whitefish and a 265 bp fragment in the Lake Superior

Table 1.03 The frequency (in percent) a scored band appeared in the 60 pygmy whitefish (6 populations) analyzed in the RAPD data set for each of the nine primers used. This shows that many of the alleles were variable and therefore are useful for analysis.

RAPD primer	Band #	% present	RAPD primer	Band #	% present	Band primer	Allele #	% present
UBC 14	1	100		28	82		55	100
	2	52		29	12		56	100
	3	57		30	5	UBC 59	57	96
	4	85		31	83		58	9
	5	48		32	97		59	9
	6	100		33	98		60	96
UBC 40	7	17		34	95		61	100
	8	85		35	92		62	96
	9	12		36	97		63	100
	10	5	UBC 134	37	88	UBC 130	64	19
	11	97		38	82		65	14
	12	100		39	25		66	69
	13	100		40	98		67	44
UBC 48	14	33		41	92		68	10
	15	15		42	83		69	7
	16	35		43	100		70	100
	17	23		44	100		71	15
	18	70		45	100		72	90
	19	88		46	100		73	78
	20	17	UBC 142	47	22		74	100
	21	12		48	8	UBC 131	75	55
	22	15		49	15		76	38
	23	60		50	12		77	3
	24	100		51	98		78	40
	25	100		52	17		79	88
	26	100		53	12		80	40
UBC 55	27	100		54	100			

Figure 1.05 Neighbour Joining tree of RAPD data, constructed using Wright's modification of Roger's distance in MEGA. Distance calculations were based on data from 10 individuals from each population.

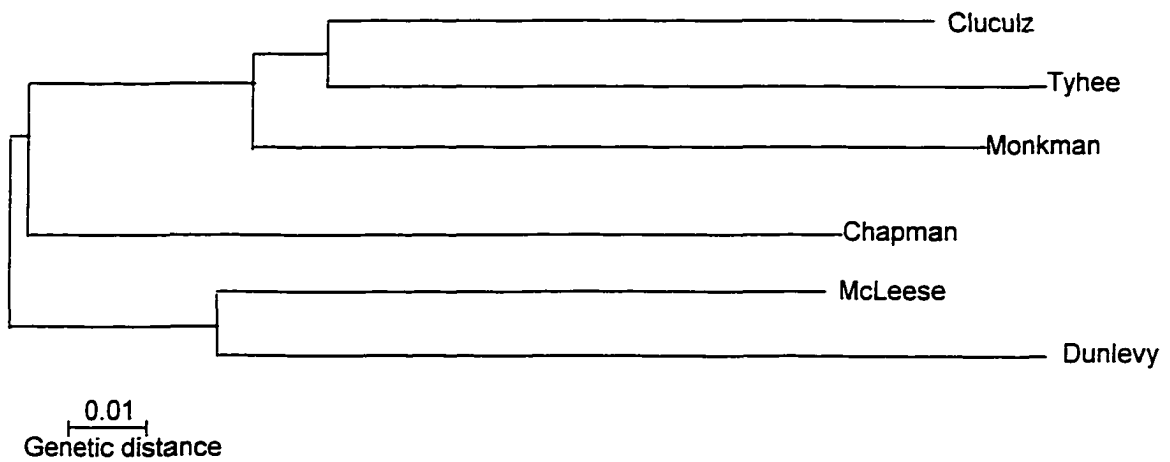


Figure 1.06 Alignment of cytochrome b sequences for 6 BC pygmy whitefish populations, a Lake Superior population and a rocky mountain whitefish (an outgroup). A "." indicates identical sequence to the first line. Changes at any particular site are indicated in bold letters.

```
            1                                                  50
Cluculz 1   CAATGTGTAT ATAGATGCAG ATAAAGAAGA AAGATGCTCC GTTAGCGTGA
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..C.......
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......C.. .......... .......... .......... ..........
Superior 2  .......C.. .......... .......... .......... ..........
Rocky       .......... ...A...... .......... .......... A..G......

            51                                                 100
Cluculz 1   ATATTTCGGA TAAGCCAACC ATAGCTAACA TCTCGACAGA TGTGGCATAC
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..A.......
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Rocky       .......... ....T..G.. G.....G... .....G..A. .A........
```

```
            101                                                   150
Cluculz 1   AGAAGAAAAA GCTGTAGAAA TGTCAGAGGT ATAGTGTATA GCCAGGAATA
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Rocky       G..G..G... .....T..G. .......A.. G.....C... ..........
```

```
            151                                                   200
Cluculz 1   GTCCTGTAAG GATTTGAGTA GCCAGACACA AGCCCAGAAG TGAGCCAAAG
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... ... ...... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Rocky       .......G.. A.....G..G ..T.....T. .......... ..........
```

```
            201                                                  250
Cluculz 1   TTTCATCAGA TTGAAATGTT AGAGGGTGCT GGAAGATCGA CTAGTGCGCC
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Rocky       .......... C...G..... T.....C... .......... ........T.
```

```
            251
Cluculz 1   ATTAG
Cluculz 2   .....
Cluculz 3   .....
Dunlevy 1   .....
Dunlevy 2   .....
Dunlevy 3   .....
Monkman 1   .....
Monkman 2   .....
Monkman 3   .....
McLeese 1   .....
McLeese 2   .....
McLeese 3   .....
Chapman 1   .....
Chapman 2   .....
Chapman 3   .....
Tyhee 1     .....
Tyhee 2     .....
Tyhee 3     .....
Superior 1  .....
Superior 2  .....
Rocky       .....
```

population as well as in the rocky mountain whitefish. Among pygmy whitefish, there were 2 transition events, between the Lake Superior pygmy whitefish and the 6 populations of BC pygmy whitefish there were 3 transitions and 1 delction. Between pygmy whitefish and rocky mountain whitefish there were 13 transitions, 8 transversions and one deletion (Figure 1.07).

In the cyt b phylogeny only the Lake Superior population branch was supported by more than 50% of the bootstraps for all distance measures (Figure 1.08). Branches not supported by 50% or more can be collapsed. The control region phylogeny shows three branches that are supported by more than 50% of the bootstraps for all distance measures (Figure 1.09). The trees shown in Figures 1.08 and 1.09, along with bootstrap values, are from the Kimura-2 parameter distance. The tree obtained when both mitochondrial regions were combined is more similar to the control region tree (Figure 1.10). This was expected as the control region contained more phylogenetic information than cytochrome b. For the bootstrap analysis 3 branches were supported by more than 50% (Figure 1.10).

There were more site changes in the GH2 intron than in cytochrome b or control region. The fragment analyzed in most individuals was 350bp (351bp for the rocky mountain whitefish; two individuals from Cluculz Lake and one from Monkman Lake). Among the BC populations sequenced, there were 3 transition events, 5 transversions and 2 insertions. Between the Lake Superior pygmy whitefish and the BC pygmy whitefish sequenced there were 2 transitions and 1 transversion (Figure 1.11). The sequence changes within the BC pygmy whitefish populations were not informative as they were randomly dispersed among several individuals from Monkman, Chapman Cluculz and Tyhee lakes. Except for two Monkman Lake fish the BC fish did not share site changes, whereas the Lake Superior

Figure 1.07 Alignment of control region sequences for 6 BC pygmy whitefish populations, a Lake Superior population and a rocky mountain whitefish (an outgroup). A "." indicates identical sequence to the first line. Changes at any particular site are indicated in bold letters, deletions/insertions are indicated by a "-".

```
                1                                                   50
Cluculz 1       ACACAGCTCT ATGTATAATA TTGCATATTA TGTACTGACC CATATATTAT
Cluculz 2       .......... .......... .......... .......... ..........
Cluculz 3       .......... .......... .......... .......... ..........
Dunlevy 1       .......... .......... .......... .......... ..........
Dunlevy 2       .......... .......... .......... .......... ..........
Dunlevy 3       .......... .......... .......... .......... ..........
Monkman 1       .......... .......... .......... .......... ..........
Monkman 2       .......... .......... .......... .......... ..........
Monkman 3       .......... .......... .......... .......... ..........
McLeese 1       .......... .......... .......... .......... ..........
McLeese 2       .......... .......... .......... .......... ..........
McLeese 3       .......... .......... .......... .......... ..........
Chapman 1       .......... .......... .......... .......... ..........
Chapman 2       .......... .......... .......... .......... ..........
Chapman 3       .......... .......... .......... .......... ..........
Tyhee 1         .......... .......... .......... .......... ..........
Tyhee 2         .......... .......... .......... .......... ..........
Tyhee 3         .......... .......... .......... .......... ..........
Superior 1      .....C.... .......... ........-. ......A... ..........
Superior 2      .....C.... .......... ........-. ......A... ..........
Rocky           TT...C.A.. .......... .......... .......... ..........

                51                                                  100
Cluculz 1       TACCAGCACG TGAGTAGTAC ATACTATGTA TTATCAACAT TAATGATTTT
Cluculz 2       .......... .......... .......... .......... ..........
Cluculz 3       .......... .......... .......... .......... ..........
Dunlevy 1       .......... .......... .......... .......... ..........
Dunlevy 2       .......... .......... .......... .......... ..........
Dunlevy 3       .......... .......... .......... .......... ..........
Monkman 1       .......... .......... .......... .......... ..........
Monkman 2       .......... .......... .......... .......... ..........
Monkman 3       .......... .......... .......... .......... ..........
McLeese 1       .......... .......... .......... .......... ..........
McLeese 2       .......... .......... .......... .......... ..........
McLeese 3       .......... .......... .......... .......... ..........
Chapman 1       .......... .......... .......... .......... ..........
Chapman 2       .......... .......... .......... .......... ..........
Chapman 3       .......... .......... .......... .......... ..........
Tyhee 1         .......... .......... .......... .......... ..........
Tyhee 2         .......... .......... .......... .......... ..........
Tyhee 3         .......... .......... .......... .......... ..........
Superior 1      .......... ..G....... .......... .......... ..........
Superior 2      .......... ..G....... .......... .......... ..........
Rocky           ..TT-..... CAG....... ..C....... .......... A.G.......
```

```
            101                                                   150
Cluculz 1   AAGCCCTCAT ACATCAGCAC CAATCCAAGG TTCACATTAA GCAAGACTCG
Cluculz 2   .......... .......... T......... .......... ..........
Cluculz 3   .......... .......... T......... .......... ..........
Dunlevy 1   .......... .......... T......... .......... ..........
Dunlevy 2   .......... .......... T......... .......... ..........
Dunlevy 3   .......... .......... T......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... T......... .......... ..........
Chapman 2   .......... .......... T......... .......... ..........
Chapman 3   .......... .......... T......... .......... ..........
Tyhee 1     .......... .......... T......... .......... ..........
Tyhee 2     .......... .......... T......... .......... ..........
Tyhee 3     .......... .......... T......... .......... ..........
Superior 1  .......... .......... T......... .......... ..........
Superior 2  .......... .......... T......... .......... ..........
Rocky       .......... .......T.. .GT....... ..T....... ..........


            151                                                   200
Cluculz 1   GATAACCACC AACGGAACCG TTCTAACTTG ATTAATTGCT AAACAACATT
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Rocky       .....T.... .......... .......C.. .......... ........AA
```

```
            201                                                 250
Cluculz 1   CCTCCAGCTA ACACGGGCTC CGTCTTTACC CACCAACTTT CAGCATCGGT
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 2   .......... .......... .......... .......... ..........
Monkman 3   .......... .......... .......... .......... .......A..
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 1  .......... .......... .......... .......... .......A..
Superior 2  .......... .......... .......... .......... .......A..
Rocky       ......A... .......... .......... ......A... ..........
```

```
            251           266
Cluculz 1   CCTGCTTAAT GTAGTA
Cluculz 2   .......... ......
Cluculz 3   .......... ......
Dunlevy 1   .......... ......
Dunlevy 2   .......... ......
Dunlevy 3   .......... ......
Monkman 1   .......... ......
Monkman 2   .......... ......
Monkman 3   .......... ......
McLeese 1   .......... ......
McLeese 2   .......... ......
McLeese 3   .......... ......
Chapman 1   .......... ......
Chapman 2   .......... ......
Chapman 3   .......... ......
Tyhee 1     .......... ......
Tyhee 2     .......... ......
Tyhee 3     .......... ......
Superior 1  .......... ......
Superior 2  .......... ......
Rocky       ..C.T..... ......
```

Figure 1.08 Neighbour Joining tree constructed using Kimura-2 parameter distance model of cytochrome b. Values above branch indicates bootstrap value using 1000 replications. Only values greater than 50% are shown. Individuals are designated with numbers and the name of the lake where they were collected.

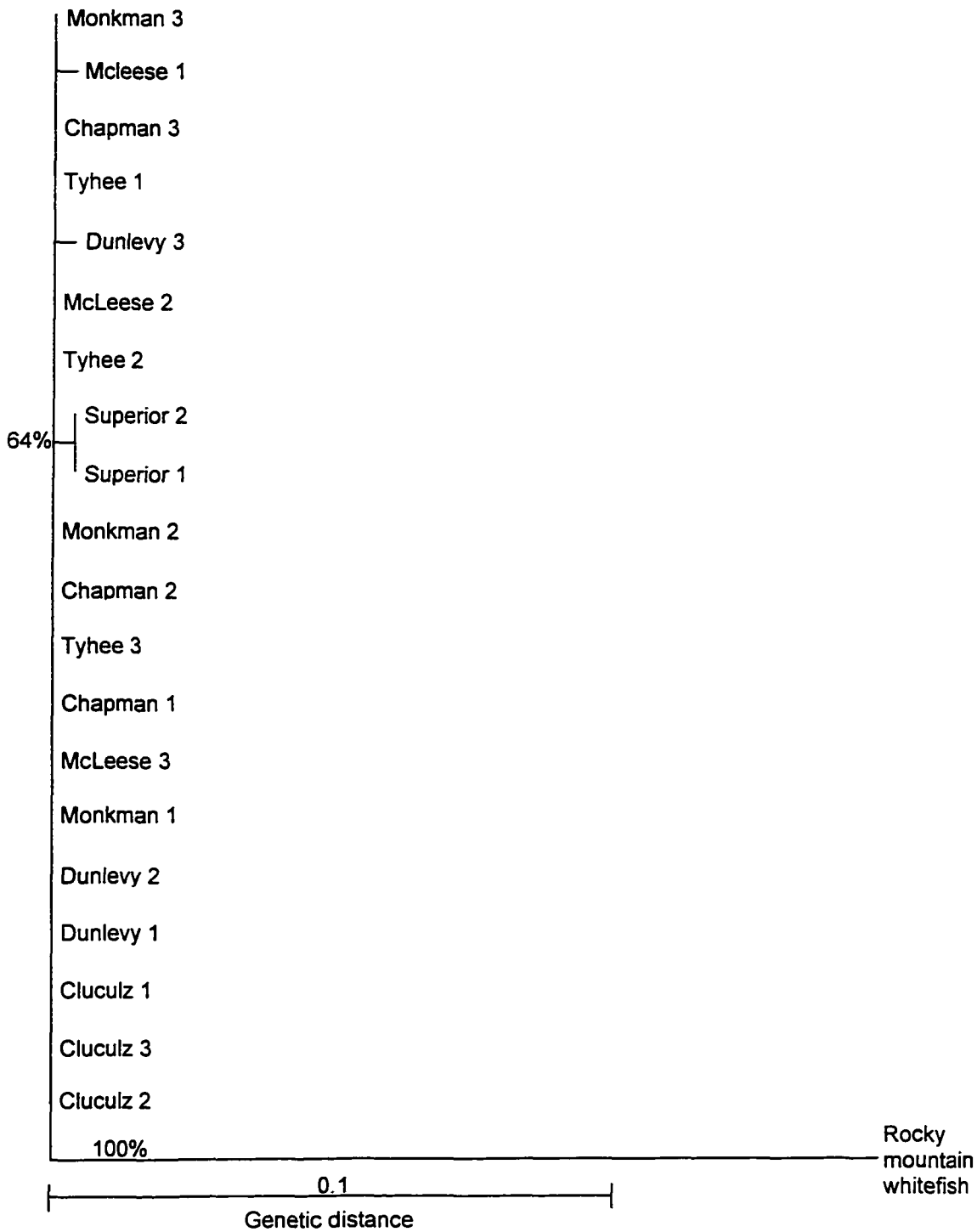


Figure 1.09 Neighbour Joining tree of control region, constructed using Kimura-2 parameter distance model. Values above branch indicates bootstrap value using 1000 replications. Only values greater than 50% are shown. Individuals are designated with numbers and the name of the lake where they were collected.

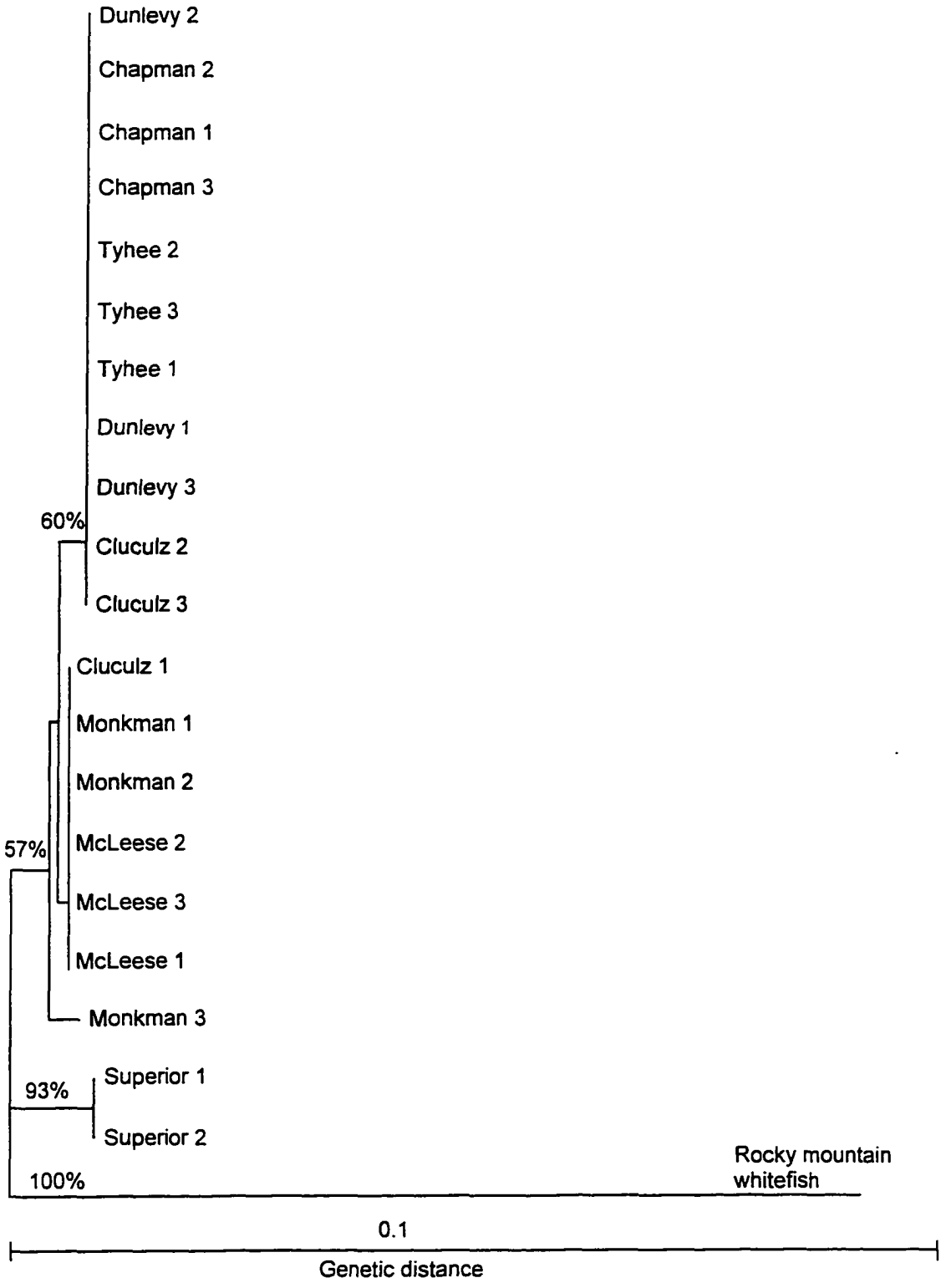


Figure 1.10 Neighbour Joining tree of combined data of cytochrome b and control region, constructed using Kimura-2 parameter distance model. Individuals are designated with numbers and the name of the lake where they were collected.

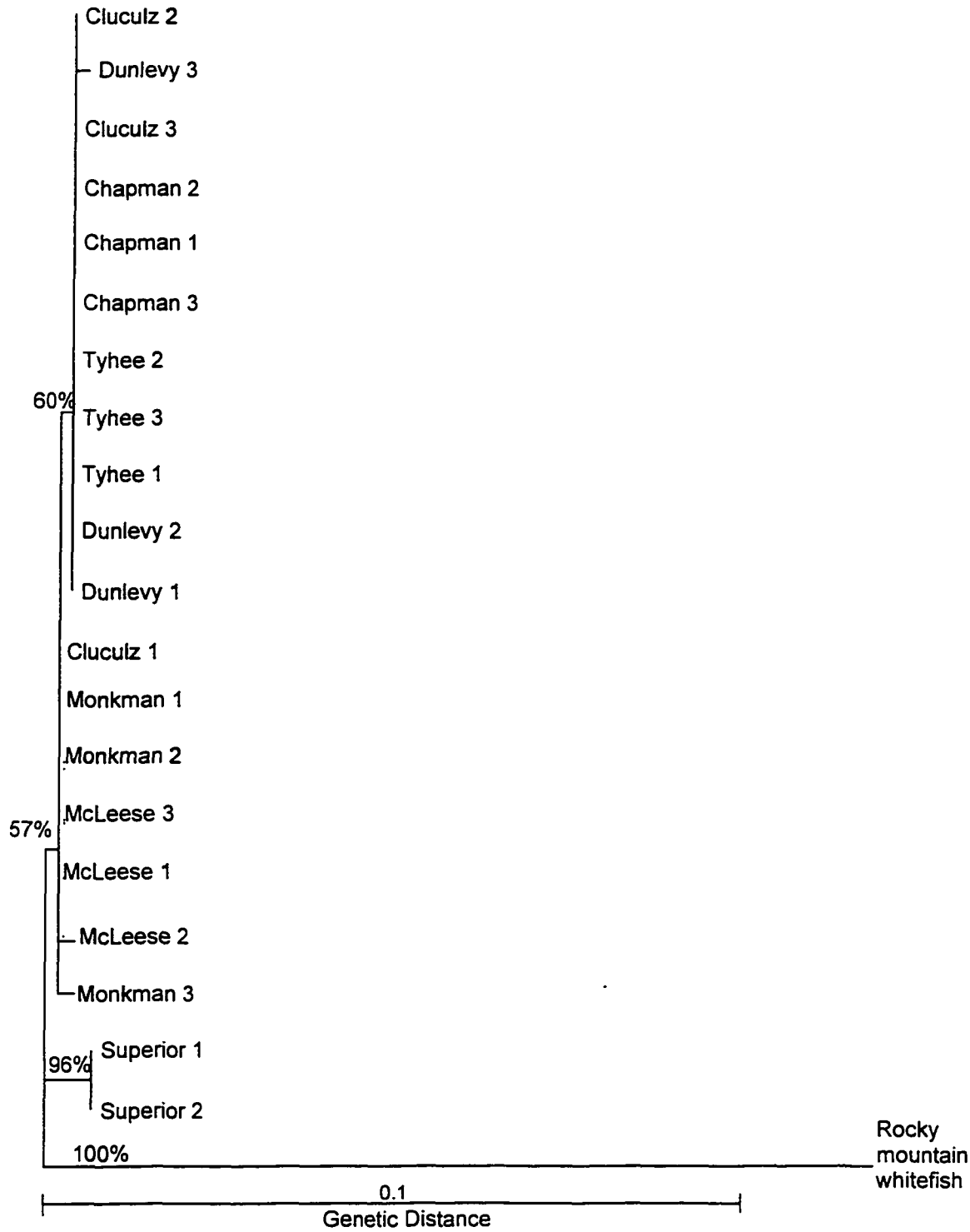


Figure 1.11 Alignment of the type-2 growth hormone intron for 6 BC pygmy whitefish populations, a Lake Superior population and a rocky mountain whitefish (an outgroup). A "." indicates identical sequence to the first line. Changes at any particular site are indicated in bold letters, deletions/insertions are indicated by a "-".

```
          1                                                  50
Cluculz 1  AGGTAAAGAA AGGAGGGAGA ACAATGACTA TTTGTGGTGC CACACTTTGT
Cluculz 2  .......... .......... .......... .......... ..........
Cluculz 3  .......... .......... .......... .......... ..........
Dunlevy 1  .......... .......... .......... .......... ..........
Dunlevy 2  .......... .......... .......... .......... ..........
Dunlevy 3  .......... .......... .......... .......... ..........
Monkman 1  .......... .......... ....A..... .......... ..........
Monkman 4  .......... .......... .......... .......... ..........
Monkman 5  .......... ........T. ....A..... .....TA... ..........
McLeese 1  .......... .......... .......... .......... ..........
McLeese 2  .......... .......... .......... .......... ..........
McLeese 3  .......... .......... .......... .......... ..........
Chapman 1  .......... .......... .......... .......... ..........
Chapman 2  .......... .......... .......... .......... ..........
Chapman 3  .......... .......... .......... .......... ..........
Tyhee 1    .......... .......... .......... .......... ..........
Tyhee 2    .A........ .......... .......... .......... ..........
Tyhee 3    .......... .......... .......... .......... ..........
Superior 2 .......... .......... .......... .......... ..........
Superior 3 .......... .......... .......... .......... ..........
Rocky      .CCA....G. ...G...... .......... .......... ..........


          51                                                 100
Cluculz 1  GCACTGTAAA CCCCAAGGCA TTTTAACTCA AATACTTCTA GTAAGTTGAA
Cluculz 2  .......... .......... .......... .......... ..........
Cluculz 3  .......... .......... .......... .......... ..........
Dunlevy 1  .......... .......... .......... .......... ..........
Dunlevy 2  .......... .......... .......... .......... ..........
Dunlevy 3  .......... .......... .......... .......... ..........
Monkman 1  .......... .......... .......... .......... ..........
Monkman 4  .......... .......... .......... .......... ..........
Monkman 5  .......... .......... .......... .......... ..........
McLeese 1  .......... .......... .......... .......... ..........
McLeese 2  .......... .......... .......... .......... ..........
McLeese 3  .......... .......... .......... .......... ..........
Chapman 1  .......... .......... .......... .......... ..........
Chapman 2  .......... .......... .......... .......... ..........
Chapman 3  .......... .......... .......... .......... ..........
Tyhee 1    .......... .......... .......... .......... ..........
Tyhee 2    .......... .......... .......... .......... ..........
Tyhee 3    .......... .......... .......... .......... ..........
Superior 2 .......... .......... .......... .......... ..........
Superior 3 .......... .......... .......... .......... ..........
Rocky      ....CACTT. .......... .......... .......... .CC.AC.TG.
```

```
          101                                                       150
Cluculz 1 CTCAA-AGTC AATGAAAAGT CATTATTACT TAAAATGTTT ATATGGTACT
Cluculz 2 .....-.... .......... .......... .......... ..........
Cluculz 3 .....-.... .......... .......... .......... ..........
Dunlevy 1 .....-.... .......... .......... .......... ..........
Dunlevy 2 .....-.... .......... .......... .......... ..........
Dunlevy 3 .....-.... .......... .......... .......... ..........
Monkman 1 .....-.... .......... .......... .......... ..........
Monkman 4 .....-.... .......... .......... .......... ..........
Monkman 5 .....-.... .......... .......... .......... ..........
McLeese 1 .....-.... .......... .......... .......... ..........
McLeese 2 .....-.... .......... .......... .......... ..........
McLeese 3 .....-.... .......... .......... .......... ..........
Chapman 1 .....-.... .......... .......... .......... ..........
Chapman 2 .....-.... .......... .......... .......... ..........
Chapman 3 .....-.... .......... .......... .......... ..........
Tyhee 1   .....-.... .......... .......... .......... ..........
Tyhee 2   .....-.... .......... .......... .......... ..........
Tyhee 3   .....-.... .......... .......... .......... ..........
Superior 2 .....-.... .......... .......... .......... ..G.......
Superior 3 .....-.... .......... .......... .......... ..G.......
Rocky     ...T.T.... .......GA. ....GA.... ..T....C.A ..........

          151                                                       200
Cluculz 1 GGC-TCAAAA CTAAATGAGA CGTCACATCA ACTCAATTTT CTAAAGTTAT
Cluculz 2 ...-...... ..T....... .......... .......... ..........
Cluculz 3 ...-...... .......... .......... .......... ..........
Dunlevy 1 ...-...... .......... .......... .......... ..........
Dunlevy 2 ...-...... .......... .......... .......... ..........
Dunlevy 3 ...-...... .......... .......... .......... ..........
Monkman 1 ...-...... .......... .......... .......... ..........
Monkman 4 ...C...... .......... .......... .......... ..........
Monkman 5 ...-...... .......... .......... .......... ..........
McLeese 1 ...-...... .......... .......... .......... ..........
McLeese 2 ...-...... .......... .......... .......... ..........
McLeese 3 ...-...... .......... .......... .......... ..........
Chapman 1 ...-...... .......... .......... .......... ..........
Chapman 2 ...-...... .......... .......... .......... ..........
Chapman 3 ...-...... .......... .......... .......... ..........
Tyhee 1   ...-...... .......... .......... .......... ..........
Tyhee 2   ...-...... .......... .......... .......... ..........
Tyhee 3   ...-...... .......... .......... .......... ..........
Superior 2 ...-...... .......... .......... .......... ..........
Superior 3 ...-...... .......... .......... .......... ..........
Rocky     ...-...... .......... A......... .......... T.........
```

```
            201                                                 250
Cluculz 1   GATAACGAAT TAACTTTTTA CCCAGCATGC TCTACTGCAG GTACATTTTT
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 4   .......... .......... .......... .......... ..........
Monkman 5   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 2  .......... .......... .......... .......... ..........
Superior 3  .......... .......... .......... .......... ..........
Rocky       .......... .......... .......... .....G.... ..........


            251                                                 300
Cluculz 1   GGGAATTGTT TTTAATATTT GTGTTTTTGC ATGTACGTTG AGGGATTTAT
Cluculz 2   .......... .......... .......... .......... ..........
Cluculz 3   .......... .......... .......... .......... ..........
Dunlevy 1   .......... .......... .......... .......... ..........
Dunlevy 2   .......... .......... .......... .......... ..........
Dunlevy 3   .......... .......... .......... .......... ..........
Monkman 1   .......... .......... .......... .......... ..........
Monkman 4   .......... .......... .......... .......... ..........
Monkman 5   .......... .......... .......... .......... ..........
McLeese 1   .......... .......... .......... .......... ..........
McLeese 2   .......... .......... .......... .......... ..........
McLeese 3   .......... .......... .......... .......... ..........
Chapman 1   .......... .......... .......... .......... ..........
Chapman 2   .......... .......... .......... .......... ..........
Chapman 3   .......... .......... .......... .......... ..........
Tyhee 1     .......... .......... .......... .......... ..........
Tyhee 2     .......... .......... .......... .......... ..........
Tyhee 3     .......... .......... .......... .......... ..........
Superior 2  .......... .........G .......... .......... ..........
Superior 3  .......... .........G .......... .......... ..........
Rocky       T.....G... .......... .......... ......A... .......G..
```

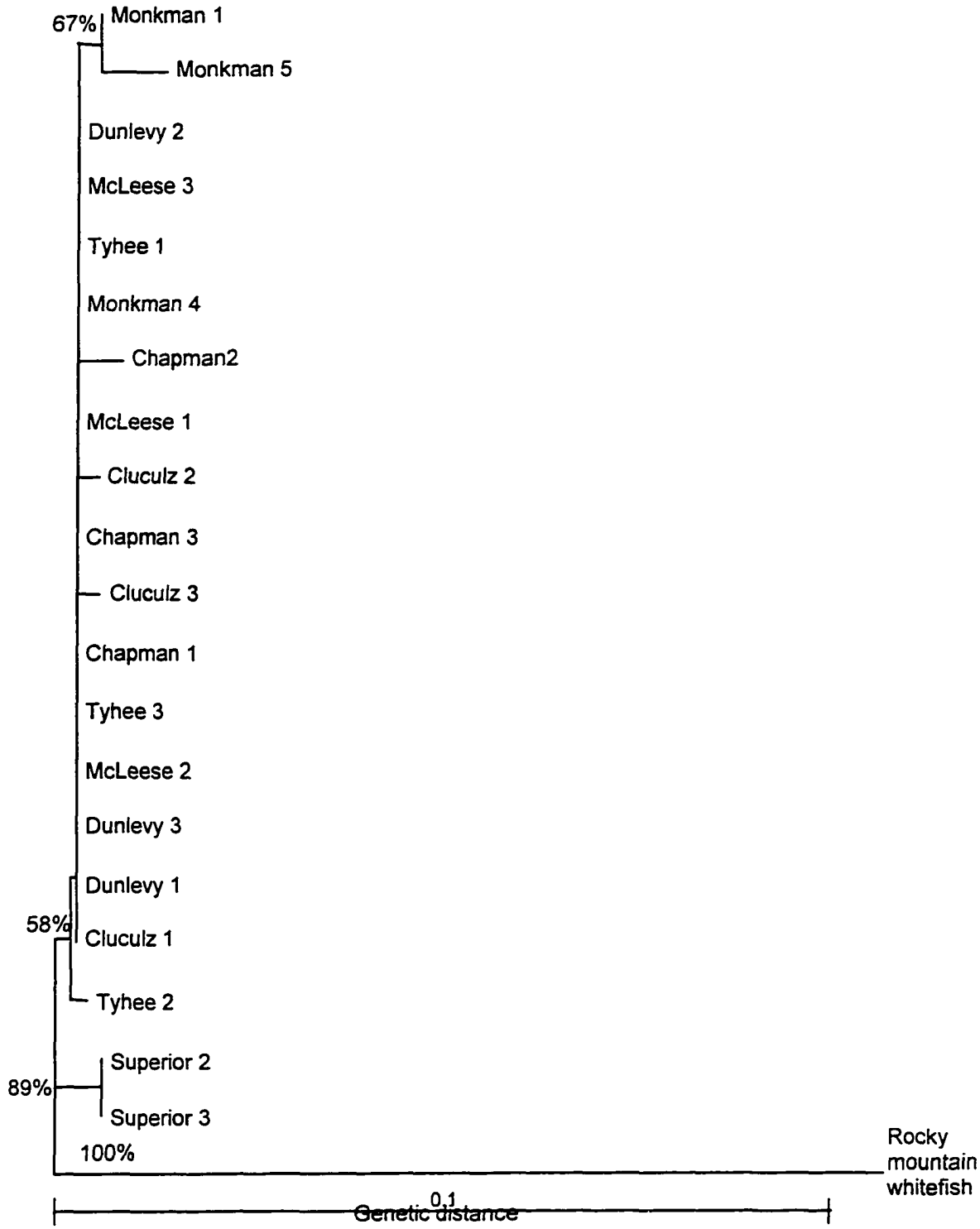
```
            301                                                      350
Cluculz 1   TGATTAATCT TAT-GCTACA CAAAGATAAA TAACATACAT TTTCCTA-CA
Cluculz 2   .......... ...-...... .......... .......... .......G..
Cluculz 3   .......... ...-...... .......... .......... .......A..
Dunlevy 1   .......... ...-...... .......... .......... .......-..
Dunlevy 2   .......... ...-...... .......... .......... .......-..
Dunlevy 3   .......... ...-...... .......... .......... .......-..
Monkman 1   .......... ...-...... .......... .......... .......-..
Monkman 4   .......... ...-...... .......... .......... .......-..
Monkman 5   .......... ...-...... .......... .......... .......-..
McLeese 1   .......... ...-...... .......... .......... .......-..
McLeese 2   .......... ...-...... .......... .......... .......-..
McLeese 3   .......... ...-...... .......... .......... .......-..
Chapman 1   .......... ...-...... .......... .......... .......-..
Chapman 2   .......... ...-...... .......... .......... .......-..
Chapman 3   .......... ...-...... .......... .......... .......-..
Tyhee 1     .......... ...-...... .......... .......... .......-..
Tyhee 2     .......... ...-...... .......... .......... .......-..
Tyhee 3     .......... ...-...... .......... .......... .......-..
Superior 2  .......... ...-...... .......... .......... .......-..
Superior 3  .......... ...-...... .......... .......... .......-..
Rocky       .......... ...T...... .......... ...-...A.A .G.T...-..
```

```
            351
Cluculz 1   TTTT
Cluculz 2   ....
Cluculz 3   ....
Dunlevy 1   ....
Dunlevy 2   ....
Dunlevy 3   ....
Monkman 1   ....
Monkman 4   ....
Monkman 5   ....
McLeese 1   ....
McLeese 2   ....
McLeese 3   ....
Chapman 1   ....
Chapman 2   ....
Chapman 3   ....
Tyhee 1     ....
Tyhee 2     ....
Tyhee 3     ....
Superior 2  ....
Superior 3  ....
Rocky       ....
```

pygmy whitefish shared two identical changes, making them informative. The number of changes between BC pygmy whitefish and rocky mountain whitefish was high; seventeen transitions, 19 transversions, 1 deletion and 1 insertion. On the final tree, three branches were supported by more than 50% of the bootstrap runs (Figure1.12) making these three branches the only reliable branches for phylogenetic information. The three branches separated the BC pygmy whitefish, the Lake Superior whitefish and the rocky mountain whitefish from one another. Lake Superior pygmy whitefish were phylogenetically separated from the BC pygmy whitefish studied. Two individuals from Monkman Lake formed a separate clade from the other pygmy whitefish in BC (Figure 1.12). The Tyhee Lake pygmy whitefish did not group separately from the other pygmy whitefish populations. Tyhee Lake individual #1 is shown as slightly separated on the Neighbour Joining tree using Kimura-2 parameter, but this was not supported by 50% bootstrap when 1000 bootstraps were done.

Since identical tree topologies were obtained using three different distance models that have different assumptions, confidence for the trees shown in Figures 1.08, 1.09, 1.10, and 1.12 is high.

Figure 1.12 Neighbour Joining tree of GH2 intron, constructed using Kimura-2 parameter distance model. Values above branches indicate % of 1000 bootstraps. Only values greater than 50% are shown. Individuals are designated with numbers and the names of the lake where they came from.



1.4 Discussion

The McLeese and Tyhee pygmy whitefish are larger than fish from any of the other populations. However, the McLeese pygmy whitefish, although large, appear to only be at the high end of the size range exhibited by pygmy whitefish and thus can not be described as "giant" pygmy whitefish, and certainly not as a separate species or sub-species based on size. The Tyhee pygmy whitefish, on the other hand, display an anomalous growth pattern that is very distinct from any other population of pygmy whitefish sampled for this study. The Tyhee "giant" pygmy whitefish are larger than other populations at every age and their size does not overlap with other populations, thus the Tyhee pygmy whitefish can be accurately described as "giant" pygmy whitefish.

The Tyhee Lake "giant" pygmy whitefish are not distinguished as genetically different based on any of the sequence data collected. Had the "giant" pygmy whitefish been genetically distinct from other pygmy whitefish (such as a sub-species would have been), the mtDNA sequence data would have shown it as the sequence data was strong enough to distinguish the Lake Superior population from the BC populations. A sub-species would be expected to distinguish itself both within BC as well as from the Lake Superior population.

The GH2 intron sequence data gave greater distance estimates than the mtDNA data, a surprising result. It has been reported that there is a greater transition to transversion ratio for mtDNA in salmonids (MacKay et al. 1996). This occurrence may not have been fully accounted for in the distance measures used, giving slightly decreased distances for the mtDNA data than would have been expected. Regardless of the relative magnitude of the distances generated by the mtDNA and GH2 intron data, neither data set gave any indication

that the Tyhee Lake population was genetically differentiated from the other BC pygmy whitefish populations.

The RAPD data, on the other hand, showed a great deal of population differentiation. This differentiation, however, distinguished all pygmy whitefish populations from one another, approximately equally. The distance of Tyhee Lake pygmy whitefish from other populations was no greater than that of distances among any other population. Although it appears that the Dunlevy Creek population and the McLeese population are in a separate clade, this should be interpreted with caution. RAPD data is prone to ties in UPGMA and NJ trees, and different computer packages give different trees using the same information depending on how the program deals with ties in the data (Backeljau et al. 1996). The tree topology given for the pygmy whitefish RAPD data may be only one of several possibilities and it can not be assumed to be the only topology

Dhar et al. (1997) used a combination of RAPD and cytochrome b data to determine genetic diversity among populations of the common loon (*Gavia immer)*. The RAPD and cytochrome b data both agreed that the populations had different amounts of genetic diversity. RAPD has sufficient resolution to address other types of population-level questions. RAPDs were sensitive enough to detect genetic changes in the European sea bass, *Dicentrarchus labrax,* before and after acclimatization to freshwater (Allegrucci et al. 1995). With such resolution within a species, it is not surprising that RAPDs were able to detect population differentiation within BC pygmy whitefish.

Some species of whales and dophins can exhibit very different morphologies but show little genetic differentiation, while other species of whales or dolphins may show little morphological differentiation but exhibit genetic differentiation at the species level (Hoelzel

1992). There are many studies which report either life history or morphological variants within a "species". In sympatric species of whitefish (*Coregonus*) representing two trophic ecotypes, mtDNA evidence suggested that the ecotypes were indeed genetically distinct (Bernatchez et al. 1996). Similar studies were done on sympatric pairs of dwarf and normal lake whitefish (*Coregonus clupeaformis*; Pigeon et al. 1997) and dwarf and normal smelt (*Osmerus*; Taylor & Bentzen 1993) using mtDNA. The dwarf and normal fish were shown to be genetically distinct from one another, yet were more closely related to each other than to the same morphotype in other lakes. This was interpreted as evidence for parallel evolution in the different lakes, meaning the dwarf forms in each lake had arisen independently from one another (Bernatchez et al. 1996; Pigeon et al. 1997; Taylor & Bentzen 1993). These studies also suggest that ecological factors may contribute significantly to phenotypic, and ultimately genetic divergence. The same type of result was also seen for two life history forms of sockeye salmon/koanee (*Oncorhynchus nerka;* Taylor et al. 1996) and brook charr (*Salvelinus fontinalis*; Jones et al. 1997) where both an anadromous and freshwater form exist. Although the two forms are genetically differentiated from one another in a geographic area, they are more similar to one another than to the same form in another geographic area (Taylor et al. 1996; Jones et al. 1997). These data were also interpreted as evidence for parallel evolution. Using cyt b, the control region and ND3 sequence (all mtDNA) populations of parasitic and non-parasitic lamprey (genus *Lampetra*) were found to be genetically differentiated from one another but were more genetically similar than the same form in different river basins (M. Docker pers comm., Biology, UNBC). Population differentiation was also detected in British whitefish (*Coregonus lavaretus*) using mtDNA. The populations separated into Scottish, English and Welsh

populations (Hartley 1995). In general, mitochondrial DNA has been useful for distinguishing 'populations'. When populations can be differentiated, however, they are not necessarily given sub-species status. They may, however, be given consideration as ESUs (Evolutionarily Significant Units) or MUs (Management Units). Given the plastic nature of whitefish it is perhaps not surprising that "giant" pygmy whitefish are not genetically distinct from other pygmy whitefish populations in BC. Although the Tyhee Lake population is not phylogenetically distinct from other pygmy whitefish populations, this does not mean that there is no genetic component to their fast growth. Hindar (1994) points out that many genes contribute to size, and can be expressed differently according to changes in environmental factors. The RAPD data does raise an important management consideration, since it showed that each population was genetically distinct from the others. Although the "giant" pygmy whitefish are not genetically distinct from other pygmy whitefish, they are one of 6 populations shown to each be genetically distinct and would require the same conservation consideration that could be given to each of the 6 populations. When RAPD data for the northern leopard frog showed that each population was genetically distinct it was recommended that each population be managed separately for conservation purposes as they are being considered as a threatened species (Kimberling et al. 1996). Although normal pygmy whitefish are not considered threatened in BC, the number of known populations is limited.

In general, mtDNA data should be used in conjunction with nuclear markers (Tessier et al. 1995; Degnan 1993), since mtDNA does not recombine and thus constitutes a single locus. Tessier's study on landlocked salmon combined mtDNA and microsatellite data to

differentiate populations, showing the potential power of such a combined marker approach for management and conservation at the genetic level.

Although my RAPD data indicated genetic differentiation among the pygmy whitefish populations, it would have been useful to confirm this finding with another marker capable of such resolution. The information generated by another marker may have given more information concerning the relationships among the BC pygmy whitefish populations. Microsatellite markers are capable of detecting such differentiation and are the subject of the following chapter.

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Chapter 2

Development of microsatellite DNA markers for pygmy whitefish

2.1 Introduction:

When dealing with a threatened species, or one for which conservation issues are important, it is imperative that managers be able to identify distinct intraspecific population units so that effective management plans can be established. In order to accomplish this objective, molecular genetic markers have been extensively utilized over the past decade. RAPD, mtDNA, and nuclear gene introns are three examples of such molecular markers, and their application was discussed in the previous chapter. In recent years, however, there has been an increased use of a different type of molecular marker based on microsatellite DNA; these markers are known as 'microsatellites'.

Microsatellites are short tandem repeats of nucleotide sequences generally between one and six base pairs long (Wright & Bentzen 1994). Microsatellites are non-coding regions, and therefore they are generally not thought to be under selective constraints (Morris et al. 1996). Microsatellite DNA is extremely abundant throughout the genome, possibly more so than previously thought as estimates were based on a single repeat unit type such as CACACA and not all possible repeat sequences (O'Connell & Wright 1997). Microsatellites usually show high levels of variation with respect to the number of repeats (Bruford & Wayne 1993). Microsatellites can be easily amplified using PCR if the sequence flanking the microsatellite is known. This makes microsatellites attractive choices for population studies since techniques that use PCR can be performed easily and efficiently. The assays can thus be done fairly quickly and without much genetic material from the animal being studied. In a

species that may need conserving, where time and animals may be limited, these points are important.

Microsatellites have been widely used in identifying intraspecific population units (Wenburg et al. 1996). When a region is not under selective constraints the mutations that occur are free to accumulate. Given time and migration among populations new alleles will eventually appear in a population. Microsatellite loci can have many alleles both within and among populations for this reason. Since microsatellites have a high rate of mutation, (estimated to be between 10^{-4} and $5x10^{-6}$) they generally show very high levels of polymorphism (Bruford & Wayne 1993). There are two models for the very high mutation process in microsatellite DNA. Currently the most widely accepted model is that of stepwise mutation; during the replication process DNA polymerase slippage occurs resulting in either a gain or loss of a single repeat unit (Bruford & Wayne 1993). The other model is the infinite allele mutation model whereby a mutation can only lead to new alleles and can involve any number of repeats (O'Connell & Wright 1997). It is unclear which model best describes how microsatellite polymorphisms occur but, either way, microsatellites can be used to detect population differentiation with high precision.

Microsatellite markers can identify genetic divergence among recently diverged populations better than other molecular genetic techniques (Wright & Bentzen 1994). For example, microsatellite markers were used to differentiate populations of coastal cutthroat trout, (*Oncorhynchus clarki clarki)* at the stream level (Wenburg et al. 1998). Researchers have been able to differentiate populations of Atlantic salmon (*Salmo salar)* within a small geographic region such as Nova Scotia (McConnell et al. 1995), as well as among different rivers in Quebec (Fontaine et al. 1997) using microsatellite loci. Estoup et al. (1998) also

used microsatellites to detect high levels of differentiation among populations of brown trout, *Salmo trutta* in a small geographic region of eastern France.

There have been several studies in which other molecular markers have been used in conjunction with microsatellite markers to compare their relative utility in population genetics. Mitochondrial DNA sequence and RFLPs have been the most widely used types of DNA-based markers in the past decade. It is thus natural that a relatively new molecular marker such as microsatellites be compared to mitochondrial DNA markers. Although mtDNA and microsatellites may both be both capable of differentiating populations, it is always best to corroborate results with several types of markers to better understand the population dynamics of a species. Different types of markers are under different constraints and conclusions drawn from only one marker type may be misleading. For example microsatellites were able to genetically differentiate two populations of broad whitefish *Coregonus nasus,* despite the lack of differentiation using mtDNA (Patton et al. 1997). For Arctic char, *Salvelinus alpinus,* populations in Europe there was no intra- or interpopulation variation using mtDNA, however, there was considerable variation when microsatellites were used (Brunner et al. 1998). In rainbow trout, *Oncorhynchus mykiss*, differentiation among populations was demonstrated using mtDNA as well as three polymorphic microsatellites (Nielsen et al. 1997). Although microsatellites will likely show genetic differentiation when mtDNA may not, it is important to have both as differentiation at the mtDNA level indicates that the differentiation developed further back in time. The differentiation at the microsatellite level may also be too great to answer certain questions. Microsatellites are promising for discriminating among wild populations of landlocked Atlantic salmon but it is

thought the most valid approach for conservation of the species is to combine the use of mtDNA and microsatellites (Tessier et al. 1995, 1997).

One early technical drawback to using microsatellites was the development of primers for species-specific microsatellite loci. The development process was a timely and costly endeavor, furthermore it was thought that primers would have to be developed for each new species under study (Bruford & Wayne 1993). However, it was shown that primers developed for one species could often be successfully used in related taxa (Bruford & Wayne 1993). For example microsatellite primers previously developed for a variety of salmonids such as sockeye salmon (*Oncorhynchus nerka)*, chinook salmon *(O. tshawytscha)*, brook trout *(Salvelinus fontinalis)*, Atlantic salmon, and brown trout also worked well in rainbow trout/steelhead (Wenburg et al. 1996; D.D. Heath, pers comm, Biology, UNBC). In another study, twelve out of sixteen primers developed for sockeye salmon amplified polymorphic loci in chinook salmon (Scribner et al. 1996). The sockeye salmon primers also successfully amplified six other *Oncorhynchus* species, as well as species in the *Salmo, Thymallus* and *Salvelinus* genera (Scribner et al. 1996). Primers developed for rainbow trout worked in four of the *Oncorhynchus* species, as well as in Atlantic salmon; in fact, the loci appeared to be polymorphic for a number of species, (cutthroat, chum salmon (*O. keta),* coho salmon *(O. kisutch)*, chinook salmon, and Atlantic salmon: Morris et al. 1996). Rainbow trout microsatellite primers were also successfully used in brown trout (Estoup et al. 1998).

Clearly microsatellite primers developed for one species can, in some cases, be used successfully in several different species. Currently, the first step in generating microsatellite primers for a new species is to screen existing primers. For population genetic analyses it is recommended that several loci are used so that adequate confidence limits are met when

analyzing the data (O'Connell &Wright 1997). Goudet (cited in O'Connell & Wright 1997) concluded that a minimum of five microsatellite loci should be used in order for the analysis to be meaningful. Those five microsatellite loci would have to meet several criteria in order to be useful: 1) The primers would have to amplify consistently for all populations. 2) The primers could only amplify one locus. 3) The amplified region should be less than 400 bp (if the microsatellite consisted of a dinucleotide repeat it has been suggested that a product size of less than 120bp would increase the scoring accuracy of the alleles; O'Connell & Wright 1997). 4) Primers that amplified multiple bands or produced streaking could not be used as they could not be reliably scored. 5) Only loci that showed polymorphisms among populations would be useful.

The objective in screening published primers was to obtain a minimum of five markers suitable for application in pygmy whitefish populations. Failing this, it was proposed that primers specific to pygmy whitefish be developed by screening a pygmy whitefish DNA library. Rather than use the traditional method of radio-labeled probes to screen the DNA library for microsatellite loci (Scribner et al. 1994), I used PCR-based techniques. Once the positive clones were identified they would be sequenced and primer pairs would be designed from the flanking sequences of the repeat unit. The primer pairs would have to be tested on the pygmy whitefish populations to evaluate them for the five criteria listed above. Polymorphic alleles would have to show intra- and interpopulation variation in order to be useful for this project.

2.2 Materials and Methods

Screening Existing Primers

The first step in generating suitable microsatellite primers to work on pygmy whitefish population genetics was to screen available primers. There were fifty-eight primer pairs available, most having been developed for various salmonid species (Table 2.01).

High quality pygmy whitefish DNA was chosen (chapter 1) and used to screen all microsatellite primer pairs. Initial PCR conditions were: 2.5 µl of 1X buffer(50mM KCL, 10mM Tris HCL and 0.1% triton X-100), 1.5 or 2.5 mM MgCl, 200 µM of each dNTP, 1 unit *Taq* polymerase, 0.05 µg of each primer, approximately 100 ng of genomic DNA and distilled water to make up a 25 µl reaction. Initially, reactions were run for one cycle at 94° C (1 min), then 30 cycles of 1 min at 94° C an annealing temperature of 48° C (1 min) and an extension temperature of 72° C (1 min, 30 s), the PCR ended with an extension cycle of 5 min at 72° C. Each primer was run using DNA from two individuals. The PCR product was visualized on a 1.8% agarose gel stained with ethidium bromide. Primers were deemed promising if the PCR product showed visible band(s) that were smaller than 400 bp and that amplified with both individuals. The selected primers were used again with various annealing temperatures to try to amplify one or two bands that could potentially be polymorphic. Several markers that showed one or two clear bands on agarose gels were run on polyacrylamide sequencing gel, using dye labeled primers and an automated DNA sequencer (Visible Genetics Gene Blaster™). Fragment sizes can be determined more accurately on sequencing gels, and some fragments that are not visible on agarose gels may be detected on the automated sequencer. The markers chosen to run on a sequencing gel

Table 2.01 Sequences of published microsatellite primers with the results of the microsatellite primers screened for use in pygmy whitefish. Astericks indicates which assays were run on a sequencing gel. $MgCl_2$ is the final mM concentration in the reaction. An arrow indicates a touchdown cycle was performed from one temperature to the next. Primers followed by "*" were used but were incorrect, the original sequences are given at the end. Primer references are identified by subscripts and are given at the end of the table.

Primers Pairs	Primer Sequence 5'-3'	PCR Conditions			Results
		$MgCl_2$	Temp (°C)	Cycles	
A: Primers which did not amplify					
Oneu6-F_1	CAG AGT GGC CTA GAT GCT TTA AT	1.5	60→52	30 @ 52	Nothing
Oneu6-R	CCA CAC ACC AAA TCC TAC CCT TA	2.5	48	30	Nothing
Oneu16-F_1	ATG CTG TAA CCA GTG AAT CCC TT	1.5	60→52	30 @ 52	Nothing
Oneu16-R	TAT CAA CAG AAT GCC AAC CTT TT	2.5	48	30	Nothing
Oneu17-F_1	ATG GCA GGA TTG TTT TAG GTT GT	1.5	60→52	30 @ 52	Nothing
Oneu17-R	GCC ATG AGG AAG ACA CAT CAA TA	2.5	48	30	Nothing
Oneu21-F_1	GGT TAC AGT GGG TTC ACT CTA CA	1.5	60→52	30 @ 52	Nothing
Oneu21-R	GTT ATG ACA ACA GTC TCT GTC GC				
Oneu22-F_1	TTC TCT ACA GGC GAT GAA CTG AT	1.5	60→52	30 @ 52	Nothing
Oneu22-R	TTC TTA CCT CCA CGA TGA CAC AA				
PuPuPy-F_2	ATG CAG CGG ATG TAG GGG GA	1.5	60-52	30 @ 52	Nothing
PuPuPy-R*	TTA AGT GAA AAG ACG TAA GTC	2.5	48	30	Nothing
Omy207-F_3	ACC CTA GTC ATT CAG TCA GG	1.5	60→52	30 @ 52	Nothing
	GAT CAC TGT GAT AGA CAT CG	2.5	48	30	Nothing
Omy207-R					
COCL-22-1_4	GAG AGG GGG TAT GTC TGT	1.5	60→52	30 @ 52	Nothing
COCL-22-2	ATC GGA GTT TAG TAA CCA C	1.5	55→46	30 @ 46	Nothing
Ots3-F_3	CAC ACT CTT TCA GGA G	1.5	60→52	30 @ 52	Nothing
Ots3-R	AGA ATC ACA ATG GAA G	2.5	48	30	Nothing
Ots5-F_3	ACA GCA GTC TAC ATT GAC C	1.5	60→52	30 @ 52	Nothing
Ots5-R	TGT TCA TTA AAA CCA AAA A	2.5	48	30	Nothing

LGLBWF1-F_5 LGLBWF1-R	TAC AGA GAA ATA CAC ACA ACG CAT CAA GAG AGG TTC CAT TAC TGA GCA C	1.5	60→52	30 @ 52	Several attempts yielded no amplification
LGLBWF2-F_5 LGLBWF2-R	CGG ATA CAT CGG CAA CCT CTG AGA CAG TCC CCA ATG AGA AAA	1.5	60→52	30 @ 52	Several attempts yielded no amplification
B: Primers that amplified multiple bands, streaks or unreliable amplification					
Ssa202-F_7	CTT GGA ATA TCT AGA ATA TGG C	1.5	60→52	30 @ 52	Nothing
Ssa202-R	TTC ATG TGT TAA TGT TGC GTG	2.5	48	30	One faint band, unreliable amplification
Fgt1-f-F_8	AGA TTT ACC CAG CCA GGT AG	2.5	48	30	Nothing
Fgt1-f-R	CAT AGT CTG AAC AGG GAC AG	1.5	60-52	30 @ 52	Multiple bands, too large
Oneu9-F_1	CTC TCT TTG GCT CGG GGA ATG TT	1.5	60→52	30 @ 52	Nothing
Oneu9-R	GCA TGT TCT GAC AGC CTA CAG CT	2.5	48	30	Multiple bands
Oneu12-F_1	ACT TAT GCT AGT CAT GGC TCT T	1.5	60→52	30 @ 52	Nothing
Oneu12-R	TCG GTC ATC GAA AGA TAC TTT T	2.5	48	30	Multiple bands
Oneu15-F_1	AGC TTG ACA TCA TAA AAT GCG TC	1.5	60→52	30 @ 52	Nothing
Oneu15-R	TTT CTT CTC TCA TTC TCA CAC GA	2.5	48	30	Multiple bands
uSat60-F_9	CGG TGT GCT TGT CAG GTT TC	1.5	60→52	30 @ 52	Multiple bands, unreliable amplification
uSat60-R	GTC AAG TCA GCA AGC CTC AC	2.5	48	30	Nothing
Omy87-F_3	TCC TGG TCT GGT GCA GG	2.5	48	30	Multiple bands with streaks
Omy87-R	ATT AAC TCC GTT CCA GCC G	1.5	60→52	30 @ 52	Multiple bands, too large
		1.5	60→52	30 @ 52	* Multiple bands
Omy325-F_3	TGT GAG ACT GTC AGA TTT TGC	2.5	48	30	Multiple bands and streaks
Omy325-R	CGG AGT CCG TAT CCT TCC C	2.5	50	30	Multiple bands
		2.5	49, 52	14, 26	Multiple bands
		2.5	49, 52	14, 26	Faint streaks
		1.5	60→52	30 @ 52	* One band or none

Oneu1-F_1	GTC TTA CCA AAT GTC TTC CTC CT	1.5	60→52	30 @ 52	Multiple bands
Oneu1-R	GCC ATT TAG CAT ACG ATT TTA TC	2.5	48	30	Multiple bands and streaks
		2.5	50	30	Multiple bands
		2.5	49, 52	14, 26	Multiple bands
		2.5	49, 52	14, 26	Multiple bands
Ots1-F_3	GGA AAG AGC AGA TGT TGT T	1.5	60→52	30 @ 52	Multiple bands, too large
Ots1-R	TGA AGC AGC AGA TAA AGC A	2.5	48	30	Multiple bands and streaks
Ots6-F_3	TCT CTT CCA GCA CCA CAC A	1.5	60→52	30 @ 52	Multiple bands, too large
Ots6-R	AGA CAG TTT TTC CAC ATC C	2.5	48	30	Multiple bands and streaks
uSat73-F_9	CCT GGA GAT CCT CCA GCA GGA	1.5	60→52	30 @ 52	Multiple bands, too large
uSat73-R	CTA TTC TGC TTG TAA CTA GAC	2.5	48	30	Multiple bands and streaks
	CTA	2.5	52	30	Faint bands with streaks
Oneu20-F_1	TCT GTG GAC AAA ACA TGA GAT TA	1.5	60→52	30 @ 52	Multiple bands
Oneu20-R	CTC CCA TTT TCC CAT TTA TTG TT				
Oneu13-F_1	TCA TAC CCC ATG CCT CTT CTG TT	1.5	60→52	30 @ 52	Multiple bands
Oneu13-R	GAT GAG TGA AAG AGA GGG AGC GA	2.5	48	30	Multiple bands, streaks
		2.5	48, 52	14, 26	One band
		2.5	49, 52	14, 26	Multiple bands
Oneu3-F_1	TCT CCT TGG TCT CTC TGT CCC TT	2.5	48	30	Multiple bands
Oneu3-R	CTA TCA GCC AAT CGC ATC AGG AC	1.5	60→52	30 @ 52	Two bands
		1.5	60→52	30 @ 52	*Multiple bands
Oneu4-F_1	TAA TTT ACA TAT CAG GTT CTG CC	1.5	60→52	30 @ 52	One or two bands
Oneu4-R	TAT GCT AGT CAT GGC TCT TAC AT	2.5	48	30	Multiple bands
		2.5	48, 52	14, 26	One band
		2.5	49, 52	14, 26	Multiple bands

Ots4-F_3	GAC CCA GAG GAC AGC ACA A	1.5	60→52	30 @ 52	Multiple bands, too large
Ots4-R	GGA GGA CAC ATT TCA GCA G	2.5	48	30	Multiple bands and streaks
		2.5	48, 52	14, 26	Two bands
		2.5	49, 52	14, 26	Multiple bands
		2.5	49, 52	14, 26	Multiple bands
		1.5	60→52	30 @ 52	Nothing
LGLPUPUPY-F_5	GGG GAG CAT GCA GCG GAT GTA	1.5	60→52	30 @ 52	One individual gave three to four bands,
LGLPUPUPY-R	GCC GGT GGG TAA AGA ATG CAG C				rest did not amplify
uSat15-F_9	TGC AGG CAG ACG GAT CAG GC	1.5	60→52	30 @ 52	Multiple bands, too large
uSat15-R	AAT CCT CTA CGT AAG GGA TTT GC	2.5	48	30	Multiple bands and streaks
		2.5	52	30	One band
		2.5	48, 52	14, 26	One band
		2.5	49, 52	14, 26	Multiple bands
Ssa197-F_7	GGG TTG AGT AGG GAG GCT TG	1.5	60→52	30 @ 52	One band, very faint
Ssa197-R	TGG CAG GGA TTT GAC ATA AC	2.5	48	30	Multiple bands with streaks
		1.5	60→52	30 @ 52	* Nothing
Oneu10-F_1	ATG GGG AAC AGA AGA GGA AT	1.5	60→52	30 @ 52	One or two bands
Oneu10-R	CTG TAG GTG TGA AAT GTA TTT	2.5	48	30	Multiple bands and streaks
	AAA	2.5	52	30	Nothing
		2.5	49, 52	14, 26	Multiple bands
		2.5	49, 52	14, 26	Two bands with streaks
Oneu14-F_1	AGA AAC ATG AGA ACA GTC TAG GT	1.5	60→52	30 @ 52	Nothing
Oneu14-R	CCT TAT GAG TTT GGT CTC CAT GT	2.5	48	30	One or two bands
		2.5	49, 52	14, 26	Faint streaking
		2.5	49,52	14, 26	One or two bands, very small and fuzzy
Oneu19-F_1	CTG GAA AGC ACA GAG AGA GCC TT	1.5	60→52	30 @ 52	Nothing
Oneu19-R	TCC AAC AGT CTA ACA GTC TAA	2.5	48	30	Multiple bands
	CCA	2.5	48, 52	14, 26	Nothing
		2.5	49, 52	14, 26	One or two bands, unreliable amplification

Sfo12-F_{10}	GGT TTT GAA GAG TGA CAG	1.5	60→52	30 @ 52	One band
Sfo12-R	CCC GTT TCA CAA TCA GAG	2.5	48	30	Nothing
		1.5	60→52	30 @ 52	*Multiple bands
		1.5	60	30	*Nothing
		1.5	58	30	*Nothing
		1.5	57	30	*Nothing
		1.5	56	30	*Nothing
		1.5	54	30	*Nothing
		1.5	53	30	*Nothing
Omy293-F_3	CAC AGA GTG CGA TCG TGG	1.5	60→52	30 @ 52	Nothing
Omy293-R	GGT ACT AAT GTT AAG CTC GAG	2.5	48	30	Very fuzzy, may be two bands
Ssa4-F_6	ATT AGG CAG CAG CAG GCT GC	1.5	60→52	30 @ 52	Nothing
Ssa4-R	TGT TCA CTC ACT GAC ACG CG	2.5	48	30	One or two bands, very small and fuzzy
Ots2-F_3	ACA CCT CAC ACT TAG A	1.5	60→52	30 @ 52	One or two bands, unreliable amplification
Ots2-R	AAT ATC CTT CAC ACT G	2.5	48	30	Nothing
Omy77-F_2	CGT TCT CTA CTG AGT CAT	2.5	48	30	Nothing
Omy77-R*	GGG TCT TTA AGG CTT CAC TCG A	1.5	60-52	30 @ 52	One or two bands, unreliable amplification
Oneu8-F_1	AAC ATT CTG GGA TGA CAG GGG TA	2.5	48	30	One or two bands, very small and fuzzy
Oneu8-R	CTG TTC TGC TCC AGT GAA GTG GA				
Oneu11-F_1	GTT TGG ATG ACT CAG ATG GGA CT	2.5	48	30	One or two bands, unreliable amplification
Oneu11-R	TCT ATC TTT CCT GTC AAC TTC CA	2.5	49, 52	14,26	Faint streaking
Ssa171-F_7	TTA TTA TCC AAA GGG GTC AAA A	1.5	60→52	30 @ 52	One faint band, unreliable amplification
Ssa171-R	GAG GTC GCT GGG GTT TAC TAT	2.5	48	30	One or two bands, barely visible
C: Primers which had potential initially but were not useful					
Ssa293-F_7	TGG TTA TTT GTT TCC AGA G	2.5	48	30	Nothing
Ssa293-R	ATC AGA TAC ACA GAG ACG G	1.5	60→52	30 @ 52	Nothing
		1.5	60→52	30 @ 52	*Two or three bands, little or no variability
		1.5	60→52	30 @ 52	* One or two bands

Omy78-F_3	ACT CCA GCA CAC CTG TCT CC	2.5	48	30	Streaks
Omy78-R		2.5	50	30	Multiple bands
	TGT CTC AGT GCT CTT TCC C	2.5	49, 52	14, 26	Multiple bands
		1.5	60→52	30 @ 52	One r two bands
		1.5	60→52	30 @ 52	* Multiple bands
		1.5	60	30	* Nothing
		1.5	58	30	* Nothing
		1.5	57	30	* Nothing
		1.5	56	30	* Nothing
		1.5	54	30	* 2 bands
		1.5	53	30	* Nothing
		1.5	54	30	* 2 or 3 bands, results not reliable or reproducible
Oneu2-F_1	GGT GCC AAG GTT CAG TTT ATG TT	2.5	48	30	One or two bands
Oneu2-R	CAG GAA TTT ACA GGA CCC AGG TT	2.5	50	30	Nothing
		2.5	49, 52	14, 26	Multiple bands
		2.5	49, 52	14,26	Multiple bands
		1.5	60→52	30 @ 52	* Two bands
		1.5	60→52	30 @ 52	* One or two bands
		1.5	60→52	30 @ 52	* One to three bands
		1.5	60→52	30 @ 52	* One band or nothing
		1.5	60→54	35@ 54	* Nothing
		1.5	55,52	5, 30	* Same band for all individuals
		1.5	60→52	30 @ 52	Multiple bands
Ssa85-F_7	AGG TGG GTC CTC CAA GCT AC	2.5	48	30	Nothing
Ssa85-R	ACC CGC TCC TCA CTT AAT C	1.5	60→52	30 @ 52	Nothing
		1.5	60→52	30 @ 52	* Nothing
		1.5	60→52	30 @ 52	* One or two bands
		1.5	55→46	30 @ 46	* Same band for all individuals, second band rare and not reliable
		1.5	60→52	30 @ 52	* One or no bands, not reproducible

Oneu7-F_1 Oneu7-R	ACA CTG CAA ACA CTC TGC TTA CT CAA GAA GAA ACC CTG TCC TCA AG	2.5 1.5 1.5 1.5	48 60→52 60→52 60→52	30 30 @ 52 30 @ 52 30 @ 52	Multiple bands * One band * two bands * One band too large, other band monomorphic
Sfo8-F_{10} Sfo8-R	CAA CGA GCA CAG AAC AGG CTT CCC CTG GAG AGG AAA	2.5 2.5 2.5 1.5 1.5 1.5 1.5	48 49, 52 49, 52 49, 52 60→52 60→52 60→52	30 14, 26 14, 26 14, 26 30 @ 52 30 @ 52 30 @ 52	Multiple bands and streaks Multiple bands Multiple bands Multiple bands * One or two bands * Monomorphic * One band or none, no variation
Sfo18-F_{10} Sfo18-R	TGG TGT ATC CTG CTC CTG TGG AAT GTG TGT CTG TTT TCT	1.5 2.5 1.5	60→52 48 60→52	30 @ 52 30 30 @ 52	One band Nothing * One band, no variation
Ssa289-F_6 Ssa289-R	CTT TAC AAA TAG ACA GAC T TCA TAC AGT CAC TAT CAT C	2.5 1.5 1.5 1.5	48 52 60→52 55→46	30 30 30 @ 52 30 @ 46	One or two bands One band * Three bands, not reliable * One band, no variation
D: Primers which may have potential but were not explored further					
Sfo23-F_{10} Sfo23-R	GTG TTC TTT TCT CAG CCC AAT GAG CGT TAC GAG AGG	1.5 2.5	60→52 48	30 @ 52 30	One band, has potential One fuzzy band, unreliable amplification
Oneu5-F_1 Oneu5-R	AAC ACA CCA GCT GTG AAA ACA AA TGT CTA TCG CCA ATC TCT CTG CT	1.5 2.5	60→52 48	30 @ 52 30	One band, has potential Multiple bands
Oneu18-F_1 Oneu18-R	ATG GCT GCA TCT AAT GGA GAG TAA AAA CCA CAC ACA CTG TAC GCC AA	1.5 2.5	60→52 48	30 @ 52 30	Two bands, very faint- has potential Nothing
Ssa14-F_6 Ssa14-R	CCT TTT GAC AGA TTT AGG ATT TC CAA ACC AAA CAT ACC TAA AGC C	1.5 2.5	60→52 48	30 @ 52 30	One band, has potential Nothing
COCL-21-1_4 COCL-21-2	GAA AGG TAA AGA GGA CAC A CTC CTT CAC TTT TTC ATC AC	1.5	60→52	30 @ 52	One band, has potential

COCL-8-1_4 COCL-8-2	GAT GCA TCA AGT CTG ACA C AGA ATG TTT TAC CCT GAG TAG	1.5	60→52	30 @ 52	One band, has potential
COCL-3-1_4 COCL-3-2	TTC AGG TTT GGT AAG CAA G AGT GTA ATA AAT CAC CCG AG	1.5	60→52	30 @ 52	One band, has potential
E: Original primer sequences, not the ones used (incorrect sequences indicated with ◆)					
PuPuPy-R_3	TTA AGT GAA AAG ACG TAA CTT				
Omy77-R_3	TGG TCT TTA AGG CTT CAC TGC A				

Primer references

[1] Scribner et al. 1996
[2] Morris et al. 1996
[3] Olsen et al. 1996
[4] Bernatchez (pers comm.)
[5] Patton et al. 1997
[6] McConnell et al. 1995
[7] O'Reilly et al. 1996
[8] Sakamoto et al. 1994
[9] Estoup et al. 1993
[10] Angers et al. 1995

were run with multiple DNA samples to determine if there was any allelic variation among the samples.

The PCR protocol was modified for greater success in amplifying microsatellites using 1.5 mM $MgCl_2$ and a "touchdown" cycle of annealing temperatures. A touchdown cycle of annealing temperatures involves a decrease by one degree Celsius in annealing temperature with every cycle until the lowest temperature is reached (Don et al. 1991). The touchdown cycle that worked best was 1° C decrease in temperature with every cycle from 60° C to 52° C, ending with 30 cycles at 52° C. As this appeared to work well with almost all the primers being used in the lab, the salmonid primers were screened on the pygmy whitefish DNA for a second time using this PCR protocol. New primers that worked well using this method were tested further as described above.

Pygmy whitefish DNA Llibrary Construction and Screening

Genomic DNA Restriction Digests: Thirty micrograms of pygmy whitefish DNA was digested with the restriction enzymes Eco RV and Hae III. The enzyme reaction consisted of 20 µL of ReAct 2 buffer (Gibco), 30µg of genomic DNA, 75 units each of Eco RV and Hae III enzymes, brought up to 200µL with distilled water. The reaction was left overnight at 37° C . Some of the restricted DNA was run on an ethidium bromide stained 1.8% agarose gel to determine the performance of the restriction enzymes. The remaining restricted DNA, about 28.2 µg, was precipitated using isopropanol and sodium acetate. rinsed with 70% ethanol and brought up in distilled water. The precipitated DNA was then cut a second time with restriction enzymes Dra I and Alu I, as described above. A sample of the digestion was again run on an agarose gel stained with ethidium bromide to determine whether the DNA

appeared to be cut. The DNA was precipitated with isopropanol and NaOAc, and reconstituted in double distilled H_2O to a concentration of 100 ng/μL.

Ligation: The pBluescript® II SK^+ vector was cut at the Sma I site prior to ligation of pygmy whitefish DNA into the vector. The following conditions were used to ligate the cut pygmy whitefish DNA into the pBluescript® II SK^+ vector: 11 μl of distilled water, 2 μl of Bluescript, 3 μl of ligation buffer, 0.1 μl Sma I, 300 ng of DNA and 1 μl of T4 DNA ligase. The reaction was incubated overnight at 15° C. The ligated DNA was then transformed into competent bacteria cells, XL1-Blue MRF, supplied by Sratagene (California). The transformation reaction included approximately 2 μg of ligated DNA added to 100 μl of competent bacteria cells. This mixture was left on ice for one hour and then heat shocked by placing it in a 42° C water bath for 45 seconds. To the bacterial cells, 350 μl of LB media (Sambrook et al. 1989) was added and left at 37° C for one hour. After an hour the cells were plated on LB agar with 100mg/L of ampicillin. To each plate, 20 μl of 100mM IPTG and 50 μl of 2% X-Gal had been added and left for one hour before plating. Once the cells were plated they were left at 37° C for approximately 15 hours. Colonies that appeared white and were not in contact with other colonies were individually picked off and placed in 100 μl of distilled water and boiled at 94° C for three minutes.

Library Screening: Sixty positive colonies were used to screen for microsatellite loci. Ten oligonucleotides were designed to screen the positive colonies, these included five dinucleotide repeats, three trinucleotide repeats and two tetranucleotide repeats (Table 2.02). A dinucleotide repeat primer consists of a number of repeated two base pair repeat motifs, a trinucleotide, a three base pair repeat and a tetranucleotide is made up of repeats of four base pairs. All the dinucleotide primers were combined so that they could be tested at one time.

Table 2.02 The di-, tri- and tetra-nucleotide primers used to screen the positive colonies.

Primer	Repeat Motif	Sequence 5'→3'
DISTAT 1	GC	GCG CGC GCG CGC GC
DISTAT 2	CA	CAC ACA CAC ACA CA
DISTAT 3	GT	GTG TGT GTG TGT GT
DISTAT 4	GA	GAG AGA GAG AGA GA
DISTAT 5	AC	ACA CAC ACA CAC AC
TRISAT 6	AAT	AAT AAT AAT AAT AAT
TRISAT 7	AAG	AAG AAG AAG AAG AAG
TRISAT 8	CAC	CAC CAC CAC CAC CAC
TETRASAT 9	GACA	GAC AGA CAG ACA GAC A
TETRASAT 10	GATA	GAT AGA TAG ATA GAT A

The same was done for the trinucleotide primers and the tetranucleotide primers. The di-, tri-, and tetranucleotide primer combinations were paired with either M13 forward, or M13 reverse, (M13 F: 5'-GT AAA ACG ACG GCC AGT-3' and M13 R: 5'-GG AAA CAG CTA TGA CCA TG- 3'). M13 forward and reverse are universal primers that anneal on either side of the Sma I restriction site within the pBluescript vector. PCR was conducted using these primer pairs and the DNA from the sixty positive colonies. Positive amplification indicated that the microsatellite primer DNA annealed to a microsatellite locus in the inserted pygmy whitefish DNA. The positive insert would then be sequenced using the M13 primers. Based on the sequence of the insert, new primers could then be designed on either side of the microsatellite locus. The M13 primers (forward and reverse) were also used alone to determine whether the white colonies did indeed have an insert. Five microlitres of the boiled colony was used in each PCR. The $MgCl_2$ concentration was 3mM and the annealing temperature was a touchdown cycle from 55° C to 46° C, with 30 cycles at 46° C.

2.3 Results and Discussion

Existing Primer Screening

Twelve of the 58 primers screened did not produce any reliable amplification (section A of Table 2.01). There are several reasons why these primers may not have amplified. Although primers developed for one species can be successful in other species, the likelihood decreases the more divergent the two species are. Loci that do not amplify (although they may be present; "null alleles"), are more likely when using primers developed for other species as the flanking sequence of the microsatellite locus may have changed (O'Connell &

Wright 1997; Morris et al. 1996). Although pygmy whitefish are a salmonid, they are not closely related to the species for which these primers were developed. Within *Prosopium*, the pygmy whitefish are considered to be the furthest diverged (Norden 1970). It was discovered that the last three bases on the 3' end of primer PuPuPy-F (Table 2.01, section E) were incorrect. The primer sequence was taken from Morris et al. (1996), but when checked with the original reference the primer sequence was found to be incorrect. This may have been why the primer did not work. Thirty-one of the 58 primers (section B of Table 2.01) successfully amplified product(s) but were judged useless for pygmy whitefish due to the occurrence of streaks, multiple bands, large bands or non-reliable amplification from one PCR run to the next. The streaking on the gel probably indicated that the primers were partially annealing to many sites within the genome. A microsatellite primer that amplifies more than one locus is not useful as one of the amplified regions may not be a microsatellite and determining which fragments are true microsatellites is difficult. Primers that amplified large molecular size products were not chosen, since the allele could not be scored efficiently on a sequencing gel. If the priming site is too far away from the repeat sequence then the allele sizes could be too large for sensitive detection of alleles (Morris et al. 1996). A large fragment may also indicate that the amplified sequence is no longer a microsatellite locus as the repeat sequences in microsatellites generally have a maximum length of only a few hundred base pairs (Morris et al. 1996). Primers that do not amplify reliably from one PCR reaction to the next are likely mispriming, and are therefore not useful for population genetic studies. Primer Omy77-R was first obtained from Morris et al. (1996), however, when checked with the original reference (Olsen et al. 1996) there were several changes from the original primer sequence (Table 2.01, section E). There may have been enough incorrect

bases that the primer could not anneal and therefore unreliable amplification or mispriming may have occurred.

Eight of the fifty-eight primers gave promising results initially and were chosen for additional testing (section C of Table 2.01). Dye labeled primers were ordered so that the markers could be run on an automated sequencer. Primers Ssa293, Omy78, Oneμ2, and Ssa85 gave one or two alleles for an individual but when repeated would produce completely different alleles. Multiple attempts compounded the lack of repeatability (Table 2.01). It is unclear what the cause of this irreproducibility was, since the allelic peaks were very strong and sharp. Controls were run with each PCR to ensure conditions were constant. Oneμ7 gave good results but did not consistently amplify and such unreliability rendered the locus useless. Finally, primers Sfo8, Sfo18, and Ssa289 amplified clear alleles but there was no allelic variation among individuals from several populations.

Primers developed for one species may amplify in another but show little or no variation (Morris et al. 1996). The sequence adjacent to the microsatellite locus can be influenced by the repeat unit. The end of the repeat unit may not always be clear. It is sometimes difficult to determine where the flanking sequence begins and the microsatellite ends. In one species, variation in sites close to the end of a microsatellite locus and within the repeat sequence can be high and can cause high rates of slippage during replication resulting in mismatching of the primer (Morris et al. 1996). There may also be other factors affecting whether a microsatellite will show variation. In a study by Brooker et al. (1994) the short simple repeats were highly polymorphic but the long imperfect repeat, combinations of different repeat motifs, was monomorphic, this was described as evidence for Wright's

suggestion that there are factors other than overall length that determine whether a microsatellite locus will show variation.

Seven of the fifty-eight primers (section D of Table 2.01) showed potential when the touchdown cycle from 60° C to 52° C was used. These primers amplified either one or two bands and were not eliminated as potential microsatellite primers for *Prosopium*. If fully explored, they may be of potential use in pygmy whitefish.

Although no microsatellite markers resulted that could be used for pygmy whitefish, the information in Table2.01 will undoubtedly be useful as a starting point for others wishing to use microsatellites for pygmy whitefish.

Microsatellite primer development

Although screening existing primers was deemed the best route, developing specific pygmy whitefish primers was judged necessary in the event that none of the primers screened were useful. The process of screening a pygmy whitefish DNA library was begun by using a PCR-based modification of the standard library screening technique. The use of PCR to directly screen colonies with inserted DNA is relatively new. Previously, colonies were screened using radioactively labeled probes of a specific repeat such as CACACA...(Patton et al. 1997; Scribner et al. 1994).

There were several hundred positive colonies on the plates, 60 of which were screened. There were no consistently amplified products. It is likely that the extraction of the ligated vector DNA from the colonies was unsuccessful. Had the cloning worked it would have been quite feasible to screen hundreds of colonies using fast capillary tube PCR protocol.

The information presented in this chapter provides important background and will be useful to future researchers working on *Prosopium.*

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Chapter 3

A general description and the ecology of pygmy whitefish

3.1 Introduction

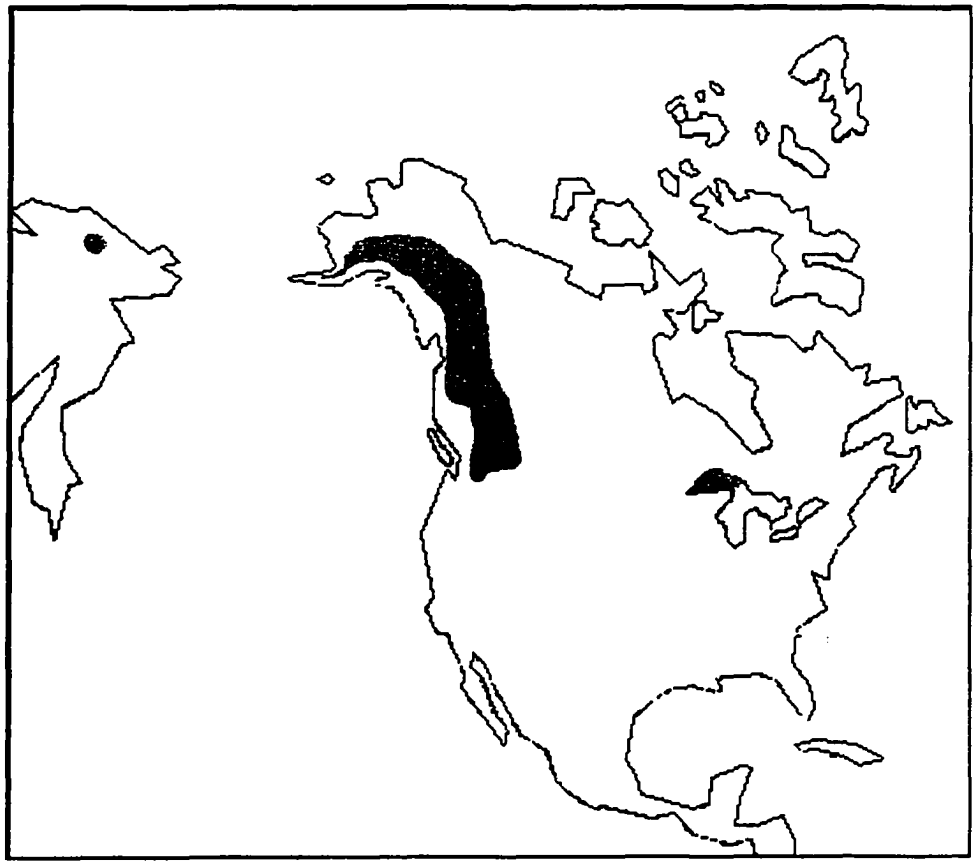
The size at age data presented in chapter one showed the Tyhee population of pygmy whitefish to be the only population of true "giant" pygmy whitefish based on their unique growth and much larger size than other pygmy whitefish. This population did not reveal itself to be genetically distinct from the other BC populations studied. Although the main objective of this thesis was to determine whether the "giant" pygmy whitefish are indeed a genetically distinct sub-species, useful ecological information was collected while sampling pygmy whitefish. The purpose of this chapter is to summarize and analyze the data collected. The first part of this chapter is an extensive review of the available literature on the ecology and life history of pygmy whitefish.

3.2 Literature Review

Geographic Distribution of Pygmy whitefish

The distribution of pygmy whitefish is discontinuous within North America and Eurasia (Figure 3.01). Previously only known to exist in North America, it has recently been documented in Eurasia in the Amuguem River basin, Chkotski Peninsula (Chereshnev & Skopets 1992). In North America it is found mainly in the northwest (Scott & Crossman 1973; Bird & Roberson 1979; Lindsey & Franzin 1972). The most easterly population recorded occurs in Lake Superior, far to the east of the other populations (Scott & Crossman 1973). Pygmy whitefish may have dispersed to these locations after glaciation from several different refugia. The pygmy whitefish in the Peace, Fraser and Skeena systems

Figure 3.01 Known distribution of pygmy whitefish. The dark area indicates approximate area where pygmy whitefish have been captured. Pygmy whitefish have been captured in the following systems; Yukon River, MacKenzie River, Copper River, Alsek River, Liard River, Skeena River, Peace River, Fraser River, Columbia River, Saskatchewan River, Naknek River, Amguem River, and Lake Superior (Scott & Crossman 1973; Bird & Roberson 1979; Heard & Hartman 1965; Lindsey & Franzin; McCart 1965; and Chereshnev & Skopets 1992)



were thought to have come from the refugium in the Columbia River basin (Lindsey & Franzin 1972). The Lake Superior population was thought to come from the upper Mississippi River refugium (Lindsey & Franzin 1972). In general, the distribution of freshwater fish in North America may have been restricted more by ecological conditions rather than land barriers (McPhail & Lindsey 1970); however those ecological conditions are unknown (McPhail & Lindsey 1970). Several researchers have been puzzled by the absence of pygmy whitefish in Russian and Alaskan lakes in what is assumed to be suitable habitat (Chereshnev & Skopets 1992; Bird & Roberson 1979). Bird & Roberson (1979) thought sampling difficulty might explain the absence of pygmy whitefish in some lakes (sampling often does not include depths that the pygmy whitefish prefer and a large mesh size would not capture them). Bird & Roberson (1979) also concluded that predatory pressures and unsuitable habitat may have eliminated the low fecundity fish in clearer, deeper lakes.

Distinguishing Characteristics

Although sampling limitations may explain the absence of pygmy whitefish from some lakes, pygmy whitefish may also be simply misidentified as juvenile rocky mountain whitefish (*Prosopium williamsoni),* the closest relative to the pygmy whitefish (Norden 1970). Reliable identification is thus essential for studies involving pygmy whitefish. The following characteristics may be of help to those identifying pygmy whitefish.

Pygmy whitefish are small cylindrical shaped fish, silver in colour. Their eye diameter is greater than the length of their snout (Scott & Crossman 1973). Their snout is blunt and rounded, unlike rocky mountain whitefish which have a pointed snout. Both rocky mountain whitefish and pygmy whitefish have a single nostril flap and can be distinguished

from the lake whitefish which has two (Wydoski & Whitney 1979). The pygmy whitefish have 50-70 lateral line scales (Scott & Crossman 1973; Wydoski & Whitney 1979) whereas rocky mountain whitefish have 74-90 scales in sympatric populations (Wydoski & Whitney 1979). These numbers can vary slightly among populations. For example, in Bull Lake, Montana, pygmy whitefish have 54-63 lateral line scales whereas rocky mountain whitefish have 74-77 (Weisel & Dillon 1954). The range of gill rakers for pygmy whitefish is 12-21, (Weisel et al. 1973) and 19-26 for rocky mountain whitefish (Norden 1970). In order to obtain more reliable distribution data it is imperative that those in the field recognize pygmy whitefish from other whitefish. If the simple characters described above are used when sampling, then pygmy whitefish can be identified reliably.

Habitat

Pygmy whitefish are described as a deep water species in Lake Superior and were found at depths of between 18.3m and 88.6m, although the majority were caught between 54.7m and 70.3m (Scott & Crossman 1973). In a few lakes in the Naknek system, Alaska, pygmy whitefish were found at depths of 168m (Heard & Hartman 1965). Although they do not usually occur as deep elsewhere, they are usually found below 6.1m. In one of the four lakes in the Amguem River basin all pygmy whitefish were caught between 18m and 25m (Chereshnev & Skopets 1992). In several Washington lakes the pygmy whitefish were caught between 7m and 92m. In Lake Chester Morse, Washington, they were caught between 1.8m and 30.4m, but in winter were caught in less than 1.8m of water (Wydoski & Whitney 1979). They were almost always captured at temperatures of 10° C or less in Washington lakes (Hallock & Mongillo 1998). In some lakes, however, pygmy whitefish

have been caught in less than 1m of water (Heard & Hartman 1965). In three of the Amguem River basin lakes, pygmy whitefish were caught in 0.5m to 1m water on a gravel or boulder bottom in the summer (Chereshnev & Skopets 1992). In the Copper River drainage system, Alaska, pygmy whitefish were taken in less than 1m of water during mid to late summer; however deeper depths were not sampled, despite the lakes being very deep, 95m to 115m (Bird & Roberson 1979).

Most pygmy whitefish are caught in benthic habitat regardless of depth. In Flathead Lake, Montana, pygmy whitefish were caught within 1m of the bottom in 18m to 82m of water (Weisel et al. 1973). Stomach content data also suggest that most pygmy whitefish are bottom feeders (see below). However, pygmy whitefish have been caught in the limnetic zone as well as at the surface, over deep water (Heard & Hartman 1965). Pygmy whitefish in Washington State have also been caught in streams and in such habitat they appear to prefer moderate to swift currents (Wydoski & Whitney 1979).

Pygmy whitefish share their deep water habitat with other salmonid species such as lake trout (*Salvelinus namaycush)* (McCart 1965) bull trout (*Salvelinus confluentus),* and kokanee *(Oncorhynchus nerka)* (Hallock & Mongillo 1998). According to McCart (1965), pygmy whitefish and rocky mountain whitefish avoid habitat overlap by utilizing different depths. That is, the lower limit of depth distribution for rocky mountain whitefish is above the upper limit for pygmy whitefish (McCart 1965).

Diet

The pygmy whitefish diet varies, but generally consists of crustaceans, aquatic insect larvae such as chironomids and plecopterans, planktonic crustaceans, and eggs of other

salmonids (Scott & Crossman 1973). Some studies have found their diet to consist primarily of ostracods, amphipods, copepods, midge larvae and pupae, and larval clams (Wydoski & Whitney 1979). In Flathead Lake, Montana, food items changed monthly, probably according to availability (Weisel et al. 1973). The most abundant food item in May and June was dipterus (chironomid larvae and pupae) in July and August, cladocera; then back to diptera for September; before returning to cladocera in October and November. In Ross Creek, Montana, pygmy whitefish were eating the eggs of other spawners in the fall (Weisel et al. 1973). During spawning, eggs were found in 75% of stomachs containing food. Pygmy whitefish usually have sand and detritus in their stomachs, indicating that they feed at or near the bottom (Weisel et al. 1973; Heard & Hartman 1965). The pygmy whitefish diet was found to be highly variable among the different lakes in southwest Alaska as well (Heard & Hartman 1965). For example, the slower growing Brooks population feeds upon plankton while the faster growing south Bay- Iliuk arm population feeds primarily on insects (Heard & Hartman 1965). The varied diet of the pygmy whitefish shows this small fish to be an opportunistic benthic feeder.

Species That Prey Upon Pygmy whitefish

Pygmy whitefish are preyed upon by piscivorous fishes such as char (Chereshnev & Skopets 1992) and Bull trout/Dolly Varden (Wyman 1975 in Hallock & Mongillo 1998). Their small size makes them suitable prey for other piscivorous species. Pygmy whitefish were one of the most abundant fish in Flathead Lake (4.8% of the catch) and it was assumed they must have been important to the food chain of the lake (Weisel et al. 1973).

Age and Size

A mature pygmy whitefish is generally between 102mm –127mm in total length (Scott & Crossman 1973; Chereshnev & Skopets 1992) although this varies widely among populations. Most pygmy whitefish are usually less than 152mm, total length (Wydoski & Whitney 1979). The largest recorded pygmy whitefish was 285mm (total length) from Horseshoe Lake in Washington State (Hallock & Mongillo 1998); however that population no longer exists. The second largest pygmy whitefish recorded was a nine year old female fish from Tyhee Lake, British Columbia which was 271mm in fork length, (this was measured after preservation and shrinkage was not accounted for; McCart 1965). The total lengths for four-year-old fish in Chester Morse Lake in Washington were between 210mm and 246mm (R2 Resource Consultants 1995).

In all age classes the female pygmy whitefish are generally larger than the males (Weisel et al. 1973; Heard & Hartman 1965). Females also tend to live longer than males (Weisel et al. 1973; Heard & Hartman 1965; McCart 1965). The oldest pygmy whitefish reported was a nine year old female from Tyhee Lake (McCart 1965). The most abundant age group is usually 2-year-olds. In most populations the oldest fish taken is age 4 (Weisel et al. 1973; Hallock & Mongillo 1998). Many pygmy whitefish populations are relatively short-lived. In some populations in the Naknek River system, no pygmy whitefish older than three years of age were caught (Weisel et al. 1973; Heard & Hartman 1965).

Growth rates for pygmy whitefish have been estimated; however most were generated by back calculation from scales (Heard & Hartman 1965; McCart 1965; Weisel et al. 1972). Within BC, McCart (1965) showed that the McLeese Lake individuals grow fastest during their first year and are larger than the Tyhee fish until age 3, at which time the

Tyhee Lake pygmy whitefish become larger and remain so. Cluculz and Tacheeda Lake pygmy whitefish growth was reported to be similar to that of Tyhee Lake pygmy whitefish for their first two years, but then growth of the Tyhee Lake population exceeds that of the other populations (McCart 1965).

Morphology

Two morphological types of pygmy whitefish were desribed by McCart (1970); "high-raker" and "low-raker" forms. McCart originally described these two forms as having separate geographical distributions, except where they occurred sympatrically in Bristol Bay, Alaska. Since then, other pygmy whitefish populations have been discovered that do not fit either the high-raker or low-raker cateogories (Lindsey & Franzin 1972; Bird & Roberson 1979) but rather are intermediate forms. All known pygmy whitefish populations in the Peace, Fraser and Skeena systems can be categorized as the low-raker form (McCart 1970). The high-raker form is usually smaller than the low-rakered form (Chereshnev & Skopets 1992). These morphological differences are not obviously correlated to ecological conditions and may have arisen before Wisconsin glaciation (Lindsey & Franzin 1972).

Reproduction

Pygmy whitefish are thought to be annual spawners as residual eggs have been found in the abdominal cavity of pygmy whitefish caught in lakes in Russia and Montana (Chereshnev & Skopets 1992; Weisel et al 1973). Pygmy whitefish generally mature at two to three years of age (Chereshnev & Skopets 1992; Bird & Roberson 1979; Hallock & Mongillo 1998; Weisel et al 1973; Weisel & Dillon 1954). Most males are mature by age

two whereas females are usually mature by age three. However Tyhee Lake and Tacheeda Lake are two exceptions where pygmy whitefish did not mature until later. Both sexes in Tyhee Lake did not mature until age 4 and in Tacheeda Lake, males did not mature until age 3 (McCart 1965).

There is no direct evidence of spawning location or timing, since spawning has not yet been observed; however, indirect evidence indicates that spawning takes place in the lake or stream between August and January, depending on geographic location (Scott & Crossman 1973; Chereshnev & Skopets 1992; Bird & Roberson 1979; Wydoski & Whitney 1979). In Montana studies it was mostly males that were caught in the fall at the mouths of inlets. It was interpreted that males were going to the spawning grounds first. Only indirect evidence for stream spawning exists, as pygmy whitefish congregated near mouths of inlets, but none have been observed spawning (Weisel et al 1973). Heard and Hartman (1965) believe that pygmy whitefish spawn at night, like rocky mountain whitefish, since pygmy whitefish were observed moving into the river about 3 hours after dark 2 nights in a row. Some sampling indicates that pygmy whitefish actively feed during their spawning period (Weisel et al. 1973).

Although pygmy whitefish fecundity is low they produce more eggs per pound of fish than rocky mountain whitefish. This is possible because of the small size of their eggs; 2.4mm to 2.6mm (water hardened) compared to 3.1mm to 4.2mm for rocky mountain whitefish (Weisel et al 1973). In Lake Chester Morse, Washington, female pygmy whitefish produced between 93-597 eggs for fish between 86mm and 150mm in length (Wydoski & Whitney 1979). Alaskan pygmy whitefish fecundity ranged from 103 to 1153 eggs which was higher than the same sized fish in Lake Superior (Heard & Hartman 1965).

Although some basic life history and ecological data exist for pygmy whitefish, there are still many unknown parameters. This chapter describes a survey of my findings on the size at age, maturation timing, depth distribution, cohabiting species, and the reliable identification of pygmy whitefish.

3.3 Materials & Methods

Sampling Area

During the months of June to September of 1996 and 1997 the fish fauna of nine northern British Columbian lakes were sampled (Figure 3.02). Samples from Monkman Lake were supplied by the Ministry of the Environment. There were three lakes in each of three drainages, with a wide range of physical parameters (Table 3.01). The three lakes not discussed in chapter one were sampled for inclusion in the microsatellite analysis discussed in chapter two. These were Jack of Clubs, Owen, and Tacheeda lakes. Two additional lakes were also sampled to check for the presence of pygmy whitefish; Tachick Lake, near Vanderhoof, and Round Lake, very close to Tyhee Lake. Tachick was chosen as the lake was eutrophic, similar to Tyhee Lake. It was thought that perhaps pygmy whitefish would be found there as well. Due to the close geographic proximity and similar eutrophic status of Round Lake to Tyhee Lake it was thought the pygmy whitefish or even "giant" pygmy whitefish could be found in Round Lake. No pygmy whitefish were caught in either lake, despite considerable effort.

Figure 3.02 Map of British Columbia indicating where the nine populations of pygmy whitefish were sampled. The location of the "giant" pygmy whitefish population is indicated by "⊙".

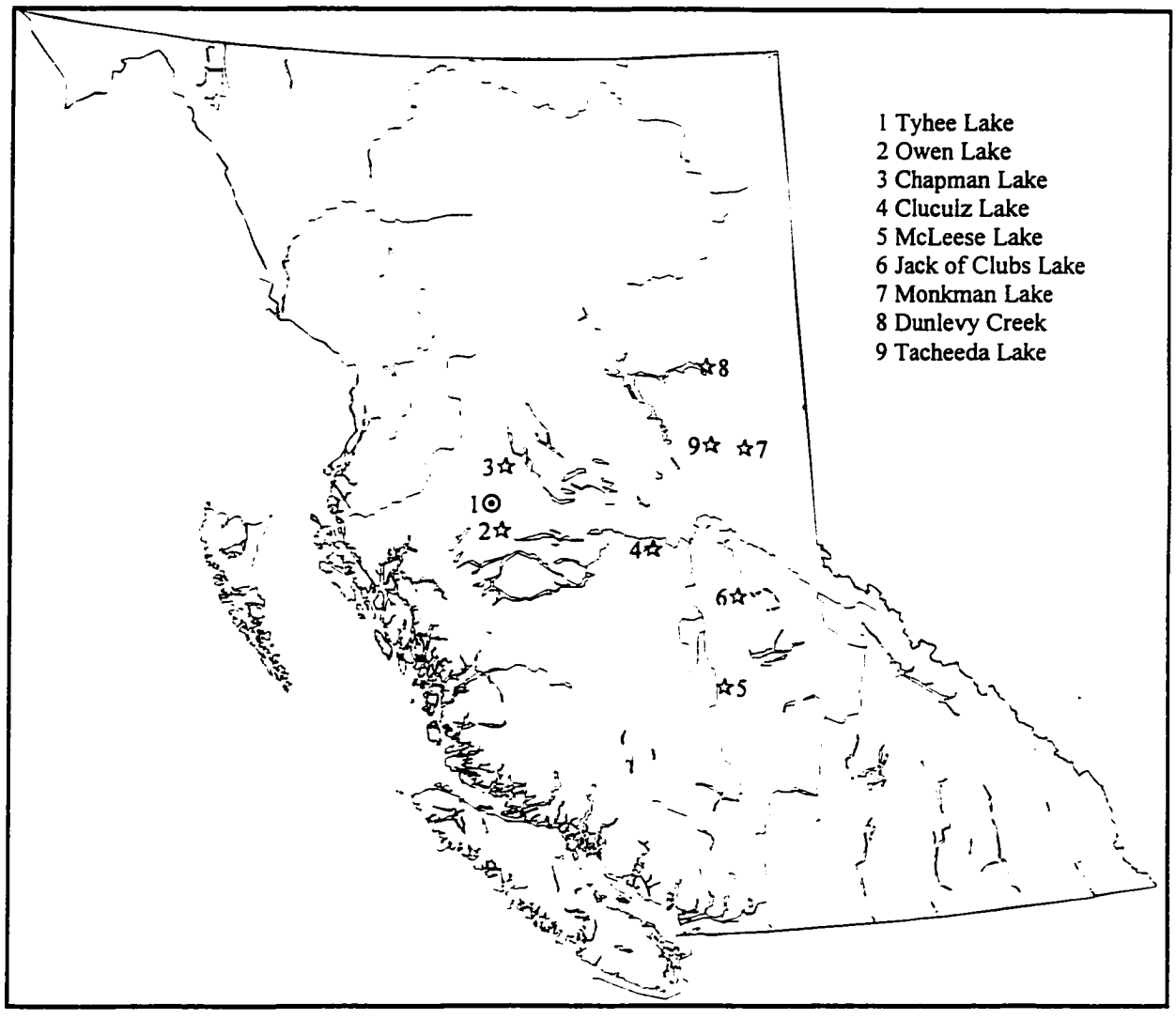


Table 3.01 Physical and biological data for each of the lakes sampled (taken from Ministry of Environment of British Columbia). Pygmy whitefish description refers to category of population reported in the literature. Location was determined using 1:50 000 maps.

Lake	Drainage	Pygmy whitefish description	Location	Elevation (m)	Surface Area (ha)	Mean Depth (m)	Maximum Depth (m)	Perimeter (m)
McLeese	Fraser	Giant	122° 18' E 52° 25' N	732	340	16	46	13,212
Jack of Clubs	Fraser	Normal	121° 36' E 53° 05' N	1232	95.31	19.1	62.5	6,553
Cluculz	Fraser	Normal	123° 35' E 53° 53' N	762	2518	30	61	NA
Monkman	Peace	Normal	121° 12' E 54° 36' N	1060	285	5.7	25	12,900
Williston	Peace	Normal	122° 35' E 56° 09' N	616	NA	NA	NA	NA
Tacheeda	Peace	Normal	122° 30' E 54° 06' N	NA	590.86	17.2	59.4	32,520
Tyhee	Skeena	Giant	127° 02' E 54° 43' N	549	318	11	22	9753
Owen	Skeena	Normal	126° 45' E 54° 05' N	762	297	16	37	163,669
Chapman	Skeena	Normal	126° 41' E 54° 55' N	914	668	13	33	19,311

Sampling Methods

Most lakes were sampled using sinking gill nets only. Two nets were made up of panels of 19mm mesh size, one 15m in length, and the other 30m in length. Two other gill nets used were experimental gang nets which consisted of two panels of 19mm mesh, two panels of 38mm mesh, one panel of 25mm mesh and one panel of 51mm mesh; each panel was 7.5m in length. A wide range of mesh sizes was used so that some indication of species composition could be determined for each lake. The nets were generally set on a steeply sloped bottom so that a wide range of depths could be sampled at one time Gill nets were left overnight or during some of the daylight hours. The number of hours that each net remained in the water was recorded (Tables 3.03-3.09). At Tyhee Lake, a beach seine was also used close to shore in hopes of catching some juvenile "giant" pygmy whitefish.

The capture depth for each fish that was caught was recorded. All live fish were released except pygmy whitefish which were euthanized by a blow to the head. Blood was taken from all pygmy whitefish and the fish and blood samples were stored on ice until transferred to a freezer (-20°C). Species composition and other lake data (see Table 3.01) were obtained from the Ministry of the Environment files in Prince George and Smithers.

Data Collection

Each fish was measured for standard length (tip of the snout to the end of the caudal peduncle). Meristic counts such as number of scales along the lateral line, number of gill rakers on the first gill arch on the left side, and number of pyloric caecae, were made. Each fish was also dissected to determine sex. Scales and otoliths were taken for aging (see chapter one)

Size Distribution

Not all pygmy whitefish could be included in the size at age graph (Figure 1.03; chapter 1). If the age determined using the scale and otolith did not agree, the age data were eliminated, therefore many of the older fish were excluded. Overall, the scale and otolith ages agreed 79.25% of the time. Some populations gave a 100% match (such as the Tyhee Lake pygmy whitefish), while other populations (such as Owen Lake) gave only a 37.25% agreement. To better understand the size distribution and age distribution of pygmy whitefish found in each population, graphs were constructed with numbers of fish versus their age for both scale and otolith aged fish. Graphs were also made for each lake with numbers of fish seen at each standard length.

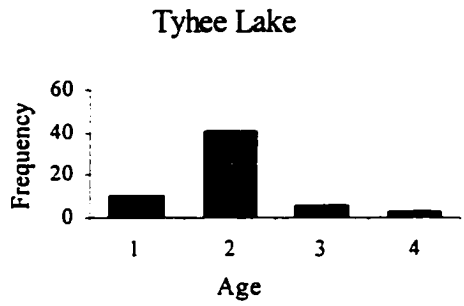
3.4 Results

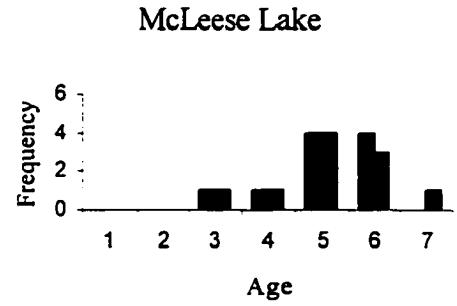
Age Data / Size Data

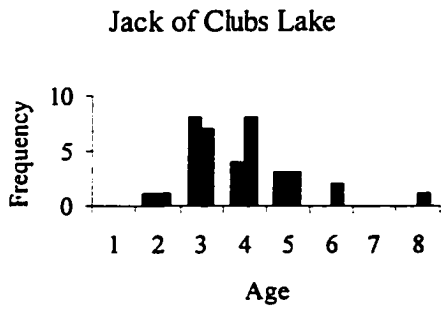
Figure 3.03 shows the number of fish sampled at each age from each population. Generally, the age estimate from the otoliths were older than that determined using scales (Figure 3.03). Most of the older fish caught were females. Otoliths showed more annuli than scales in older fish. Scales become less reliable after the age of 6, before age 6 the scale and otolith usually concur. The oldest pygmy whitefish was 16 years old from Owen Lake, according to the otolith, although it was only 10 years old based on its scales. This fish was therefore the oldest pygmy whitefish documented (Table 3.02).

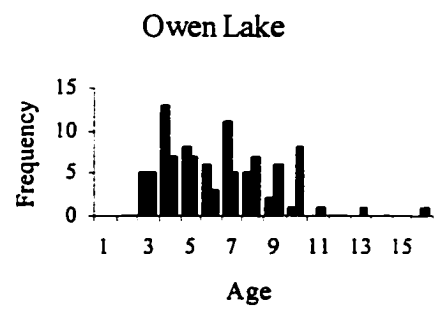
Figure 3.04 shows the number of individuals that were measured in each size class. These graphs show the maximum standard length for each population. Some of these

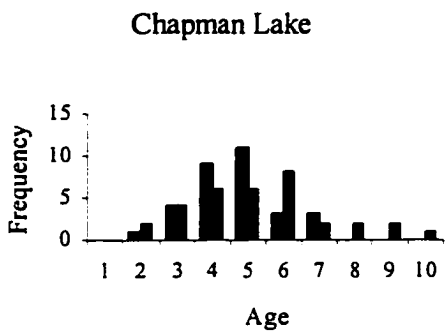
Figure 3.03: The frequency of each age using scales (solid) and otoliths (striped) that appear in 8 of the lakes sampled. Total numbers of fish are given in table 3.02.

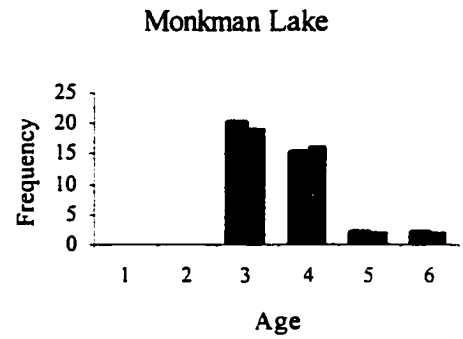


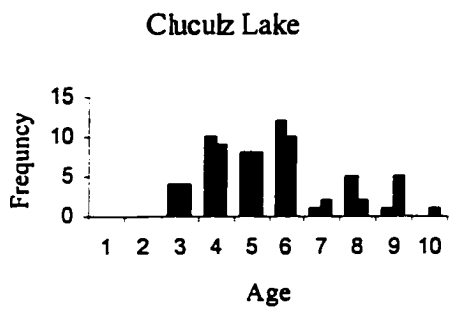












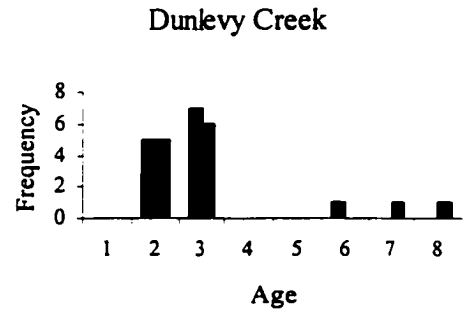
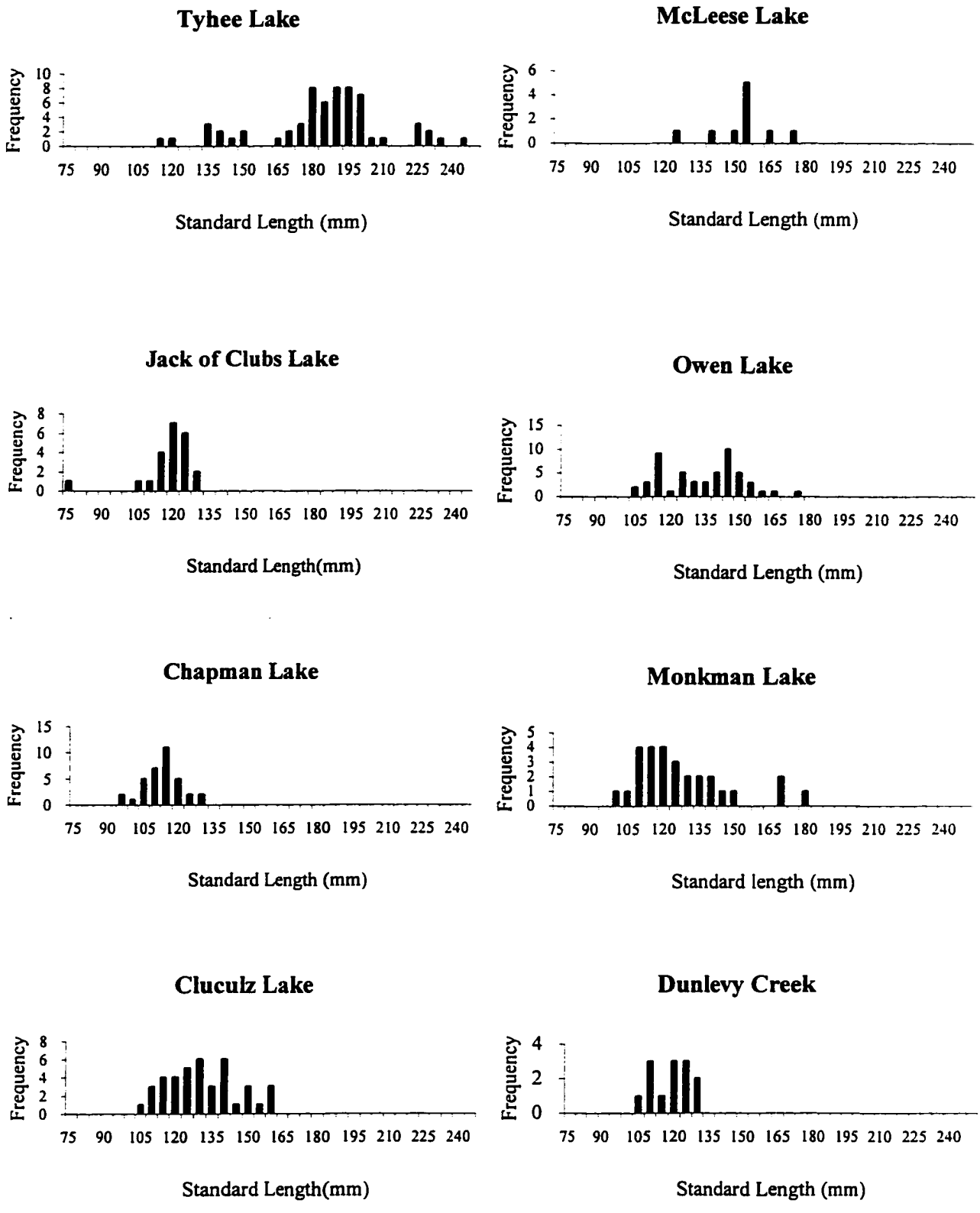


Table 3.02 Maximum age (in years) observed for each population as determined by scale and otolith analysis. Note that maximum age by scale and otolith did not usually agree. The sex of the oldest fish is shown by ♀(female) or ♂ (male)

Lake	Age (scale)	Age (otolith)	Number analyzed
McLeese	6 ♀	7 ♀	10
Jack of Clubs	5 ♀	8 ♀	22
Cluculz	9 ♂	10 ♀	40
Monkman	6 ♀	6 ♀	28
Williston	6 ♂	8 ♂	13
Tyhee	4 ♀	4 ♀	62
Owen	10 ♀	16 ♀	52
Chapman	7 ♀	10 ♀	35

Figure 3.04 Frequency of pygmy whitefish taken from each population of a particular standard length. Individuals were binned into groups of approximately the same length.



fish were not aged or were not included in the size age at graph in chapter one. Thus Figure 3.04 gives a better indication of the lengths that pygmy whitefish can attain in other populations. Clearly, Tyhee Lake has the longest pygmy whitefish. This is particularly impressive as the oldest Tyhee Lake pygmy whitefish is only four years old. Pygmy whitefish from other lakes usually do not reach the size of a one and two year old Tyhee Lake pygmy whitefish until the age of five.

Meristic data

The number of scales found along the lateral line was between 52 and 69 for all populations. The number of pyloric caecae was between 13 and 26 and the number of gill rakers was between 12 and 17 for all populations. These numbers were consistent between "giant" pygmy whitefish and regular pygmy whitefish. The juvenile rocky mountain whitefish caught in Owen Lake had 80-81 scales along the lateral line and 22 gill rakers, thus confirming that they had initially been sampled and misidentified as pygmy whitefish. This misidentification was also confirmed with RAPDs data (see chapter 1).

Sampling/Lake Data

Most of the lakes sampled could be described as oligotrophic while Tyhee and McLeese lakes could be described as eutrophic. The lakes ranged in maximum depth from 22m (Tyhee Lake) to 62.5m (Jack of Clubs Lake) (Table 3.01). Pygmy whitefish were caught at depths between 4.6m to 41m. Pygmy whitefish were caught in Chapman and Tacheeda lakes below 20m. The sampling data for the 8 lakes sampled can be seen in Tables 3.03 to 3.10. The location of nets in which pygmy whitefish were caught can be seen in

Figures 3.05 to 3.12. Pygmy whitefish were always caught at depths below both rocky mountain whitefish and lake whitefish except in Dunlevy Creek. Juvenile rocky mountain whitefish, however, were caught in the same panels as pygmy whitefish in Owen Lake and at Dunlevy Creek, and were difficult to distinguish. All the pygmy whitefish were caught on 19mm mesh size. In general, pygmy whitefish were caught in small schools; pygmy whitefish were rarely caught alone. Pygmy whitefish were the most abundant fish caught in Cluculz, Jack of Clubs, Tacheeda, and Owen lakes. Pygmy whitefish were also numerous in Tyhee and Chapman lakes. The only lake in which pygmy whitefish appeared to be scarce was McLeese Lake. Only one juvenile pygmy whitefish was caught in Jack of Clubs Lake.

Many of the female pygmy whitefish were gravid. Slight pressure released eggs at Dunlevy Creek, Williston Reservoir, which was sampled in late summer (August and September). Most of the pygmy whitefish were caught in net 6 closest to Dunlevy Creek (Figure 3.08). Eggs were also released from pygmy whitefish caught in Chapman Lake, which was also sampled at the end of August. Most of the pygmy whitefish caught in Chapman Lake were also close to a small inlet. At the mouth of the inlet was a large amount of sand and gravel which dropped off quickly to the depths that the pygmy whitefish were caught. The location of the nets 3,7 and 11 that caught the ripe pygmy whitefish in Chapman Lake can be seen in Figure 3.12.

The only salmonid present in all lakes was rainbow trout. In Tyhee Lake rainbow trout were the only other salmonid species, while McLeese Lake had both rainbow trout and kokanee. All lakes except McLeese and Tyhee, had either rocky mountain whitefish or lake whitefish. Rocky mountain whitefish were found in Jack of Clubs, Tacheeda, Owen,

Table 3.03 McLeese Lake sampling results June, July 1996.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
June 11, day set	1	?	19mm/ 15m	?	nothing
	2	?	19mm/ 30m	?	1 Rainbow trout 1 Squawfish 5 Peamouth chub
	3	~ 18.2	19mm/ 15m	?	Nothing
	4	4.6-18.2	19mm/ 30m	2	2 Squawfish
	5	6 -15.2	exp. gang/ 46m	2:15	13 Peamouth chub 10 Redside shiner
	6	3-11	19mm/ 15m	2:30	Nothing
July 22-23, overnight set	7	6-11.6	19mm/ 30m	20	4 Kokanee 2 Squawfish
	8	7.6-15.5	exp. gang/ 46m	19	9 Kokanee 1 Squawfish 1 Rainbow trout
	9	10-19.8	19mm/ 15m	18	2 Pygmy whitefish
	10	4-12.2	exp. gang/ 46m	18:30	6 Kokanee 5 Sucker 29 Squawfish 2 Redside shiner 1 Rainbow trout
July 23, day set	11	6-21.3	19mm/ 30m	7:05	1 Rainbow trout
	12	12.2-21.3	exp. gang/ 46m	9:20	Nothing
	13	9.1-19.8	19mm/ 15m	9:35	Nothing
July 23-24, overnight set	14	6-21.3	19mm/ 30m	13:50	1 Pygmy whitefish 1 Rainbow trout 1 Kokanee
	15	6-18.2	exp. gang/ 46m	12:20	6 Pygmy whitefish
	16	6-21.3	19mm/ 15m	12:30	Nothing
July 24, day set	17	12.2-21.3	19mm/ 30m	8:45	1 Pygmy whitefish
	18	10.6-19.8	exp. gang/ 46m	9:15	Nothing
	19	10.6-19.8	19mm/ 15m	10:25	Nothing

Figure 3.05 McLeese Lake, with net sets indicated by a •—• . This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.03.

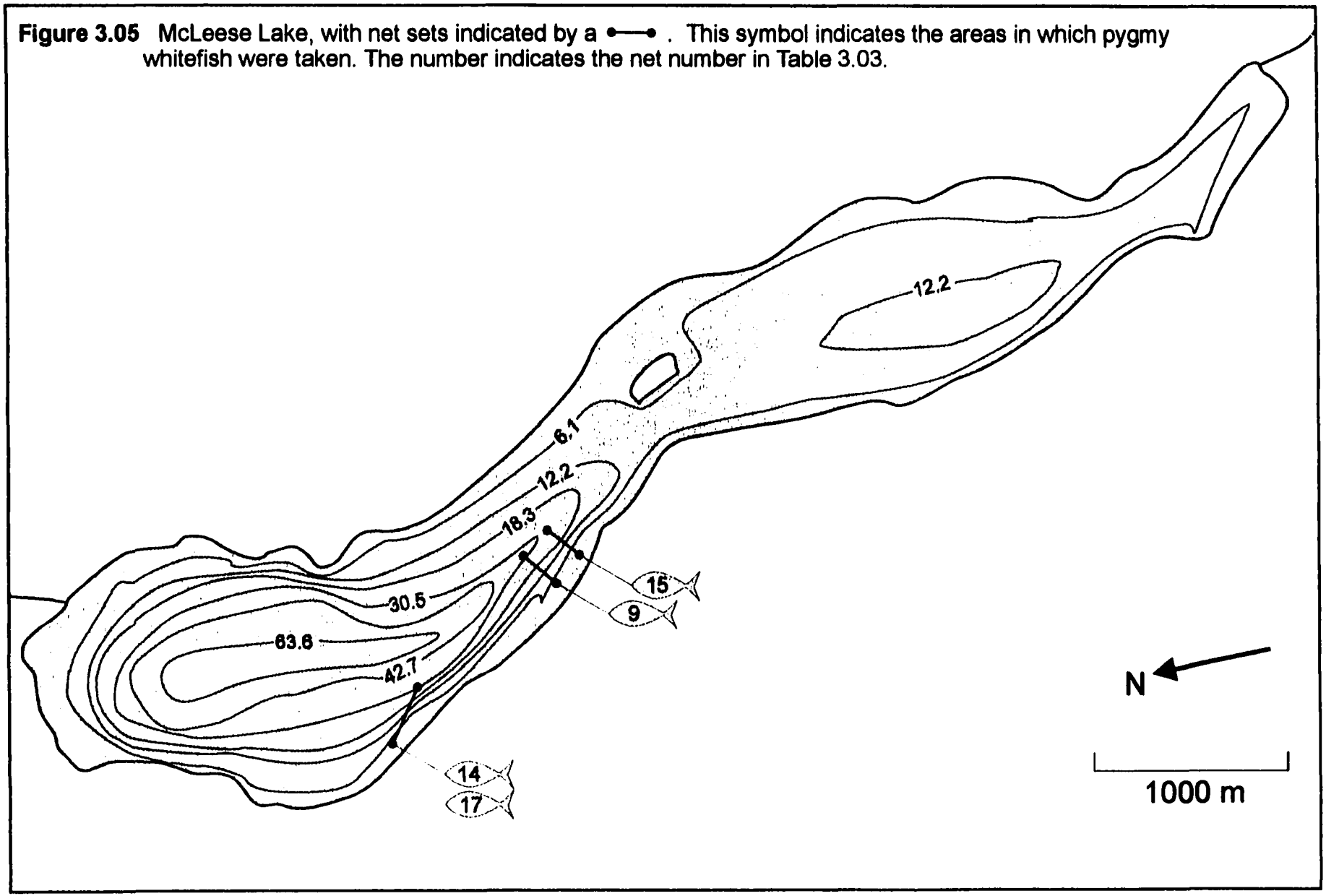


Table 3.04 Jack of Clubs Lake Sampling Results July, 1996.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
July 8, day set	1	7.3-10.9	19mm/ 30m	7:00	1 Bull trout
	2	19.8-38	exp. gang/ 46m	5:40	2 Bull trout 1 Pygmy whitefish (at 24m)
	3	3.7-18.2	19mm/ 15m	5:00	nothing caught
July 9-10, overnight set	4	5.5-31.9	exp. gang/ 46m	19:25	5 Lake trout 2 Pygmy whitefish
	5	6-18.2	19mm/ 15m	19:25	1 Lake trout 1 Pygmy whitefish
	6	15.2-28.9	19mm/ 30m	19:50	nothing caught
July 15-16 overnight set	7	?	19mm/ 30m	22:20	1 Lake trout
	8	?	19mm/ 15m	21:45	10 Pygmy whitefish
	9	?	exp. gang/ 46m	20:50	1 Lake trout 1 Pygmy whitefish
	10	?	exp. gang/ 46m	19:20	5 Lake trout 1 Pygmy whitefish (at 4.6m)
July 16, day set	11	?	19mm/ 15m	4:45	1 Lake trout 1 Rocky mountain whitefish
	12	?	19mm/ 30m	4:50	nothing caught
July 16-17, overnight set	13	?	19mm/ 15m	16:50	1 Bull trout 1 Lake trout 3 Pygmy whitefish
	14	?	19mm/ 30m	16:05	1 Pygmy whitefish 1 Rainbow trout 2 Rocky mountain whitefish 3 Lake trout
	15	?	exp. gang/ 46m	15:15	4 Lake trout

Figure 3.06 Jack of Clubs Lake, with net sets indicated by a ⊶ This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.04.

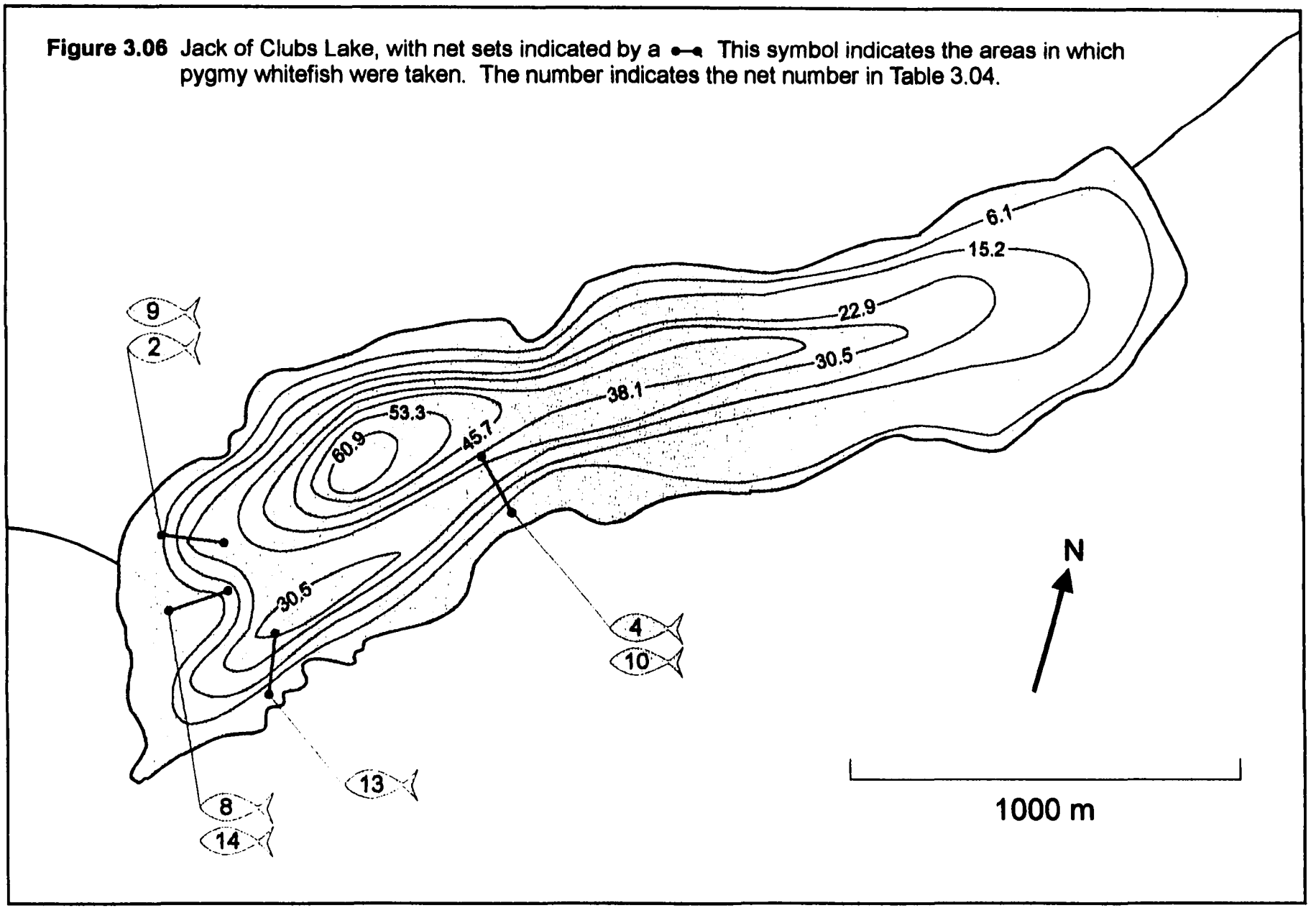


Table 3.05 Cluculz Lake Sampling Results July 26, 1996.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
July 26, day set	1	10.6-21.3	19mm/ 30m	6:45	1 Pygmy whitefish 9 Lake trout
	2	10.6-39.5	exp. gang/ 46m	5:40	3 Pygmy whitefish 3 Lake trout 1 Lake whitefish
	3	6-27.4	19mm/ 15m	5:20	9 Pygmy whitefish
	4	9.1-41	exp. gang/ 46m	6:15	5 Pygmy whitefish 5 Lake trout 2 Lake whitefish

Figure 3.07 Cluculz Lake, with net sets indicated by a •—•. This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.05.

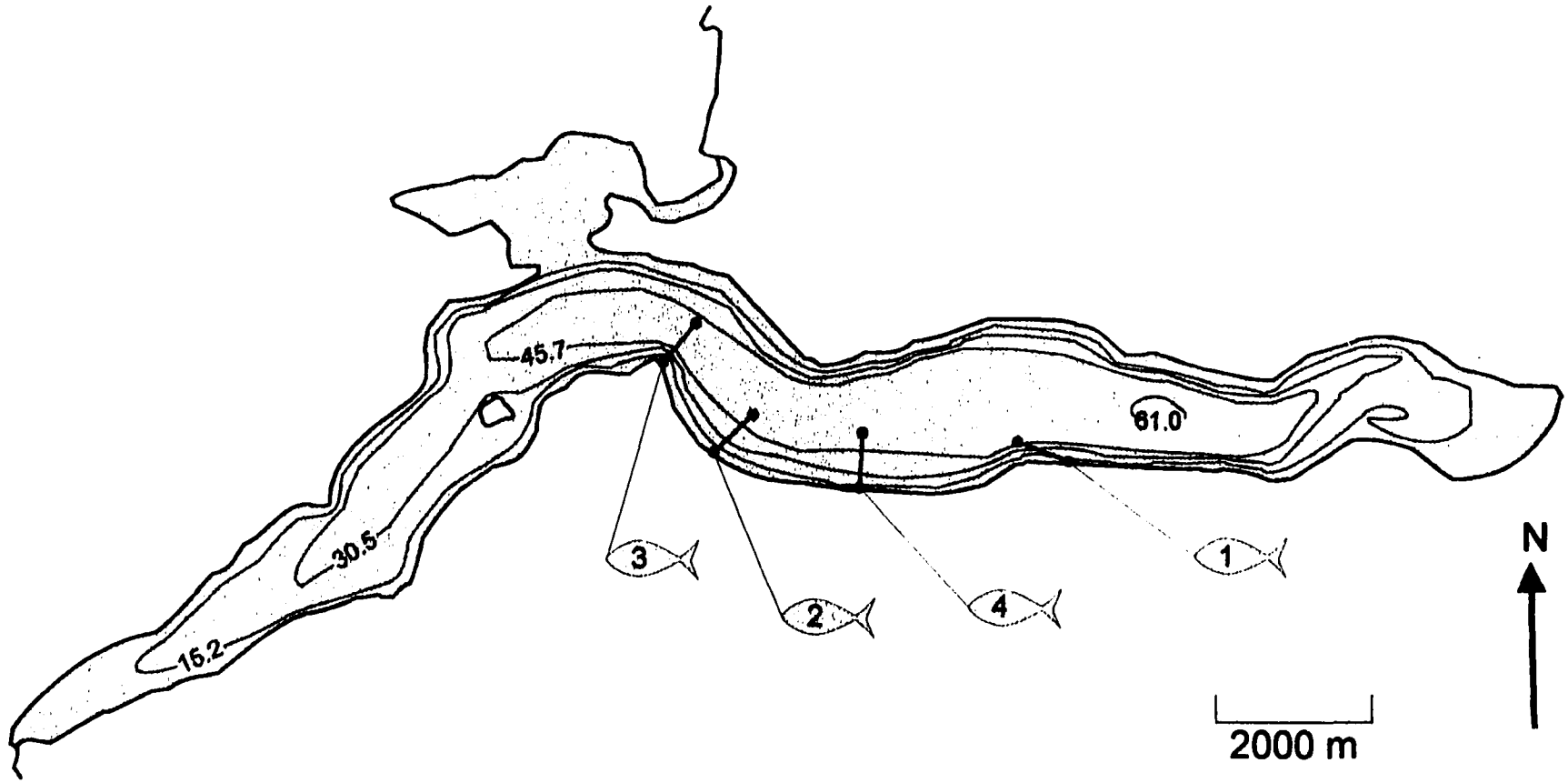


Table 3.06 Sampling Results Dunlevy Creek, Williston Lake Sept. 21, 22, 1996 and August 6-7, 1997.

	Net	Depth(m)	Mesh/ Length	#of hours Hours:Minutes	Species and # captured
Sept 21 day set 1996	1	6-10.6	19mm/ 15m	7:55	5 Pygmy whitefish 8 Rocky mountain whitefish* 2 Bull trout 12 Peamouth chub
	2	4.6-17.6-6	exp. gang/ 46m	8:10	1 Pygmy whitefish 1 Rocky mountain whitefish 2 Bull trout
	3	6.-25.9	19mm/ 30m	7:35	126 Peamouth chub 4 Bull trout 3 Rocky mountain whitefish 5 Suckers
Sept. 21-22 Overnight set 1996	4	9.1	19mm/ 15m	15:10	7 Pygmy whitefish 3 Bull trout 2 Kokanee
	5	6-15.2	19mm/ 30m	15:40	31 Peamouth chub 8 Bull trout 3 Suckers 12 Rocky mountain whitefish 3 Squawfish
August 6-7, 1997 overnight set	6	12.2-13.1	19mm/ 15m	15:20	33 Pygmy whitefish 1 Squawfish
	7	8.8-18.5	19mm/ 30m	15:45	3 Rainbow trout 2 Burbot 3 Rocky mountain whitefish
	8	7.6-16.1-4.6	exp. gang/ 46m	16:00	36 Pygmy whitefish 1 Rainbow trout 10 Rocky mountain whitefish 7 Suckers 3 Burbot

* Captured in the same panel as the pygmy whitefish

Figure 3.08 Dunlevy Creek with net sets indicated by a —• . This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.06.

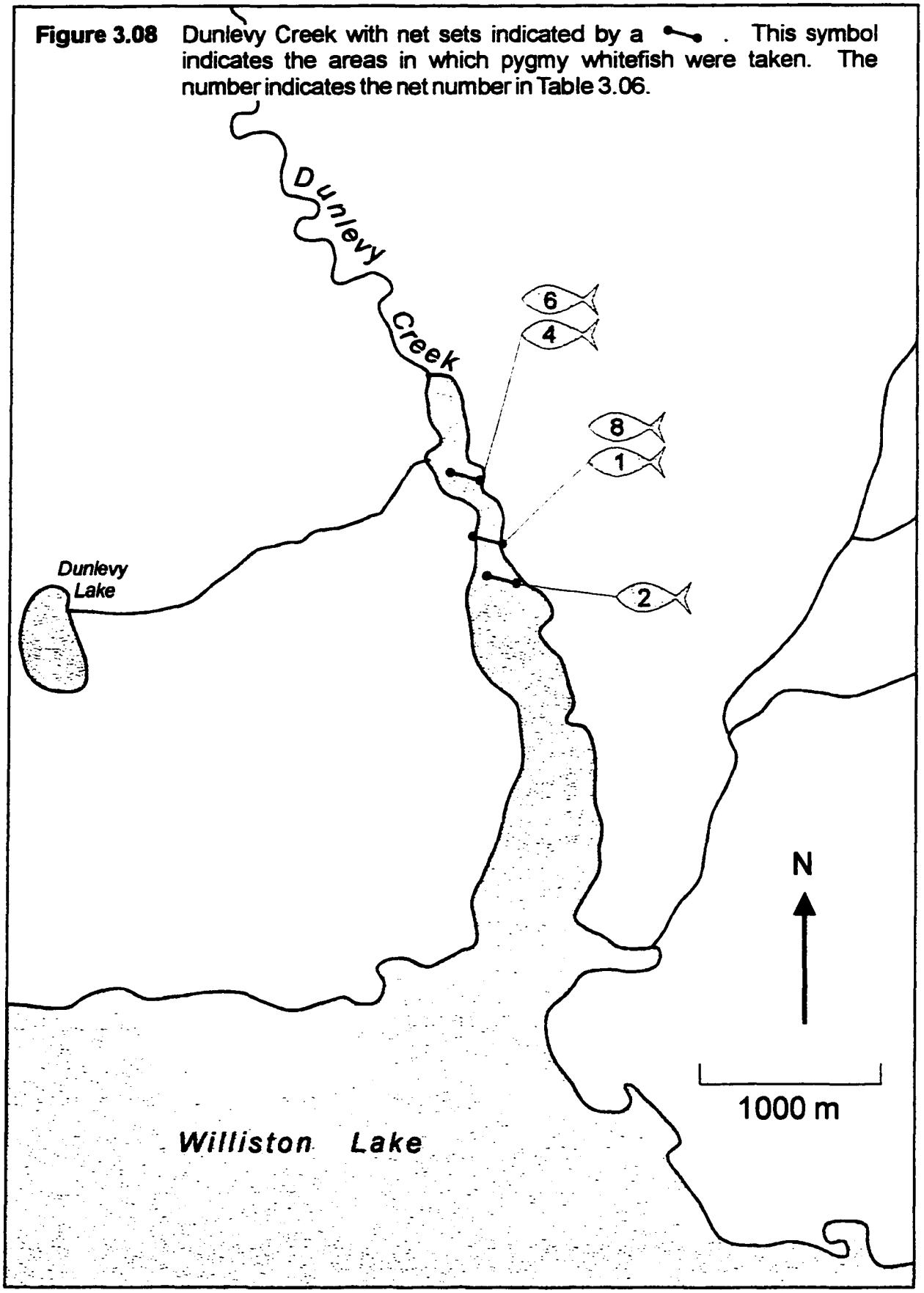


Table 3.07 Sampling of Tacheeda Lake August 11, 12, 13 & 14, 1997.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
August 11-12, overnight set	1	6-16.1	19mm/ 30m	13:10	1 Rocky mountain whitefish 3 Lake whitefish 1 Lake trout
	2	3.7-28	exp. gang/ 46m	14:40	6 Pygmy whitefish 12 Lake whitefish 2 Lake trout 6 Kokanee 22 Rocky mountain whitefish 1 Squawfish 3 Rainbow trout 2 Sucker
	3	7.6-16.4	19mm/ 15m	14:45	no fish
Aug.12, day set	4	23.7-33.4	19mm/ 15m	5:40	no fish
August 12-13, overnight set	5	20.7-30.4	19mm/ 15m	16:35	1 Pygmy whitefish
	6	12.2-28.9	exp. gang/ 46m	15:15	3 Pygmy whitefish 1 Lake trout
	7	15.2-36.5	19mm/ 30m	15:25	2 Pygmy whitefish
August 13, day set	8	19.8-33.4	19mm/ 15m	6:55	1 Pygmy whitefish
	9	19.8-35.6	19mm/ 15m	7:05	no fish
	10	27.4-29.5	exp. gang/ 46m	7:17	4 Pygmy whitefish
August 13-14, overnight set	11	21.3-33.4	19mm/ 15m	16:15	2 Pygmy whitefish
	12	20.4-33.4	19mm/ 15m	16:30	2 Pygmy whitefish 1 Lake whitefish
	13	28.6-29.2	exp. gang/ 46m	16:45	6 Pygmy whitefish 2 Lake whitefish

Figure 3.09 Tacheeda Lake, with the net sets indicated by a •—•. This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.07.

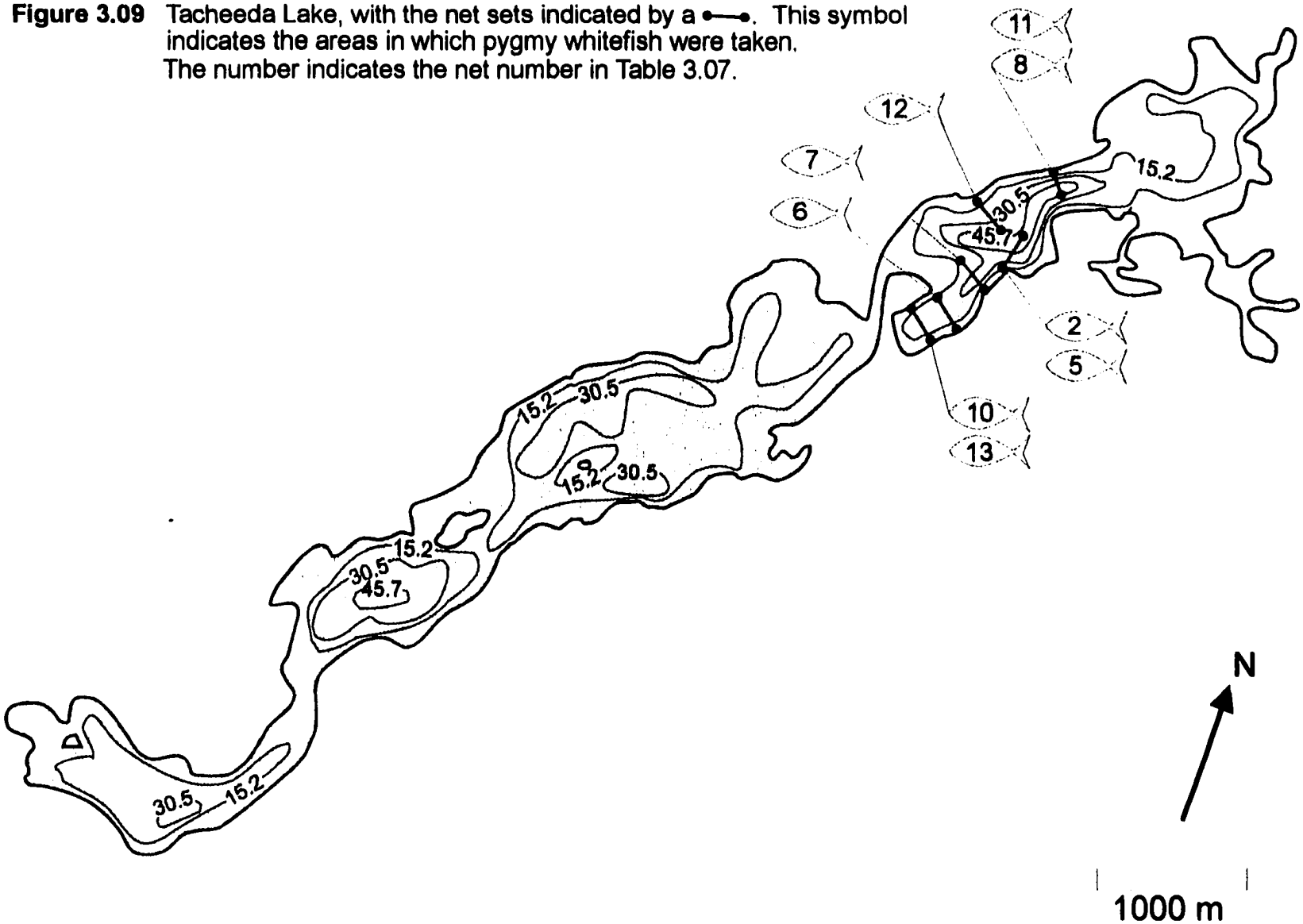


Table 3.08 Tyhee Lake Sampling Results: Aug. 9,10,11, 1996.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
August 9-10, overnight set	1	7.9-16.7	exp. gang/ 46m	16:45	4 Rainbow trout 12 Squawfish 6 Sucker 1 Pygmy whitefish (at 7.3m) 9 Peamouth chub
	2	19.5-21.3	19mm/ 15m	17:55	Nothing caught
	3	7.6-14.3	exp. gang/ 46m	17:45	45 Pygmy whitefish 17 Peamouth chub 9 Sucker 8 Squawfish
	4	13.6-15.2	19mm/ 30m	18:35	Nothing caught
August 10, day set	5	16.7-21.3	exp. gang/ 46m	1:45	Nothing caught
August 11, day set	6	shallow	19mm/ 15m	2:45	combined with 7
	7	shallow	19mm/ 30m	3:30	28 Squawfish 65 Peamouth chub 2 Suckers 7 unknown minnow
August 11	8	Beach Seine		Three attempts	4 Squawfish 8 Sculpin 3 Unknown minnow (silver with dark stripe)

Figure 3.10 Tyhee Lake, with the net sets indicated by a •—•. This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in Table 3.08.

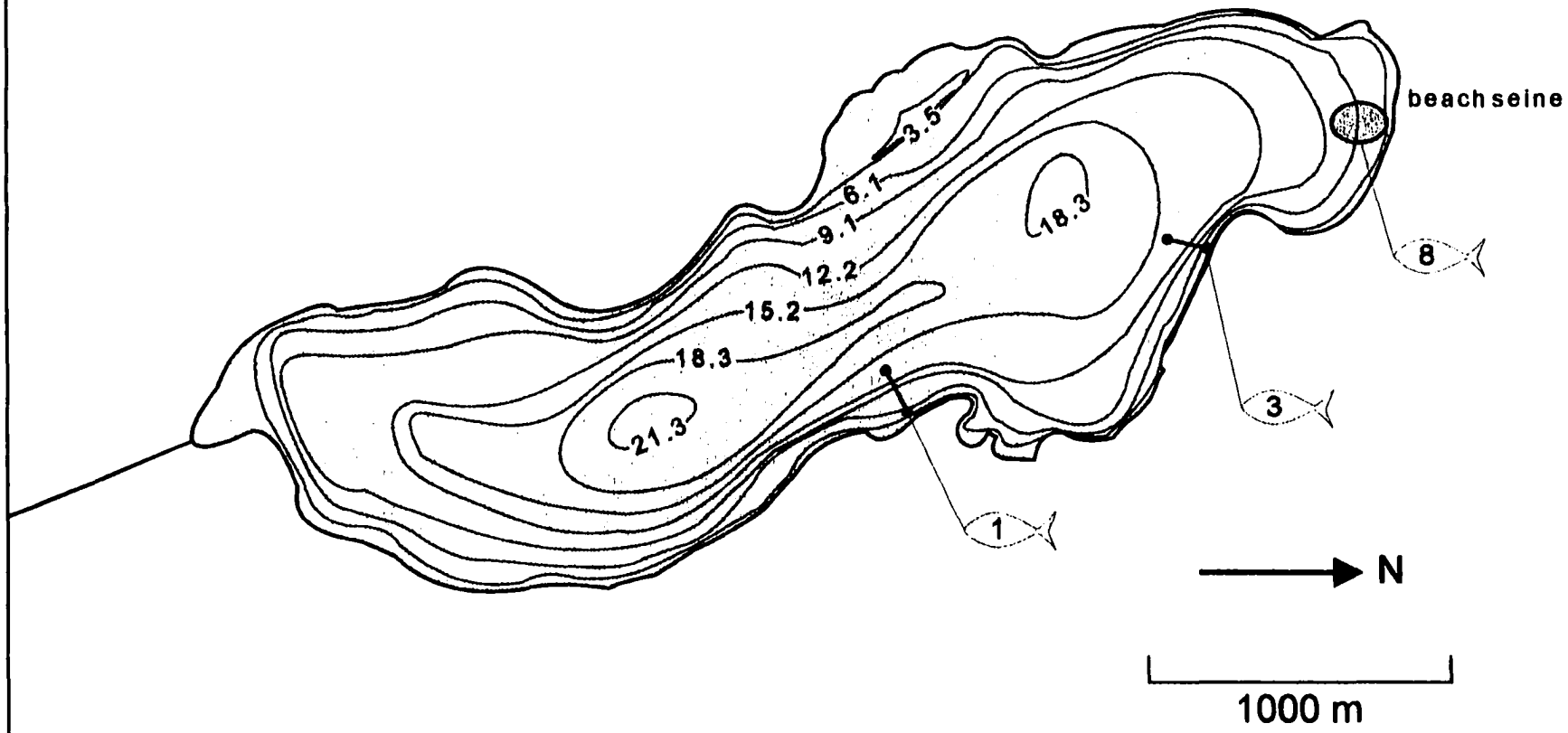


Table 3.09 Owen Lake Sampling Results Aug. 3,4 & 5, 1996.

	Net	Depth(m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
August 3, day set	1	7.9-23.7	19mm/ 15m	7:30	nothing caught
	2	7.6-27.4	exp. gang/ 46m	7:15	1 Pygmy whitefish
	3	7.6-22.8	19mm/ 30m	7:15	5 Lake trout 1 Rainbow trout 5 Pygmy whitefish
	4	8.2-24	exp. gang/ 46m	7:40	1 Lake trout 1 Pygmy whitefish
August 3-4, overnight set	5	9.1-16.7	19mm/ 15m	15:00	5 Pygmy whitefish
	6	12.2-28.3	exp. gang/ 46m	15:30	nothing caught
	7	9.1-23.7	19mm/ 30m	15:30	3 Pygmy whitefish 3 Lake trout
	8	9.1-24.9	exp. gang/ 46m	15:20	nothing caught
August 4, day set	9	11.3-16.1	19mm/ 15m	9:30	4 Pygmy whitefish
	10	10.6-21.3	exp. gang/ 46m	9:15	3 Pygmy whitefish
	11	9.7-21.3	19mm/ 30m	9:20	3 Pygmy whitefish 1 Lake trout 1 Sucker
	12	9.1-19.8	exp. gang/ 46m	9:10	12 Lake trout 20 Pygmy whitefish 4 Rainbow trout
August 4-5, overnight set	13	9.7-19.2	19mm/ 15m	12:55	7 Pygmy whitefish
	14	10.6-20.4	exp. gang/ 46m	12:55	5 Pygmy whitefish 1 Sucker
	15	10.6-21.3	19mm/ 30m	12:45	2 Pygmy whitefish 3 Rocky mountain whitefish 1 Sucker 6 Lake trout

Figure 3.11 Owen Lake, with net sets indicated by a •—• . This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in table 3.09.

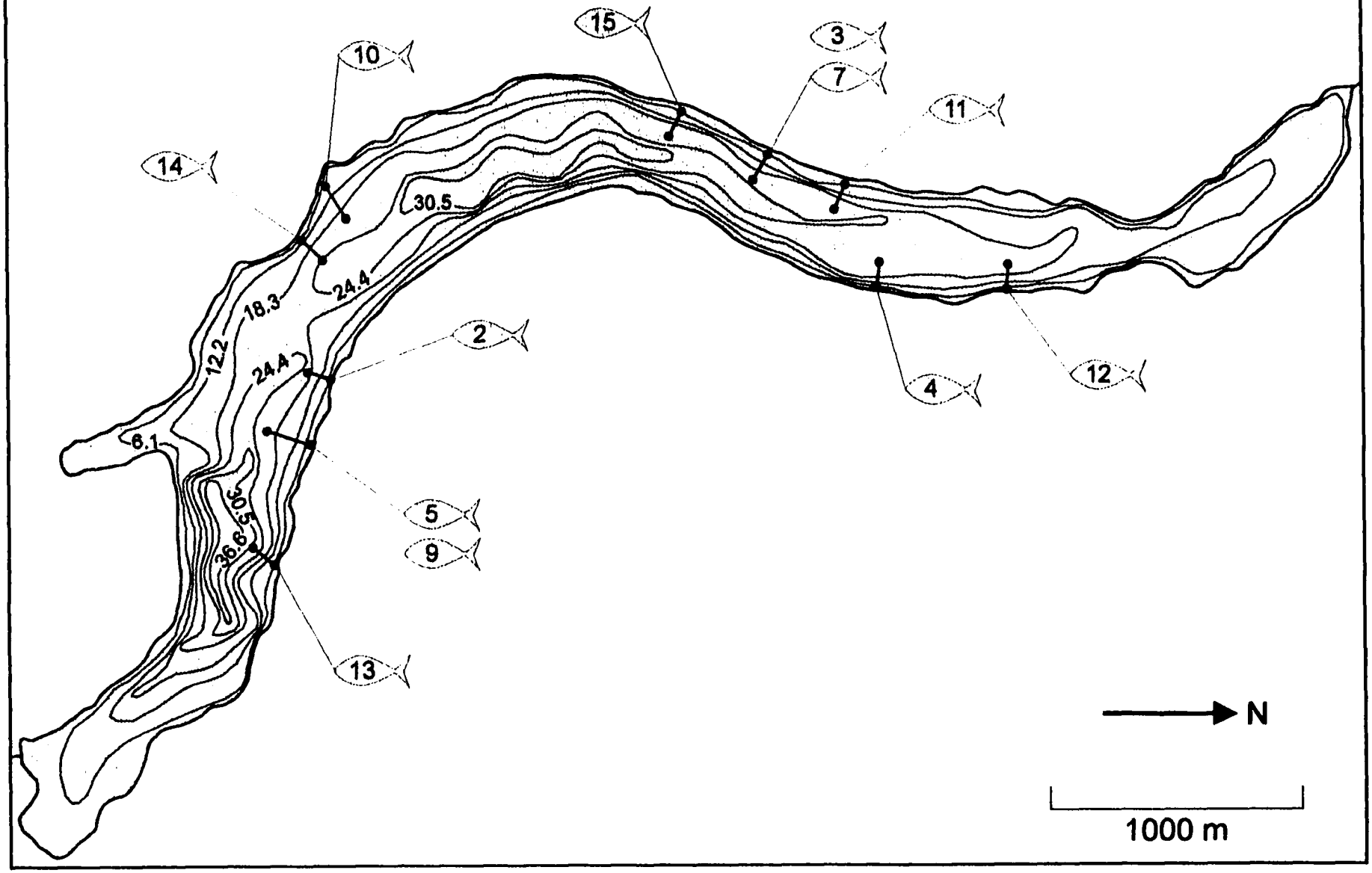
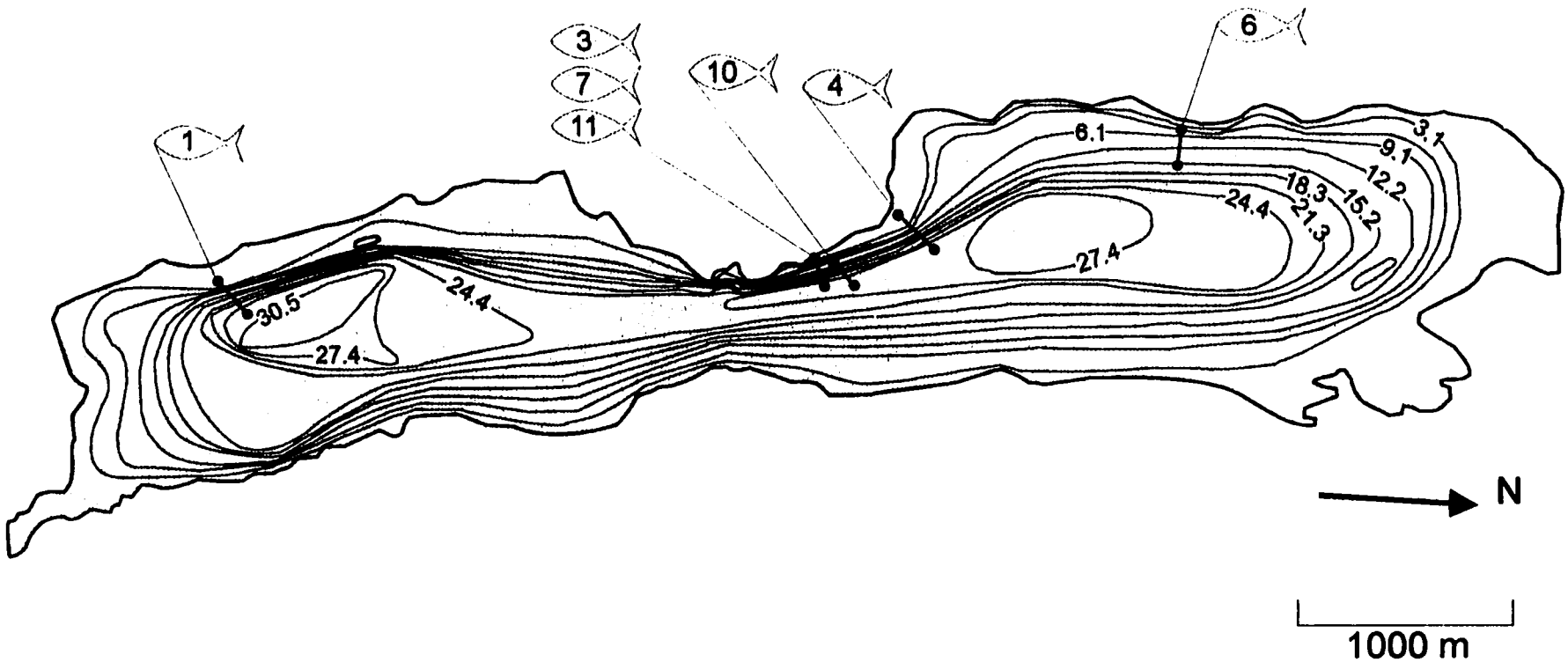


Table 3.10 Sampling results from Chapman Lake August. 27,28,29,30, 1996.

	Net	Depth (m)	Mesh/ Length	# of hours Hours:Minutes	Species and # captured
August 27-28, Overnight set	1	6-27.4	exp. gang/ 46m	16:45	4 Suckers 2 Burbot 7 Lake trout 2 Rocky mountain whitefish 2 Pygmy whitefish 11 Peamouth chub
	2	9.1-21.3	19mm/ 30m	17:30	27 Peamouth 5 Lake trout 17 Rocky Mountain Whitefish 5 Suckers
	3	7.6-19.5	19mm/ 15m	18:25	7 Peamouth chub 6 Pygmy whitefish 1 Rainbow trout 2 Unknown minnow (same as in Tyhee) 1 Sculpin 2 Rocky mountain whitefish
	4	6-25.8	exp. gang/ 46m	18:45	1 Pygmy whitefish 1 Sucker 2 Peamouth chub 6 Rocky mountain whitefish 4 Lake trout
August 28, day set	5	13.4-16.7	exp. gang/ 46m	5:10	2 Pygmy whitefish 25 Peamouth chub 20 Rocky mountain whitefish 6 Suckers 2 Lake trout
	6	9.1-16.7	exp. gang/ 46m	5:45	56 Rocky mountain whitefish 75 Peamouth chub 3 Suckers 4 Lake trout
August 28-29, overnight set	7	10.6-21.3	19mm/ 15m	20:50	6 Pygmy whitefish 1 Lake trout 3 Peamouth chub
	8	10.6-25.5	19mm/ 30m	19:25	1 Peamouth chub 1 Sucker 2 Lake trout
August 29, day set	9	10.6-19.8	19mm/ 15m	7:50	6 Peamouth chub 1 Rocky mountain whitefish
	10	9.1-18.2	19mm/ 30m	7:45	7 Pygmy whitefish
August 29-30, overnight set	11	15.2-24.3	19mm/ 15m	14:45	1 Peamouth chub 15 Pygmy whitefish -caught @ ~24m
	12	15.2-30.4	19mm/ 30m	15:05	2 Lake trout

Figure 3.12 Chapman Lake, with the net sets indicated by a ⟷ This symbol indicates the areas in which pygmy whitefish were taken. The number indicates the net number in table 3.10.



Chapman lakes and in Dunlevy Creek. Only Tacheeda and Cluculz lakes had lake whitefish. The fish caught at the same depth or deeper than pygmy whitefish were lake trout, bull trout and burbot. Salmonids found at shallower depths than pygmy whitefish were rocky mountain whitefish, lake whitefish, kokanee and occasionally rainbow trout.

In Jack of Clubs Lake some of the pygmy whitefish were partially eaten in the gill net. Both bull trout and lake trout were caught at similar depths to the pygmy whitefish so it was assumed that the pygmy whitefish had been preyed on by either of these two species. When retrieving nets from Dunlevy Creek bull trout were actively feeding on the pygmy whitefish. A lake trout caught in Chapman Lake was in the process of swallowing a fish close in size to itself. Although not a pygmy whitefish, it was assumed that lake trout would also eat pygmy whitefish as they are found at the same depths.

Sex Ratio

Table 3.11 shows the sex ratio of fish for each lake. There were more female fish caught than male fish in all of the lakes sampled, with the exception of Dunlevy Creek. When this sex ratio is broken down by age it can be seen that males only rarely outnumber females and only at or below age 5 (Table 3.11)

3.5 Discussion

Although many of the other populations of pygmy whitefish had fish larger than the 120mm given as their average adult size, this is a result of their greater age. Thus pygmy whitefish can grow to larger sizes if given enough time. It is the unusual growth in the first few years of life in the Tyhee population and their overall larger size that sets them apart as "giant" pygmy whitefish.

Table 3.11 Sex ratio of males to females caught in each lake, by age of the otolith. The numbers in the first row are age in years.

	>1	2	3	4	5	6	7	8	9	10	11	13	16	Total males	Total females
Monkman	1:2	-	4:15	2:13	1:1	0:1	-	-	-	-	-	-	-	9	33
Dunlevy	-	4:1	1:4	-	-	-	1:0	1:0	-	-	-	-	-	7	5
Chapman	-	0:2	2:2	0:6	0:5	0:8	0:3	0:2	0:2	0:1	-	-	-	3	32
Cluculz	-	-	1:3	0:8	4:4	2:7	0:2	1:1	1:4	0:1	-	-	-	9	31
Jack of Clubs	-	0:1	0:7	0:8	0:3	0:2	-	0:1	-	-	-	-	-	0	23
McLeese	-	-	1:0	0:1	1:3	0:3	0:1	-	-	-	-	-	-	2	8
Owen	-	-	0:3	2:4	4:3	1:2	1:4	2:4	0:6	1:7	0:1	0:1	0:1	11	37
Tyhee	6:4	17:24	3:5	0:3	-	-	-	-	-	-	-	-	-	26	37

The ecological and life history data collected generally agrees with data described in the literature. Although some of the literature indicates that pygmy whitefish are not solely a deep water species most of my data show them to be found to be at or below 20m in BC. The exceptions to this were McLeese and Tyhee Lake pygmy whitefish populations and the Dunlevy Creek population. Dunlevy Creek is a small inlet on Williston Reservoir, and the reservoir's water level changes quite dramatically over the course of a year, at least 5m difference between sampling years. The location where the pygmy whitefish were found would have a changing depth profile, thus a less stable environment than the deeper depths. The deeper locations had more vegetation than the depths which remain dry and rocky for periods of time throughout the year. This was the only example of a pygmy whitefish population found at more shallow depths than rocky mountain whitefish. The anomaly may be explained by the unusual nature of the water body, which is a reservoir and not a true lake.

The large size and fast growth of the "giant" pygmy whitefish in Tyhee Lake may be due to a combination of several factors. One explanation may be that environmental factors are driving this population to have such a different life history. Whitefish are known to be a very phenotypically plastic group, thus environmental factors are a reasonable explanation for the "giant" pygmy whitefishes' large size and faster growth. Although it was concluded in Chapter 1 that the Tyhee pygmy whitefish are not genetically differentiated from the other pygmy whitefish populations studied, it is important to note that there may still be a genetic component to their fast growth. A phylogenetic study would not detect a few genes that might contribute to faster growth; rather a quantitative genetic study would be necessary.

Therefore a combination of environmental and genetic factors may be contributing to the unusual growth of the "giant" pygmy whitefish.

Species of dwarf or giant fish often exist where unusual conditions in the lake persist over time. There may be one or more environmental variables in Tyhee Lake that contributes to the rapid growth of the "giant" pygmy whitefish, and the fairly large size of McLeese Lake pygmy whitefish. These variables, including temperature (Pettit & Wallace 1975), TDS (total dissolved solids) (McCart 1963), diet (Dawidowicz & Gliwicz 1983) and species composition (Donald & Alger 1986), have all been documented as being correlated with the occurrence of dwarf or giant forms of various fish species. It may be these variables that contribute to the Tyhee Lake pygmy whitefish being "giants".

A unique feature of Tyhee and McLeese lakes is the description of the lakes as eutrophic (ie more productive lakes). The lakes are also small and are therefore likely to have warmer temperatures, which adds to the productivity (and TDS) of the lake. It is thought that pygmy whitefish survived in the deep lakes after the glaciers retreated. Small size and early maturation are thought to be adaptations developed for survival in cold and nutrient-poor water during glaciation (Weisel et al. 1973). It is interesting to note that in the warmer more productive Tyhee Lake the "giant" pygmy whitefish grow to a larger size and mature later. The warmer more productive Tyhee Lake would presumably have more food available for the pygmy whitefish.

The size of pygmy whitefish in each population could also be affected by their diet. It has been discussed that their diet varies with prey/food availability. The slower growing Brooks Lake population in Alaska is a plankton feeder and the faster growing south Bay-Iliuk arm population feeds on insects (Heard & Hartman 1965). These diet differences are

probably related to growth, but again there are many other ecological factors to take into account (Heard & Hartman 1965). Heath & Roff (1996) thought stunting in yellow perch (*Perca flavescens*) may be due a trophic bottleneck which is a low occurrence of certain types of food, necessary for growth. Size is not only affected by the type of food available, but also by how much is available. In a more productive lake, there would be a higher availability of food items in comparison to a colder oligotrophic lake. Therefore the Tyhee and McLeese lakes may have a more abundant food supply for pygmy whitefish than the other lakes. However, the Tyhee Lake pygmy whitefish are "giant" pygmy whitefish while those in McLeese are large but not "giant" pygmy whitefish. Therefore there must be other factors in addition to diet that contribute to the large size of Tyhee Lake pygmy whitefish.

McCart (1965) proposed the lack of other whitefish as a contributing factor to the large size of the pygmy whitefish in McLeese and Tyhee lakes. Other salmonids besides whitefish may also affect the growth, either by competing for food and space or by preying on the pygmy whitefish. Rocky mountain whitefish, lake whitefish and kokanee (found at shallower depths) may be keeping the pygmy whitefish at deeper depths. There is less light and colder temperatures at deeper depths, and therefore it is a less productive environment. The pygmy whitefish caught in Dunlevy Creek were at shallower depths than the rocky mountain whitefish, however, the same argument of less productive waters may also apply as some of the areas they are found in can be exposed for months, and the plant fauna can not survive. The difference in salmonid species composition between McLeese and Tyhee lakes is the presence of kokanee in McLeese Lake. Perhaps the kokanee compete with the pygmy whitefish for space and food, affecting the pygmy whitefish growth. It is speculated that when fish populations are exposed to different predatory pressures they respond with

evolutionary shifts in age and size at maturity (Abrams & Rowe 1996). Tyhee and McLeese lakes do not have bull trout or lake trout, both known to prey on pygmy whitefish. Both lakes, however, do have rainbow trout which can prey on smaller fish. Known piscivorous rainbow trout broods have been introduced to Tyhee Lake. Perhaps the "giant" pygmy whitefish in Tyhee Lake exceed a size threshold that allows them to escape predatory pressures, thus reducing the potential impact of the introduced predators.

It may be that the variation in growth is a result of the phenotypic plastic nature of whitefish in general, responding to varied ecological factors (Lindsey & Woods 1970). Plasticity in pygmy whitefish is highlighted by their ability to utilize many different environments within the lake and by their varied diet (Heard & Hartman 1965).

To better understand which of the ecological factors may have an impact on size it is best to compare to another population of large pygmy whitefish. Chester Morse Lake in Washington state appears to also have a population which could be considered "giant" pygmy whitefish according the criteria used to distinguish the Tyhee Lake population as "giant" pygmy whitefish, namely their larger size at all age classes as well as an unusual size at age curve (R2 Resource Consultants Inc. 1995). Chester Morse Lake is also a fairly small lake (681 ha, max depth 35m), and would be expected to be a productive lake as well, however biologists in the region do not consider it an eutrophic lake (B. Pfeifer, pers. comm). Although not eutrophic, there may still more food available to the pygmy whitefish as they share the lake with only two other fish species. If a small eutrophic lake is important for producing "giant" pygmy whitefish, then Bull Lake, Montana, should have larger pygmy whitefish, but they are within the normal range (under 150mm in length; Wydoski &

Whitney 1979). Bull Lake, however, does have sympatric rocky mountain whitefish, unlike Tyhee, McLeese, and Chester Morse lakes.

Since pygmy whitefish are too small to be a sport fish and are too rare for any other use, there is no economic value associated with these fish. The pygmy whitefish populations in McLeese and Tyhee are considered a threatened species due to the low number of populations documented as "giant" pygmy whitefish, however, other pygmy whitefish populations are not considered threatened in British Columbia. In Washington State, pygmy whitefish were historically known to occur in 15 lakes, they now inhabit only nine lakes and thus it was recommended that pygmy whitefish be listed as a sensitive species. The loss of the six populations of pygmy whitefish is thought to be due to intentional application of piscicides, the introduction of exotic piscivorous species, and decline in water quality. The lakes in which populations were lost were the smaller, shallower lakes (Hallock & Mongillo 1998).

In BC, the Ministry of the Environment, Lands and Parks, had thought the population of pygmy whitefish in McLeese Lake was extinct. McLeese Lake is also undergoing eutrophication, and has had species introduced for sport fishing. I found ten pygmy whitefish in McLeese Lake so the population is not extinct, but is clearly not abundant. The loss of pygmy whitefish in the other lakes studied is unlikely, as the pygmy whitefish were usually abundant and the lakes were generally oligotrophic. The abundance reported in sampling results of pygmy whitefish is not relative numbers since the sampling methods used targeted pygmy whitefish (setting the nets deep and on the bottom). The populations perhaps most vulnerable, according to the conditions that led to the loss of pygmy whitefish populations in Washington State lakes, are Tyhee and McLeese lakes. The lakes are small,

eutrophic, and exotic species of rainbow trout are introduced every year for the sport fishery. Tyhee Lake has been undergoing eutrophication and exotic piscivorous fish have been present since at least the 1960's. These factors do not appear to have affected the numbers of Tyhee Lake "giant" pygmy whitefish, and they do not appear to be in any danger of extinction at present.

Other researchers have found unbalanced sex ratios in pygmy whitefish. In some cases there were more males. In those cases, the researchers were catching the fish in the fall close to streams. It has been suggested that male pygmy whitefish may move to the spawning grounds before the females (Weisel et al. 1972). Thus observed sex ratio may depend on the timing of the sampling. Most of the fish caught in this study were found quite deep and it is possible that the males may have already moved elsewhere to spawn. Some sampling was done in mid-summer so it is unlikely in those cases that the males had already moved to the spawning grounds. The study done by R2 Resource Consultants (1995) also showed many more females. They attributed this to sampling bias; the female pygmy whitefish were larger in girth and got trapped by the gill nets much more easily than the smaller males. This could also explain the absence of age 1 and 2 fish. The exception to the skewed sex ratio in this study was the Dunlevy Creek population which had approximately equal numbers of males and females. Both the males and females were close to the stream. The female pygmy whitefish caught in Dunlevy Creek and Chapman Lake were both noted to have ripe females, and were both noted to be caught at the mouths of streams. These two sites were sampled in August and September. It is likely that the females were waiting to enter the stream to spawn. According to the females' condition in Dunlevy Creek and Chapman Lake it is estimated that spawning takes place in September. In September1996,

when not many pygmy whitefish were caught at Dunlevy Creek, sampling may have taken place too late in the season. It could be that most of the pygmy whitefish had already moved closer to, or into, the stream and were therefore not captured.

The information collected on pygmy whitefish in this study agrees with that already in the literature. From the size at age information presented in chapter one it can be seen that the Tyhee Lake "giant" pygmy whitefish are unique in their life history from other pygmy whitefish populations studied. Although my data do not show their habitat as unique, there may still be some ecological factors contributing to the "giant" pygmy whitefishes' unusual growth. Presented in this discussion are some factors which may be related to the "giant" pygmy whitefishes' growth, however these ideas are speculative and are not conclusive. There is probably not one ecological variable that is responsible for the growth of the "giant" pygmy whitefish, but rather a combination of variables. Those most important factors are likely those which contribute to availability of food items, such as warmer eutrophic lakes, the lack of potential competitors such as rocky mountain whitefish, lake whitefish and kokanee, and the lack of aggressive predatory fish species.

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General Conclusion

The main objective to determine whether the "giant" pygmy whitefish from McLeese and Tyhee lakes are phylogenetically distinct was met. "Giant" pygmy whitefish are not phylogenetically distinct from other pygmy whitefish. Furthermore, the McLeese Lake pygmy whitefish, reported to be "giant" pygmy whitefish, can not be given the distinction of being "giant" pygmy whitefish. The Tyhee "giant" pygmy whitefish do, however, show a distinct size at age curve, and are clearly larger than pygmy whitefish from other populations studied. Neither population can be assigned sub-species status. All the populations studied should remain as *Prosopium coulteri*.

When addressing concerns about conservation of a species, both genetic data and ecological data have to be taken into consideration. This study has addressed the question of genetic distinctiveness. As the "giant" pygmy whitefish do not distinguish themselves genetically, questions remain as to what environmental components may contribute to their large size.

Clearly more research needs to be done to identify the factors contributing to the fast growth of the Tyhee Lake pygmy whitefish if full consideration is to be given to this population for conservation. Conservation decisions are not only made based on genetic information, ESU's, or whether a population enjoys sub-species status but may also be based on personal opinions that different life history variants are worth conserving in their own right.

Each of the six populations studied using the RAPD analysis were genetically distinct. Given this information, each population could be described as an ESU, or at least an MU. The question remains whether directing conservation efforts towards each of these

populations is an efficient and wise use of resources. The only effort required is that managers make careful decisions when it comes to changing the lakes' environment. Although not genetically distinct, Tyhee Lake and McLeese Lake, the two populations that were the basis for this study, should be given special consideration.

Management Implications for Tyhee and McLeese Lakes

Although McLeese Lake pygmy whitefish are not "giant" pygmy whitefish, they are a distinct population and likely to be in danger of extinction. The same stresses believed to be the responsible for the demise of pygmy whitefish in Washington State lakes, could be at work in McLeese and Tyhee lakes. The residents around the lakes should try to minimize their contribution to the lakes' natural eutrophication, to the benefit of pygmy whitefish and other salmonids in the lake. Although it was stated that warmer lakes may contribute to the growth of pygmy whitefish by increasing the food supply, there is a threshold where an increase in temperature becomes detrimental to fish since as temperature increases, oxygen content decreases. Other salmonids that may potentially share the same habitat as pygmy whitefish, such as rocky mountain whitefish, lake whitefish and kokanee, should not be introduced to these lakes. If these fish compete with pygmy whitefish for space and food the pygmy whitefish may not have as much food available to them. If foraging becomes more difficult the fish uses more energy, taking away energy that may be used for growth. Energy may also be lost avoiding predators. In both lakes any introductions of aggressive or large piscivores, such as lake and bull trout, should be avoided.

Appendix A RAPD data collected from 9 RAPD primers. There are ten pygmy whitefish from six BC populations scored . Bands were scored as present (1) or absent (2). Eighty variable bands were scored.

Appendix A

Bands

Lake	Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Cluculz	1	1	2	2	1	2	1	2	1	2	2	1	1	1	2	2	1	2	1	1	2	2	2	1	1	1	1	1	1	2	2
	2	1	2	2	2	2	1	2	1	2	2	1	1	1	1	2	1	2	1	1	2	2	2	1	1	1	1	1	2	2	2
	3	1	2	2	1	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	2	2	2	1	1	1	1	1	1	2	2
	4	1	2	2	1	1	1	2	1	2	2	1	1	1	1	2	1	2	1	1	2	2	2	1	1	1	1	1	1	2	2
	5	1	2	1	1	1	1	2	1	2	2	1	1	1	1	2	2	1	1	1	2	2	2	1	1	1	1	1	1	2	2
	6	1	2	2	1	1	1	2	1	2	2	1	1	1	2	2	2	2	1	2	2	2	2	2	1	1	1	1	2	2	2
	7	1	2	1	1	1	1	2	1	2	2	1	1	1	2	1	1	2	2	1	2	2	2	1	1	1	1	1	1	2	1
	8	1	2	1	1	1	1	2	1	2	2	1	1	1	1	2	1	2	1	1	1	2	2	1	1	1	1	1	2	2	1
	9	1	2	2	1	1	1	2	1	2	2	1	1	1	2	2	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2
	10	1	2	2	1	1	1	2	1	2	2	1	1	1	1	2	1	2	1	1	1	2	2	1	1	1	1	1	1	2	1
McLeese	1	1	1	1	2	1	1	2	1	2	2	2	1	1	2	2	2	1	2	1	2	2	2	1	1	1	1	1	1	1	2
	2	1	2	2	1	2	1	2	1	2	2	1	1	1	2	2	1	2	1	1	2	1	2	1	1	1	1	1	1	1	2
	3	1	1	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	2	2	1	2	1	1	1	1	1	2	2
	4	1	1	2	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	2	2	2	1	1	1	1	1	1	1	2
	5	1	1	2	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	2	2	1	1	2	1	1	1	1	1	1	2
	6	1	1	1	1	2	1	2	1	2	2	1	1	1	2	2	2	2	1	1	2	2	2	2	1	1	1	1	1	1	2
	7	1	1	1	1	2	1	2	2	2	2	1	1	1	2	2	1	2	1	1	2	2	2	2	1	1	1	1	1	2	2
	8	1	1	1	1	2	1	2	1	2	2	1	1	1	2	2	1	2	1	1	2	2	2	1	1	1	1	1	1	1	2
	9	1	1	1	2	2	1	1	1	2	2	1	1	1	2	2	2	2	2	1	2	1	2	2	1	1	1	1	1	1	2
	10	1	1	1	1	2	1	2	1	2	2	1	1	1	1	2	2	2	1	1	2	2	2	1	1	1	1	1	1	2	2
Tyhee	1	1	2	2	1	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	2	2	2	1	1	1	1	1	1	2	2
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		Bands																													
Lake	**Fish**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
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McLeese	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	2	1	1	1	1	2	2	1
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	9	1	2	1	1	1	1	2	2	2	1	1	2	1	1	1	1	2	1	2	2	2	2	2	1	1	1	2	1	2	1
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Tyhee	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	2	2	2	2	1	1	2	1	1	1	1	2	2	1
	2	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	2	2	2	2	1	2	2	1	1	1	1	2	2	1
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	9	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	1	1	1	2	2	1
	10	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	1	1	1	2	2	1

		Bands																		
Lake	**Fish**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**
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	4	1	1	1	2	2	2	2	2	2	1	2	1	1	1	2	2	2	2	1	2
	5	1	1	1	2	2	1	1	2	2	1	2	1	1	1	2	2	2	2	1	2
	6	1	1	1	2	2	1	2	2	2	1	1	1	2	1	1	1	2	2	1	2
	7	1	1	1	2	2	1	1	2	2	1	2	1	1	1	1	2	2	1	1	1
	8	1	1	1	2	2	1	1	2	2	1	2	1	1	1	1	1	2	2	1	1
	9				2	2	1	1	2	2	1	1	1	1	1	1	1	2	1	1	1
	10	1	1	1	2	1	1	2	2	2	1	2	1	1	1	1	2	2	1	1	1
McLeese	1	1	1	1	2	2	2	1	2	2	1	2	1	1	1	2	2	2	1	1	2
	2	1	1	1	2	2	1	1	2	2	1	2	1	1	1	1	1	2	1	1	2
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	4	1	1	1	1	2	1	2	2	2	1	2	1	1	1	1	1	2	1	1	2
	5	1	1	1	1	2	1	2	2	2	1	1	1	2	1	1	2	2	2	2	1
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	9	1	1	1												2	2	2	2	2	1
	10	1	1	1	2	2	2	2	2	2	1	2	1	1	1	1	1	2	1	1	2
Tyhee	1	1	1	1	2	2	1	1	2	2	1	2	1	1	1	2	2	2	2	1	2
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		Bands																													
Lake	**Fish**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Chapman	1	1	2	2	2	2	1	1	1	2	2	1	1	1	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	2	2
	2	1	1	2	2	2	1	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	1	1	2	2
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Monkman	1	1	2	2	1	2	1	2	1	2	2	1	1	1	1	2	1	2	2	1	2	2	1	1	1	1	1	1	1	2	2
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Williston	1	1	1	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	2	1	1	1	1	1	2	2
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		Bands																													
Lake	**Fish**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
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Monkman	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	2	2	2	2	1	2	1	1	1	1	1	2	2	1
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Williston	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2	2	1	2	2	1	1	1	1	2	2	1
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	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	2	2	1	1	1	1	1	2	1
	8	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	2	2	2	1	2	2	1	1	1	1	1	1	1
	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	1	1	2	1	1	1
	10	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	1	1	1	2	2	1

		Bands																		
Lake	**Fish**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**
Chapman	1	1	2	1	2	2	1	2	2	2	1	2	1	1	1	2	2	2	1	1	2
	2				2	2	2	2	2	2	1	2	1	1	1						
	3	1	2	1	2	2	2	2	1	2	1	2	1	1	1	2	2	2	2	1	2
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	7				2	2	1	1	2	1	1	2	1	1	1	2	1	2	2	1	2
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Monkman	1	1	1	1	2	2	1	1	2	2	1	2	1	1	1	2	2	2	2	1	2
	2	1	1	1	2	1	2	2	2	2	1	2	1	1	1	1	1	2	2	1	1
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	10	1	1	1	2	2	1	2	2	2	1	2	2	2	1	1	1	2	2	1	1
Williston	1	1	1	1	1	2	1	2	2	2	1	2	1	1	1	1	2	2	2	2	2
	2	1	1	1	1	1	1	2	2	2	1	2	1	1	1	1	2	2	2	2	2
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