A GENETIC AND BEHAVIOURAL ANALYSIS OF THE DISTINCTIVENESS OF THE CULTUS PYGMY SCULPIN (*COTTUS ALEUTICUS*) AND IMPLICATIONS FOR ITS CONSERVATION

by

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Abstract

The Cultus pygmy sculpin (*Cottus aleuticus*) is endemic to Cultus Lake, southwestern BC, and is listed as threatened under the federal *Species at Risk Act* (SARA). The Cultus pygmy sculpin was first discovered by Ricker in the 1930's (Ricker 1960) and was described as a dwarf coastrange sculpin (*C. aleuticus*). It matures at a smaller size than the "normal" *C. aleuticus*; has a lacustrine rather than a fluvial life history; and appears to undertake diurnal feeding migrations into the water column to feed on the abundance of *Daphnia*. The Cultus pygmy sculpin also has different morphological features, including larger cephalic pores, shorter pelvic fins, and a greater number of pectoral fin rays, which may be advantageous for an open-water, planktivorous lifestyle. Little else, however, is known of the biology of this fish. I used mtDNA and microsatellite analyses to determine whether there is any genetic distinction between the pygmy sculpin and the coastrange sculpin. Potential behavioural differences between the two forms were also examined by comparing vertical depth selection in the laboratory, with and without *Cottus asper*, a benthic predator found in Cultus Lake. Results from combined microsatellite and mtDNA analyses indicate that there is no clear phylogeographic separation between pygmy and coastrange sculpin, but that there is genetic differentiation at the population level. Pelagic sculpin from Lake Washington were compared with the Cultus pygmy sculpin and showed divergence from it, which suggests that the pelagic life history has evolved independently in each lake. The Cultus pygmy sculpin was found significantly higher in experimental depth selection tanks than the stream forms of *C. aleuticus*, but only when *C. asper* was also present in the tanks. Taken together, molecular and behavioural results indicate that the Cultus pygmy sculpin is discrete from the coastrange sculpin, and validate its recognition as a

conservation unit distinct from "typical" *C.aleuticus*. The presence of abundant

planktonic prey within the water column of Cultus Lake, combined with the presence of

predators on the lake's bottom, may be important features of the lake that have

promoted the evolution of pelagic behaviour by the Cultus pygmy sculpin.

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Chapter 1: General introduction

British Columbia's (BC) glacial history involved repeated glacial advances and retreats during the Pleistocene (~ the last 2 million years), and the province is home to a fish fauna that is relatively depauperate taxonomically; BC has only about 70 taxonomically recognized species (McPhail 2007). During Pleistocene glacial advances the BC fish fauna was either eliminated or restricted to ice-free refugia peripheral to the main ice sheets. Consequently, the contemporary freshwater fish fauna of BC consists almost entirely of postglacial immigrants (McPhail and Carveth 1992). Even after the climate began to warm and the glaciers started to retreat at the end of the Wisconsinan glaciation about 12,000 years ago, there were only a few connections between different drainages and these passages were only temporary (McPhail and Carveth 1992). The geography of drainage systems and their interconnections heavily influenced dispersal and range expansion of freshwater fishes (Lindsey and McPhail 1986; McPhail and Lindsey 1986; Bernatchez and Wilson 1998). Consequently, these historical and biological factors have resulted in many opportunities for allopatry between fish populations. These factors, coupled with the generally low species diversity and potentially unexploited niches, have probably promoted postglacial genetic divergence and speciation (McPhail and Carveth 1992; Taylor 1999). The BC freshwater fish fauna, therefore, is characterized by relatively low taxonomic diversity, but contains many examples of highly divergent "ecotypes", intraspecific phylogenetic groups, and biological species that have yet to be described formally (e.g. McPhail and Taylor 1999; McPhail 2007).

One way in which adaptive radiation occurs is when species begin to exploit a variety of different resource types (Futuyma 1986). Geographic isolation and new,

harsh ecological conditions present during deglaciation (McPhail and Carveth 1992)

provided the opportunity to expand into new niches, possibly due to intraspecific

competition for limited resources (Schluter 1993). As morphology and habitat use are

strongly correlated with each other within and between populations, different selection

pressures can promote changes in morphology (Schluter 1993). These selection

pressures are thought to have promoted diversification of various "species complexes"

in freshwater fishes (Behnke 1972; Schluter and McPhail 1992).

Many fishes, such as members of the Salmonidae (salmon, trout, char, grayling,

and whitefishes) that inhabit post-glacial lakes, frequently exhibit two or more closely

related, sympatric forms within a single taxon (Behnke 1972). These differences could

be due to a single species adopting different life history traits, or they could be separate

species (Schluter 1993). Freshwater fishes such as threespine sticklebacks

(*Gasterosteus* spp.; Schluter 1993) and cichlids (*Amphilophus* spp*.;* Barluenga et al.

2006) have shown evidence of rapid speciation owing, in part, to interactions in

sympatry. My study focused on a distinctive population of a freshwater sculpin

(Cottidae) currently only observed in open water habitats of one lake in southwestern

BC that is thought to be another example of rapid, postglacial divergence. The

population is presumed to have been recently derived from a geographically more

widespread form that typically has a stream-dwelling and benthic life history (Scott and

Crossman 1973).

The coastrange sculpin

The coastrange sculpin (*Cottus aleuticus*) is found in coastal watersheds of

western North America from Bristol Bay in Alaska, south to San Luis Obispo, California

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(Scott and Crossman 1973) (Figure 1). As is the case with all sculpins, it lacks a swim bladder and is, therefore, primarily benthic. Except in a few large river systems such as the Fraser and Skeena rivers, it is typically restricted to within about 100 km of the coast. Sculpins invaded freshwater from the marine environment during the Pliocene or late Miocene (Yokoyama and Goto 2005). In BC, *C. aleuticus* is found in all coastal watersheds, including Vancouver Island and the Queen Charlotte Islands (McPhail 2007). It is believed that it survived the most recent glaciation in both northern and southern refugia, but all BC populations probably originated from a single southern refugium (McPhail and Lindsey 1970). Due to its extensive range, it is likely that ecological specializations exist (e.g., Whitely et al. 2009). Ricker (1960) described one such specialization: a population of dwarf coastrange sculpins known only from Cultus Lake, southwestern BC, and recognized informally as the "Cultus pygmy sculpin".

The young of the coastrange sculpin become planktonic in lakes or estuaries after hatching, and then take up a benthic lifestyle approximately one month later, before making their way back into the streams (Scott and Crossman 1973). The coastrange sculpin favours a flowing water environment when they become bottom-dwelling (Scott and Crossman 1973). While the larvae feed on microplankton during their pelagic stage, adult fluvial coastrange sculpin primarily forage on the nymphs and larvae of aquatic insects (McPhail 2007).

The Cultus Lake pygmy sculpin is different from "typical" coastrange sculpin in that it appears to reside and mature at a smaller size in the lake's pelagic environment (Cannings 1993; McPhail 2007; Figure 2). A close relative of the coastrange sculpin, the prickly sculpin (*C. asper*) is a voracious piscivore (McPhail 2007) and is also found in Cultus Lake, but apparently only in the littoral and benthic habitats of the lake.

Coastrange sculpin found in Frosst Creek (a tributary of Cultus Lake) and Cultus pygmy sculpin appear to be smaller on average than *C. aleuticus* caught in Norrish Creek (lower Fraser River) and the Little Campbell River (Semiahmoo Bay just south of the Fraser River estuary). Cultus pygmy sculpin are thought to occupy only the deeper, offshore waters of the lake, based on lack of observation of Cultus pygmy sculpins during shore and tributary sampling. In Ricker's (1960) study, the Cultus pygmy sculpin was not captured in shore seining or in traps set for juvenile Pacific salmon (*Oncorhynchus* spp.), and was only found in significant numbers in the stomachs of bull trout (*Salvelinus confluentus*), which prefer deeper, offshore waters. Since 1970, Fisheries and Oceans Canada (DFO) have captured sculpins in their pelagic trawling for sockeye salmon (*Oncorhynchus nerka*) in Cultus Lake (J. Hume, DFO, pers. comm.). It has been proposed that the Cultus pygmy sculpin undertakes diurnal feeding migrations, following the zooplankton on which it feeds (McPhail 2007). Limnetic life histories are extremely rare in the Cottidae. In fact, only three species of the 250 or so described taxa are limnetic and these belong to two highly divergent genera endemic to Lake Baikal in central Siberia (Kontula et al. 2003).

The Cultus Lake pygmy sculpin is a provincially red-listed species (meaning that it is an indigenous species listed as threatened in BC) as its range is restricted to one heavily utilised lake: Cultus Lake received three million recreational visitors in 2009 (Cultus Lake Park Board minutes, September 2009). The Cultus pygmy sculpin has also been recognized as a "designatable unit" (DU) separate from the coastrange sculpin by the Committee for the Status of Endangered Wildlife in Canada (COSEWIC, e.g. COSEWIC 2010). A designatable unit is an intraspecific population, or assemblage of populations, within a species that is recognized as separate from other members of

the taxon if it is both "discrete", and if this discreteness is "significant" in terms of the evolutionary or ecological legacy of the taxon (COSEWIC 2009). Consequently, a DU can be recognized as a "wildlife species" for legal listing and protection under Canada's *Species at Risk Act* (*SARA*). Various genetic, biogeographic, and ecological criteria are used to assess the "discrete" and "significance" criteria for DU recognition by COSEWIC (2009). The Cultus pygmy sculpin is legally listed under *SARA* on Schedule 1 as a threatened species, which means that recovery planning and critical habitat protection are mandatory and a recovery strategy for this DU has been completed (National Recovery Team for the Cultus Pygmy Sculpin 2007). Despite the status of the Cultus pygmy sculpin as a DU, the only studies on this fish were conducted by Ricker in 1932-37 (Ricker 1960) and the status reports of COSEWIC (e.g. COSEWIC 2010). Although COSEWIC (2010) reported some differences in morphology between Cultus pygmy sculpin and typical coastrange sculpin (primarily in cephalic pore sizes), there has been no rigorous assessment of the status of this fish as a valid DU under the discrete and significant criteria of COSEWIC. For instance, it is unclear how genetically divergent the lake form is from the stream-dwelling form in Cultus Lake. It is possible, for example, that the two forms are part of the same genetic population and that the lake form results from dispersal from the stream-spawning habitats of coastrange sculpin. Alternatively, the lake form may be a genetically discrete population(s) that, coupled with its ecological specializations, is behaving as a species distinct from coastrange sculpin. These possibilities have direct bearing on the status of the lake form as a distinct DU under COSEWIC and *SARA* (COSEWIC 2010), yet, the Cultus pygmy sculpin's validity as a DU has never been objectively assessed against the criteria used by COSEWIC (COSEWIC 2009 Operations and Procedures Manual Appendix F5). This is an

important limitation because although the Cultus pygmy sculpin is currently listed by *SARA*, such listings are reviewed at least every 10 years and the status may change, and eligibility of a wildlife species as a DU is one basis on which the legal status may be altered. Further, there have been no ecological studies on this fish since Ricker's (1960) original work, and little of its life history is known. It is believed that the population is stable and that reproduction is sufficient to replace the population, although spawning has not been observed, and the location of spawning and fate of the newly-hatched young is not known. The Cultus Lake situation could be unique and significant from a conservation standpoint in that the Cultus pygmy sculpin is apparently endemic to BC, and it provides an example of rapid diversification within a species. A similar fish, however, has been found in Lake Washington near Seattle, WA (Larson and Brown 1975). The evolutionary origin of these similar ecotypes, however, is uncertain. This other pelagic sculpin could share a common ancestor with the Cultus pygmy sculpin or it could have arisen through parallel evolution.

In summary, the eligibility of the Cultus pygmy sculpin as a DU under *SARA* as well as the evolutionary origins of this fish are uncertain. My thesis sought to address these uncertainties and had the following overall objectives:

1. Determine the genetic distinctiveness of the Cultus pygmy sculpin from *C. aleuticus* found in tributary streams to Cultus Lake and elsewhere throughout its range, through the use of mtDNA and microsatellite (nuclear) DNA.
2. Map the habitat of the Cultus pygmy sculpin.
3. Using Fisheries and Oceans Canada trawl catch data and lab experiments, determine whether there are any behavioural differences between the Cultus pygmy sculpin and typical stream-dwelling coastrange sculpin.

Chapter 2: Evolutionary relationships and population structure of the Cultus pygmy sculpin, *Cottus aleuticus*

Introduction

Over the last thirty years, there has been an increase in the use of molecular genetic information to assess the conservation status of intraspecific units (Avise 2004). Although the morphology of the Cultus pygmy sculpin has been examined (Ricker 1960; COSEWIC 2010), no genetic assessments of its distinctiveness from typical coastrange sculpin or of its evolutionary origin have been conducted. Genetic distinctiveness, including differences in mtDNA sequences or allele frequency differences in microsatellites, is one of the criteria used to determine whether a population is a Designtatable Unit (COSEWIC 2009). My study used mitochondrial DNA (mtDNA) and microsatellite (nuclear) DNA to determine the phylogenetic relationships and population structure of the Cultus pygmy sculpin, in comparison with *C. aleuticus*.

Mitochondrial DNA is a small, haploid molecule that is inherited maternally (Avise et al. 1987; Moritz et al. 1987; Allendorf and Luikart 2007). The analysis of mtDNA can be used to obtain information about the relationships between individuals and populations (Moritz et al. 1987). There is no recombination (Aquadro and Greenberg 1983; Avise et al. 1987; Allendorf and Luikart 2007), and so it provides a clear record of genealogical descent (Allendorf and Luikart 2007). The ultimate source of variation in the mtDNA sequence is mutation (Aquadro and Greenberg 1983; Avise 2004), and evolution at the nucleotide sequence level is rapid compared to most single copy

nuclear loci (Avise et al. 1987), which often allows for the resolution of intraspecific variation (Yokoyama and Goto 2002).

Within the genus *Cottus,* mtDNA has been used on a broad scale to examine the relationships among species and related taxa (Kinzinger et al. 2005; Yokoyama and Goto 2005) and to explore the diversification of a monophyletic group (Kontula et al. 2003).   At the species level, mtDNA has been used to examine genetic fragmentation, variation and phylogeography in *Cottus nozawae* (Okumura et al. 1996; Yagami et al. 2002; Yokoyama and Goto 2002). The mtDNA sequences that have been used for *Cottus* include the ATPase 8 and 6 genes, the cytochrome b gene (Kontula et al. 2003), the control region, and 12S rRNA (Yokoyama and Goto 2005). For my study, I used the displacement loop (D-loop) or the "control region". This sequence functions in replication, and can change very rapidly (Moritz et al. 1987). Sequences in the control region have been used successfully in *Cottus* to estimate genetic differentiation (Okumura et al. 1996) and reveal phylogenetic relationships (Yokoyama and Goto 2002; Kontula et al. 2003).

If a population is genetically distinct from other populations, this satisfies one of the criteria for recognition as a DU under *SARA* (Guidelines for recognizing Designatable Units, COSEWIC 2009). For example, sequences from the control region of mtDNA were used to examine the phylogeography of the sand goby, *Pomatoschistus minutus* (Stefanni and Thorley 2003). The Adriatic population of the sand goby was found to be highly distinct from other populations and was therefore determined to be an Evolutionarily Significant Unit, or ESU, a concept analogous to that of DUs for many jurisdictions outside Canada (Moritz 1994). Sequences including those from the mtDNA control region were used to distinguish the deepwater sculpin (*Myoxocephalus*

thompsonii) from the fourhorn sculpin (*M. quadricornis*); these results also identified two separate DUs for the deepwater sculpin in Canada (COSEWIC 2006).

The mutation rate of mtDNA is typically more rapid than that of many nuclear DNA loci, which has proven useful for resolving major evolutionary units within species (Moritz 1994); however, this rate of mtDNA mutation is relatively too low to disclose processes on a more recent time scale, such as since around the end of the last glacial maximum (~ 15,000 years before present, McPhail and Lindsey 1986; Hanfling et al. 2002). Comparatively, microsatellites are more rapidly evolving loci that can be more informative over such short time scales (Hanfling et al. 2002). Microsatellites are variable, non-coding regions of nuclear DNA (Hansen et al. 2001), which are composed of tandemly-repeated simple sequences (e.g., CACACA, GTTTGTTT, Jarne and Lagoda 1996). Microsatellite loci are commonly used to understand population structure within species (Slatkin 1995), because they are highly polymorphic, even in small populations, due to a high mutation rate and because it is relatively easy to assay many unlinked loci (Allendorf and Luikart 2007). The evolutionary divergences within species are assessed and the distribution of genetic variation within and between populations is analyzed using F-statistics (Jarne and Lagoda 1996; Rico et al. 1996). In populations that display low genetic diversity (e.g., Canadian polar bears, *Ursus maritimus*, Paetkau et al. 1995; European bullhead, *C. gobio*, Hanfling et al. 2002), microsatellites have been used to examine genetic population structure with greater success than mtDNA. Englbrecht et al. (1999) successfully developed microsatellite loci for *C. gobio*, and these loci were also variable in related taxa. These loci have been used successfully to look at genetic subdivision among populations (Whiteley et al. 2009) and diversity within populations (Taylor et al. 2010) in *C. aleuticus.*

In this chapter, I used a combined approach to assess the genetic distinctiveness of the Cultus pygmy sculpin. First, I used mtDNA data to reconstruct the relationships between sequences of the Cultus pygmy sculpin relative to that of the coastrange sculpin. I expected that if the Cultus pygmy sculpin is a valid DU, it should represent a monophyletic assemblage distinct from the coastrange sculpin or, failing this, that it should be significantly different in terms of the frequencies of shared mtDNA haplotypes. To these ends, I compared the mtDNA D-loop sequences of Cultus pygmy sculpin with coastrange sculpin from the inlet stream and other tributaries to Cultus Lake. I also included the Lake Washington pelagic sculpin, as well as samples from throughout the range of *C. aleuticus* to get an idea of how much sculpin samples from within the Cultus Lake drainage differ from one another relative to sculpins sampled from outside the drainage and to assess how different the Cultus pygmy sculpin is from a similar phenotype in Lake Washington.

Second, I used eight microsatellite loci to determine whether the Cultus pygmy sculpin is a population genetically distinct (i.e., with different allele frequencies; COSEWIC 2009) from *C. aleuticus* from the inlet stream and other tributaries to Cultus Lake. For comparison with other coastrange sculpin populations, I included samples from Norrish Creek in the lower Fraser River (which is also part of the Fraser system, but is located on the north side of the Fraser River); three different rivers in the Squamish River watershed (to provide information on population differentiation in a small, interconnected geographic area); and pelagic sculpin from Lake Washington (to compare two different pelagic sculpin populations). The use of microsatellites should provide a more sensitive test of the genetic "discreteness" criterion of DU status for the

Cultus pygmy sculpin, given that they probably have diverged from *C. aleuticus* only postglacially.

Materials and methods

Study area

Cultus Lake is located approximately 10 km south of Chilliwack, in the eastern Fraser River Valley, BC (Shortreed 2007) (Figure 3). The surface area of the lake is 6.3 km^2 and it has a mean depth of 31 m, with a maximum depth of 44 m (Shortreed 2007). Frosst Creek is the lake's inlet stream and largest tributary; the lake discharges into Sweltzer River, which itself empties into the Chilliwack River (a tributary of the lower Fraser River) approximately 3 km downstream from the lake (Shortreed 2007). Cultus Lake is warm, monomictic, and thermally stratified from May-November (Shortreed 2007). Cultus Lake is mesotrophic and there has been an increase in lake temperature over the past 70 years which has been accompanied by an increase in productivity (Shortreed 2007). Pelagic *C. aleuticus* are also found in Lake Washington, which is located at Seattle, WA, and has a maximum depth of 65.2 m, with an average depth of 32.9 m (Ikusemiju 1975).

Sculpin samples

A total of 46 different samples from 12 different locations were used to obtain mtDNA sequences, including three from *C. asper* that was used as the outgroup (Table 1). Ten populations consisting of a total of 350 samples from nine different locations

were used for microsatellite analysis (Table 2). Samples were captured by trawl, minnow trap or electrofishing.

Mitochondrial DNA

Tissue samples were subject to Qiagen spin column-based DNA extraction procedures and the DNA was stored at -20°C. The entire control region was amplified by PCR using the primers L-Thr (5'-AGCTCAGCGYCAGAGCGCCGGTCTTGTAA-3') and H12Sr5 (5'-TGATAATAAAGTCAGGACCAAG-3') (Yokoyama and Goto 2002). Amplification reactions contained 50-300 ng of genomic DNA template (Ilves 2007). The control region of mtDNA was amplified in a 50 μL PCR containing: 10 x buffer (New England Biolabs, NEB), 5 mM of dNTPs, 10 μM of primers L-Thro and H12Sr5 (Yokoyama and Goto 2002), and 1.5 units of *Taq* DNA polymerase (NEB). Thermal cycling conditions began with 3 minutes at 95°C, 1 minute at 58°C, 1 minute at 72°C, with 5 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C, 33 cycles of 92°C for 45 seconds, 56°C for 30 seconds, and 72°C for 1 minute, followed by a final extension step of 72°C for 10 minutes.

PCR products were purified using the Qiagen QIAquick PCR purification kit and sequenced at the NAPS unit at the University of British Columbia on an ABI Prism 377 automated sequencer using L-Thr as the sequencing primer.

Sequences were edited using *BioEdit* v7.0.9.0 (Hall 1999) and aligned using *ClustalX* (Thompson et al. 1997). *MrModeltest* v2.3 (Posada and Crandall 1998) was used to select a model for sequence evolution employing the Akaike Information Criterion (AIC) to select the best model (Posada and Buckley 2004). *PAUP** (Swofford 2002) was used for neighbour-joining tree analysis of pairwise genetic distances.

MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) was used for Bayesian estimates of phylogeny, with 1,000,000 generations run. Due to three samples (Lake Washington and two from Alaska) having shorter sequences, the analysis was run once with the more complete sequences (using 775 base pairs) and then again using all the sequences, with the longer ones truncated to 340 base pairs.

Microsatellite analysis

A total of 350 fish were genotyped at eight *Cottus gobio* dinucleotide microsatellite loci (Nolte et al. 2005; Whiteley et al. 2009; Taylor et al. 2010). These loci were amplified by PCR using fluorescently-labelled primers (Table 3a) according to Qiagen's Multiplex PCR protocol for amplification of microsatellite loci using multiplex PCR (Taylor et al. 2010). An annealing temperature of $60^{o}C$ and 25 cycles were used in the amplifications (Table 3b, Taylor et al. 2010). The PCR products were then diluted 1:20 before being assayed using a CEQ 8000 Genetic Analysis System and software (Beckman Coulter) with CEQ DNA Size Standard Kit (400 base pairs) used as internal size standard (Taylor et al. 2010).

Convert (Glaubitz 2004) was used to convert the raw microsatellite data into the formats required by the different programs. *Microchecker* v2.2.3 (Van Oosterhout et al. 2004) was used to test for the presence of null alleles and to detect any technical problems with the microsatellite results. To obtain descriptive statistics, *Tools for Population Genetic Analysis* (TFPGA) (Miller 1997) provided observed (H_O) and expected (H_E) heterozygosities, sample size (N) and number of alleles. *Fstat* (Goudet 2001) was used to determine allelic richness (A_R), which is a measure of allelic diversity that takes sample size into account (Allendorf and Luikart 2007); *Fstat* was also used to compare allelic richness among groups. *Genepop* v3.4 (Raymond and Rousset 1995)

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was used to detect any deviation from Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium, and also to test for differences in F_{ST} values and genotypic differentiation between all pairs of populations. *Geneclass* v2.0 (Piry et al. 2004) was used to investigate the origins of the fish caught in minnow traps by comparing them to reference samples from all sample localities within Cultus Lake. *Structure* v2.3.3 (Pritchard et al. 2000) was used to estimate the number of distinct genetic clusters in the microsatellite data. The burnin period was 50,000 iterations while the Markov Chain Monte-Carlo (MCMC) length was also 50,000. There were ten separate runs conducted for each population estimate (*K*), and the average of each log likelihood value was used for each *K* estimate. All eight populations were first tested, and then a nested analysis including only the three populations within the Cultus Lake drainage was performed. *Arlequin* (Schneider et al. 2000) was used to produce an analysis of molecular variance (AMOVA) that compared all sculpin populations sampled and those within the Cultus Lake drainage. Factorial Correspondence Analysis (FCA) in *Genetix* (Belkhir et al. 2001) was used to visualize similarity among the *C. aleuticus* multilocus microsatellite genotypes (Taylor et al. 2010). The sequential Bonferroni correction was used to determine significance for tests that involved many simultaneous tests (Rice 1989).

Results

Mitochondrial DNA

The best model selected by AIC using *MrModelTest* was determined to be GTR + I + G (General Time Reversible model (GTR) (Tavaré 1986), with proportion of

invariable sites (I) and gamma distribution (G)). The GTR model assumes that the probability of the data is independent of the placement of the root on the tree; that the relative rates of substitutions remain constant across the tree; and that the four nucleotides remain at the same frequencies (Waddell and Steel 1997). The substitution rate varies among sites in a gamma distribution (with my data, the shape of the gamma distribution was 1.0277) (Li 1997). The proportion of invariable sites is the expected frequency of sites that do not evolve (with my data, I = 0.7046) (Waddell and Steel 1997). This model was used to develop the Bayesian trees with likelihood values (Figure 4). Two samples, Cultus 214 and Frosst 73, were more closely related to the *C. asper* group than to *C. aleuticus*; these two fish were removed from further analysis. The Cultus pygmy sculpin samples formed a paraphyletic grouping, and did not form a monophyletic grouping distinct from *C. aleuticus* (Figure 4). The average pairwise genetic distance amongst all samples and the outgroup *C. asper* was 0.048. The average pairwise divergence between *C. aleuticus* sampled from outside the Cultus Lake drainage and Cultus pygmy sculpin sequences was 0.010. The average sequence divergence between Cultus pygmy sculpin and coastrange sculpin sampled from other areas of the Cultus Lake drainage was 0.005. When the truncated sequences, including Lake Washington and Alaska sculpins, were used (Figure 5), the sequence divergence of the Lake Washington fish was 0.014 from all other *C. aleuticus*, and 0.013 from Cultus pygmy sculpin. Sequence divergence of *C. aleuticus* sequences from Alaska were 0.009 both from Cultus pygmy sculpin and all other *C. aleuticus*.

Microsatellite DNA

Across loci there were between four and thirty-seven alleles detected, with individual populations having between four and twenty-four alleles at each locus (Table 4). *Cottus aleuticus* from the Cultus Lake streams and the Cultus pygmy sculpin both had lower allelic richness ($p < 0.05$) when compared to other *C. aleuticus* populations (Table 5). No population failed to meet Hardy-Weinberg assumptions across all loci (Table 6). Only 12 out of 280 tests showed evidence of departures from linkage equilibrium, but such departures tended to be idiosyncratic and not concentrated at particular loci or within particular populations (Table 7). The lack of linkage disequilibrium could be due to the number of low frequency alleles as well as the high amount of variation observed in the limited sample size; to observe linkage disequilibrium, it would be necessary to compare areas matched for allele frequency (Eberle et al. 2006). *Microchecker* identified locus CottES19 to be a possible locus with null alleles, associated with cases of heterozygotic deficiency/homozygote excess. The analyses were conducted with and without this locus, and no differences between results were observed; therefore, this locus was included in the analyses.

F_{ST} values across population comparisons ranged from 0.001 to 0.06 (Table 8), while the overall F_{ST} value was 0.03. There was no significant difference in F_{ST} between coastrange sculpin sampled from Watt and Windfall creeks, and so the two populations were combined. There was no significant difference between Cultus pygmy sculpin caught using trawl or minnow traps, and so those two groups were also combined (Table 8). Consequently, subsequent tests consisted of three groups of fish from the Cultus Lake drainage: Cultus pygmy sculpin sampled from the lake, the

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Windfall and Watt creeks combined samples ("tributaries"), and the Frosst Creek sample. There was a significant difference in F_{ST} values between Cultus Lake pygmy sculpin and sculpin from Frosst Creek and the tributary samples although these differences were much smaller than F_{ST} values between populations sampled from different watersheds (Table 8).

Assignment tests showed that approximately half of the fish caught by minnow traps were assigned to the Cultus pygmy population, while the other half were assigned to the tributary populations (Table 9).

The results from the *Structure* analysis indicated that the most likely number of genetic groups across all samples was three (Table 10). Group 1 consisted of Cheakamus, Mamquam, Squamish rivers and Norrish Creek; Group 2 consisted of Cultus Lake, Frosst, Watt and Windfall creeks; and Group 3 consisted of the Lake Washington sample (Figure 6). The nested *Structure* analysis indicated that within the Cultus Lake drainage, the most likely number of populations was two (Table 11). Some individuals in the Cultus pygmy sculpin population (Group 1) are more strongly assigned to one population, compared to the more symmetrical assignments seen in the sculpins found in Frosst Creek (Group 2) and Watt and Windfall creeks (Group 3; Table 11; Figure 7). Therefore, there does appear to be a difference between the Cultus pygmy sculpin population with the stream populations and population structure is typically concluded to be present when the proportions of the sample assigned to each population is asymmetric (Hubisz et al. 2009).

The hierarchical analysis of genetic structure using AMOVA showed that about 2.0% (P < 0.001) of the total genetic variability could be attributed to differences among the Squamish River, Cultus, and "other" (Norrish Creek, Lake Washington) drainages

and the vast majority of remaining variation resided within populations (Table 12). The subtle genetic differences between Cultus pygmy sculpin and other *C. aleuticus* within the Cultus Lake drainage were also indicated when the AMOVA analyses were conducted within the Cultus Lake drainage alone. Here, Cultus pygmy sculpin were partitioned between trawl and minnow trap samples and were compared to stream fish partitioned across Windfall, Watt, and Frosst creeks. In the Cultus Lake drainage, only 1.2% of the variation ($P > 0.05$) was attributable to differences between lake and stream sculpins (Table 12).

The FCA analysis amongst all samples (Figure 8) showed that the populations from the same drainages grouped together, while the Lake Washington and Norrish Creek populations formed two separate clusters. These results agreed with the *Structure* analysis (Figure 6), in that the Lake Washington population was different from the other populations, while Norrish Creek was intermediate between the Squamish and Cultus drainage populations. When the analysis was restricted just to the Cultus Lake samples, the FCA also suggested that there is genetic differentiation between the Cultus pygmy sculpin and the Frosst Creek and tributary sculpins (Figure 9). The three populations do not form completely distinct clusters, but do show some segregation in FCA space (i.e., there is little overlap between the populations). This association between genetic identity and geographic location is similar to the results from the *Structure* analysis (Figure 7), in that the three populations are distinct from each other, but the amount of divergence between the three populations is low.

Discussion

mtDNA inter-relationships

The results from the mtDNA analysis did not show genetic differentiation between the Cultus pygmy sculpin and other *C. aleuticus* populations. Fish from northern populations in North America that recolonized habitats from southern refugia have lower genetic diversity, as well as broader geographic distributions than southern populations (Bernatchez and Wilson 1998; Hewitt 2000). Cultus Lake (and nearby Lake Washington) only became accessible to fishes within the last 8 – 10, 000 years, after the Wisconsinan ice sheets retreated (COSEWIC 2010). The recent emergence of the only known habitats presently occupied by pygmy populations of coastrange sculpin implies that these fish have had a postglacial origin as well. My mtDNA results are consistent with this idea. Sequence divergence estimates between Cultus pygmy sculpin and parapatric coastrange sculpin were very low and consistent with differences between populations that have only recently diverged from one another (e.g., Bernatchez and Wilson 1998; Taylor and McPhail 1999). In addition, the mtDNA haplotypes of Cultus Lake and coastrange sculpin were not reciprocally monophyletic, again, suggesting a recent divergence.

The phylogeny of mtDNA in recently diverged species may be affected by retained ancestral polymorphisms in that after a divergence event, the relationships among the alleles proceed from polyphyly, through various paraphyletic conditions to monophyly, as ancestral polymorphisms are replaced by derived states (Moritz 1994). If the parent population is larger than the daughter population, some lineages in the parent population may share a more recent common ancestor with those in the

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daughter population than in the rest of the parent population (Paetkau 1999). Other species, including polar bears (*U. maritimus*, Talbot and Shields 1996), song sparrows (*Melospiza melodia*, Zink and Dittman 1993), and juncos (*Junco spp.*, Mila et al. 2007) have demonstrated differences in phenotypes, which were not evident in their mtDNA haplotypes.

To qualify as a Designatable Unit under COSEWIC guidelines, a population needs to show genetic distinctiveness, including, but not limited to, differences in DNA sequences and microsatellite allele frequencies (COSEWIC 2009). For a population to be significant in terms of neutral loci, there must be qualitative genetic differences at slowly evolving markers (COSEWIC 2009). The relatively recent, postglacial divergence of the Cultus pygmy sculpin from the parapatric *C. aleuticus*, as demonstrated by the mtDNA analysis, is such that there are no marked genetic differences that would qualify the Cultus Lake population as significant. Therefore, I used microsatellite loci to look at population-level differentiation, as well as differences in behaviour and habitat.

Interestingly, of the forty-three samples that I identified by morphology as *C. aleuticus*, two samples were designated as *C. asper* by mtDNA. While I used multiple criteria in order to identify each individual to species (Scott and Crossman 1973; McPhail 2007), it is possible that those two specimens could have been misidentified (i.e., if anal fin rays had been broken off). It is also possible that this mismatch could result from hybridization, specifically *C. asper* females mating with *C. aleuticus* males (Taylor et al. 2010). One of these fish was from Frosst Creek; the sampling site in Frosst Creek had a predominantly riffle habitat, preferred by *C. aleuticus* (Taylor 1963), but small numbers of *C. asper* were also caught during sampling. The low numbers of

C. asper in the area would influence hybridization dynamics in that one might expect hybridization to be biased in favour of *C. asper* females mating with *C. aleuticus* males (Wirtz 1999; Taylor et al. 2010). The second fish was from Cultus Lake; *C. asper* is present in large numbers in all areas throughout the lake, and matures at a much larger size than *C. aleuticus* and the Cultus pygmy sculpin (McPhail 2007). Hybridization can result from differences in sizes at maturity, with small males of one species mating with larger females (Wirtz 1999; Taylor et al. 2010). While hybridization events between sympatric species pairs of *Cottus* have been reported elsewhere in North America (McPhail 2007), the only other report of *C. aleuticus* x *C. asper* hybridization has been from the Cheakamus River (Taylor et al. 2010). As exemplified by the suggested cases of hybridization described above, due to the peculiarities of mtDNA inheritance (no recombination, maternally-inherited), mtDNA-based phylogenies may not reflect the situation in the rest of the genome and I conducted microsatellite DNA analyses to address this limitation (Coyne and Orr 2004).

Microsatellite DNA differentiation

The F_{ST} values of the Cultus pygmy sculpin in comparison with sculpins from tributary streams within the drainage were less than 0.05, but statistically significant and exhibit genetic differentiation at the population level. The samples were characterized by abundant genetic variation within samples, but low levels of divergence between samples. These general patterns are consistent with the few other studies that have been conducted on freshwater sculpins. For instance, Whitely et al. (2009) found little evidence for genetic subdivision in the Alaska populations separated by about 50 km

($F_{ST} < 0.001$), while Taylor et al. (2010) found the fish in the three Squamish Rivers to have an overall F_{ST} of 0.003. Consequently, compared with the low values observed in other systems, the differences that I have documented between Cultus pygmy sculpin and tributary fish are relatively large.

There are several reasons why sculpins may exhibit a general pattern of low interpopulation variability. For instance, as newly hatched larvae, the coastrange sculpin (and many other species) have a pelagic dispersal stage in which they enter a larger body of water (such as an estuary, lake or ocean) and remain in the water column for up to a month before re-entering freshwater streams (Mason and Machidori 1976). This highly dispersive stage has been suggested to constrain the development of population subdivision at neutral loci (Whiteley et al. 2009). Combined with the relatively recent time for colonization and the elevated level of dispersal, the amount of gene flow can be high enough to obscure the effects of genetic drift in driving molecular divergence at neutral loci (Whiteley et al. 2009). The low level of sequence divergence between the coastrange sculpins in BC and Alaska also suggests that they survived the last glaciation in one refugium (McPhail and Lindsey 1970; Mila et al. 2007). Lake Washington fish, however, were highly distinct in the microsatellite analyses and this suggests that they have had a separate origin from those in Cultus Lake. If so, this would suggest that the two known occurrences of pygmy sculpin are a result of parallel evolution, in that similar traits have evolved in similar habitats (e.g., Rundle et al. 2000). Both the Cultus pygmy sculpin and the Lake Washington pelagic sculpin live in the open waters of productive lakes, and have enlarged cephalic pores, shorter pelvic fins, and a higher number of pectoral fin rays (Larson and Brown 1975; COSEWIC 2010). Other northwestern North American fishes have exhibited parallel evolution after glaciation,

including sockeye/kokanee (*Oncorhynchus nerka*, Taylor et al. 1996), threespine stickleback species pairs (*Gasterosteus aculeatus*, Taylor and McPhail 1999), and dwarf and normal lake whitefish (*Coregonus clupeaformis*) pairs (Pigeon et al. 1997).

The F_{ST} values and results from *Structure* and the FCA showed that the Cultus Lake pygmy sculpin were genetically differentiated from the adjacent stream samples. The one apparently anomalous result, however, was that sculpins that I captured in minnow traps were found by the assignment tests to be comprised of both Cultus Lake and fish from the two tributaries, Watt and Windfall creeks. The fish that were assigned to the tributaries had been sampled in July and tended to be from traps located near the shoreline, when the tributaries were either dry or the depth and flow were both greatly reduced. One possible explanation for this result is that these fish actually belonged to the stream populations, but they had taken refuge in the lake. Despite this spatial overlap in the summer, it is unlikely that there is gene flow between the stream and lake fish; the stream fish lay their eggs in spring, generally from late March to early April (Ricker 1960), allowing time for the young to hatch before the streams dry up, while the Cultus pygmy sculpin fish have a summer spawning season (mature fish were caught May–late July; also Ricker 1960). Alternatively, the assignment of some minnow trap fish both to Cultus Lake and tributary populations could stem from the relatively low assignment scores of the fish identified to the Cultus Lake population. These low assignment scores could be a result of the lower sample size of the Cultus reference population (n=30, compared with the tributary population, n=55). In populations with low F_{ST}, good assignment scores require larger samples of individuals and loci (Cornuet et al. 1999; Bjornstad and Roed 2002). The low F_{ST} between the Cultus Lake and tributary populations, combined with the high level of diversity observed in each

population, would mean that more of the variation would be captured with a larger reference population and would thus result in higher assignment scores (in this case, for the tributary population).

In summary, this chapter has presented evidence that Cultus pygmy sculpin shows genetic differentiation (at least at microsatellite DNA loci) from stream-dwelling fish and that this divergence has occurred postglacially. In addition, my data present the first evidence that the pygmy forms in Cultus Lake and Lake Washington have had independent origins and represent another example of parallel evolution in postglacial fishes. In the next chapter, I discuss the results from behavioural assays conducted in laboratory conditions and I then use Fisheries and Oceans Canada trawl data to define potential habitat requirements of pelagic sculpin.

Table 1. Location, species and number of samples of *Cottus* used for mtDNA D-loop analysis.

Location	Species	Watershed code	Drainage	N
Cultus Lake	*Cottus aleuticus*	100-065700-09700-13300	Chilliwack	14
	C. asper	100-065700-09700-13300	Chilliwack	1
Frosst Creek	*C. aleuticus*	100-065700-09700-13300-9760	Chilliwack	10
Windfall Creek	*C. aleuticus*	100-065700-09700-13300-5370	Chilliwack	5
Watt Creek	*C. aleuticus*	100-065700-09700-13300-9100	Chilliwack	5
Sweltzer River	*C. aleuticus*	100-065700-09700-13300	Chilliwack	1
Lake Washington	*C. aleuticus*	17110012	Lake Washington	1
Norrish Creek	*C. aleuticus*	100-06400	Harrison	1
Alouette Reservoir	*C. aleuticus*	100-026700-06000	Lower Fraser	1
Skeena River	*C. aleuticus*	400	Skeena	1
Bella Coola River	*C. aleuticus*	910-290700	Bella Coola	2
Bartlett River, Alaska	*C. aleuticus*		Glacier Bay	2
Kinbasket Reservoir	*C. asper*	300	Columbia	2

Table 2. Population location and number of samples of *Cottus* used for microsatellite analysis.

Location	Watershed code	Drainage	N
Cheakamus River	900-097600-12900	Squamish	67
Mamquam River	900-097600-05100	Squamish	48
Squamish River	900-097600	Squamish	30
Cultus Lake (trawl caught)	100-065700-09700-13300	Chilliwack	30
Frosst Creek	100-065700-09700-13300-9760	Chilliwack	30
Lake Washington	17110012	Lake Washington	30
Windfall Creek	100-065700-09700-13300-5370	Chilliwack	25
Watt Creek	100-065700-09700-13300-9100	Chilliwack	30
Norrish Creek	100-06400	Harrison	29
Cultus Lake (minnow trap caught)	100-065700-09700-13300	Chilliwack	31

Table 3a. PCR strategy: repeat motifs and sequences of the primers used for microsatellite analysis.

Locus	Repeat motif	Primer sequences (5'-3')	Size bp
Cott100	$(TG)_{15}$	CTCATCGTGGTTTGATCGGTG CCGAGCGTGAGTCAGGCGTG	176
CottES19	$(CT)_{11}$	CCCCTTCCGTGAGCGACG ATATCACAGCACTTTTGGGGATG	144
Cott127	$(AC)_{14}$	TTCAGCTGCCTGTGTAAGGC TGTATGGAGCACTTGGGCTG	194
Cott687	$(CT)_{17}$	TGTACCTAGTGAGCCTGCTG CGTCAGCATCTACTGGGCAG	144
LCE29	$(TG)_9(T)(TG)_{22}(A)(GT)_6$	GTGGGGAGAATGACGGATGG TACACATGCATTTGGATTGACC	221
Cott635	$(TC)_8$	AGAAGGGCAACATAAGGGCC GAGGCGAGTGCGATGCCACC	169
Cott255	$(CT)_{10}$	TCACTACAGCCAGGTGTCTG GCATGTGCATGCCTCCAGAG	172
Cott582	$(GT)_{39}$	AGCTCTGGATGAGGACTGTC TCTGTGAGGTTCAGTGCGAC	233

Table 3b. PCR strategy: the multiplex technique used for microsatellite analysis.

Multiplex	Locus	Beckman dye	Range of sizes in base pairs
Quadplex I	Cott100	Cy5	165-233
	CottES19	Cy5	130-166
	Cott127	Cy5.5	183-211
	Cott687	Cy5.5	129-153
Diplex I	LCE29	Cy5.5	167-269
	Cott635	Cy5	166-352
Diplex II	Cott255	Cy5.5	148-214
	Cott582	Cy5	173-207

Table 4. Number and range of sizes (in base pairs, bp) of alleles by locus and population for microsatellite analysis of *Cottus* samples.

Locus	Cheakamus River	Squamish River	Mamquam River	Cultus Lake trawl	Frosst Creek
Cott100	22(165-229)	16 (169-231)	21 (165-233)	11 (179-209)	10 (173-209)
CottES19	12(132-166)	10 (132-158)	11 (132-166)	10 (130-158)	6 (132-146)
Cott127	7(183-203)	5 (183-191)	6 (183-193)	5 (183-193)	5 (183-203)
Cott687	8(129-147)	7 (131-153)	6 (131-145)	7 (137-145)	4 (139-145)
Cott255	11(148-176)	7 (152-178)	9 (152-184)	4 (164-174)	7 (162-204)
Cott635	14 (166-352)	11 (166-240)	13 (166-352)	11 (166-266)	10 (166-352)
LCE29	23 (173-247)	21 (173-241)	24 (167-247)	14 (173-259)	9 (181-249)
Cott582	9 (173-195)	6 (177-189)	8 (173-191)	7 (173-189)	6 (177-189)

Locus	Lake Washington	Windfall Creek	Watt Creek	Norrish Creek	Cultus Lake traps
Cott100	17 (173-229)	12 (175-209)	13 (173-213)	15 (169-225)	14 (175-211)
CottES19	6 (132-158)	5 (132-146)	9 (132-158)	11 (132-162)	8 (132-154)
Cott127	8 (183-211)	5 (183-193)	5 (183-195)	5 (183-191)	5 (183-195)
Cott687	6 (129-145)	5 (137-145)	3 (139-145)	4 (131-145)	4 (139-145)
Cott255	9 (162-192)	5 (162-174)	7 (162-184)	10 (152-214)	6 (152-174)
Cott635	13 (166-320)	8 (166-352)	11 (166-236)	8 (166-236)	13 (166-352)
LCE29	12 (173-259)	8 (181-249)	10 (179-249)	15 (173-269)	11 (175-251)
Cott582	8 (177-191)	8 (175-189)	8 (177-195)	10 (173-207)	7 (177-189)

Table 5. Descriptive statistics by locus and population for microsatellite analysis of *Cottus* samples. N is the number of samples, H_E is the expected heterozygosity, H_O is the observed heterozygosity, and A_R is allelic richness.

Population	Cott100	CottES19	Cott127	Cott687	LCE29	Cott635	Cott582	Cott255	Ave.
Cheakamus									
N	66	67	65	66	61	64	66	63	65
H_E	0.908	0.800	0.745	0.777	0.878	0.856	0.784	0.838	0.823
Ho	0.879	0.672	0.754	0.758	0.787	0.844	0.788	0.762	0.780
A_R	13.850	8.055	5.955	6.580	13.872	9.993	6.496	8.461	9.158
Mamquam									
N	48	48	48	48	44	47	48	46	47
H_E	0.919	0.823	0.720	0.734	0.893	0.873	0.821	0.812	0.824
Ho	0.833	0.750	0.688	0.729	0.909	0.766	0.708	0.848	0.779
A_R	14.555	8.512	4.898	5.643	15.371	10.461	6.902	7.325	9.208
Squamish									
N	31	31	30	30	32	32	31	31	31
H_E	0.882	0.830	0.618	0.796	0.904	0.868	0.805	0.775	0.810
Ho	0.774	0.613	0.733	0.733	0.906	0.750	0.742	0.839	0.761
A_R	12.657	8.584	4.739	6.523	16.098	10.061	5.911	6.059	8.829
Cultus trawl									
N	27	27	27	27	27	28	30	30	28
H_E	0.865	0.826	0.521	0.620	0.821	0.830	0.757	0.748	0.749
Ho	0.852	0.556	0.630	0.704	0.778	0.786	0.600	0.933	0.730
A_R	9.985	8.501	4.254	4.579	11.162	9.567	6.302	4.000	7.294
Frosst									
N	27	27	21	21	28	29	27	26	26
H_E	0.844	0.758	0.571	0.514	0.722	0.669	0.748	0.771	0.700
Ho	0.815	0.407	0.524	0.760	0.750	0.759	0.704	0.923	0.676
A_R	9.049	5.861	4.614	3.935	7.371	7.347	5.495	6.192	6.233
Lake Wash									
N	26	25	21	25	27	27	28	27	26
H_E	0.891	0.786	0.722	0.658	0.783	0.865	0.792	0.826	0.790
Ho	0.808	0.800	0.524	0.760	0.852	0.852	0.679	0.889	0.770
A_R	13.835	5.679	7.206	5.040	9.512	11.059	7.630	8.315	8.535
Windfall									
N	23	24	23	23	22	23	23	23	23
H_E	0.876	0.751	0.637	0.598	0.721	0.622	0.710	0.738	0.707
Ho	0.957	0.583	0.609	0.478	0.864	0.652	0.609	0.609	0.670
A_R	10.883	4.708	4.478	4.475	7.223	7.187	7.090	4.936	6.373
Watt									
N	29	28	29	29	28	28	30	29	29
H_E	0.878	0.783	0.596	0.561	0.736	0.656	0.779	0.754	0.718
Ho	0.931	0.607	0.655	0.690	0.750	0.714	0.667	0.724	0.717
A_R	10.839	7.515	4.169	2.975	7.829	8.660	6.914	6.103	6.876
Norrish									
N	26	28	24	26	17	19	27	25	24
H_E	0.896	0.847	0.578	0.654	0.863	0.850	0.833	0.810	0.791
Ho	0.923	0.714	0.625	0.615	0.824	0.842	0.593	0.720	0.732
A_R	12.802	9.582	4.412	3.885	15.000	7.982	8.468	8.593	8.841
Cultus traps									
N	27	28	26	26	24	26	24	24	26
H_E	0.892	0.769	0.663	0.570	0.771	0.792	0.736	0.760	0.744
Ho	0.963	0.607	0.769	0.539	0.667	0.846	0.708	0.792	0.736
A_R	12.168	7.037	4.617	3.617	9.219	10.420	6.315	5.627	7.378

Table 6. Results of tests of Hardy-Weinberg Equilibium by locus and population for microsatellite analysis of *Cottus* samples. Underlined probability values indicate significant differences from Hardy-Weinberg expectations.

Population	Cott100	CottES19	Cott127	Cott687	LCE29	Cott635	Cott582	Cott255
Cheakamus	0.5628	<u>0.0333</u>	0.7998	0.2940	0.3135	<u>0.0466</u>	0.4442	0.1249
Squamish	0.3926	0.2005	0.7923	0.6288	0.3620	<u>0.0149</u>	0.0900	0.5229
Mamquam	0.2071	<u>0.0014</u>	0.3019	0.0848	0.7268	0.2310	0.1623	0.9311
Trawl	0.5396	<u>0.0024</u>	0.5419	0.4077	0.7882	<u>0.0392</u>	<u>0.0445</u>	0.1181
Frosst	0.2417	<u>0.0002</u>	0.5498	0.3199	0.4550	0.4240	<u>0.0017</u>	0.3131
Lake Wash	<u>0.0124</u>	0.0889	0.0924	0.1924	0.4880	0.3292	0.0237	0.2422
Windfall	0.9103	0.1130	0.8011	0.1720	0.7390	0.8853	0.1839	0.4222
Watt	0.1301	<u>0.0181</u>	1.0000	0.4333	0.6977	0.6532	0.0738	0.1526
Norrish	0.6473	<u>0.0472</u>	0.6549	0.8342	0.4457	0.1885	<u>0.0270</u>	<u>0.0209</u>
Traps	0.4819	0.1223	0.3585	0.7819	0.3594	0.5462	0.5091	0.5056

Table 7. Results from tests of linkage disequilibrium for all populations, locus by locus, for microsatellite analysis of *Cottus* samples. There was no significant result over all populations, following Bonnferroni correction.

Locus	Locus	P value
Cott100	CottES19	0.071
Cott100	Cott127	0.380
CottES19	Cott127	0.895
Cott100	Cott687	0.881
CottES19	Cott687	0.361
Cott127	Cott687	0.036
Cott100	LCE29	0.987
CottES19	LCE29	0.956
Cott127	LCE29	0.980
Cott687	LCE29	0.416
Cott100	Cott635	0.986
CottES19	Cott635	0.943
Cott127	Cott635	0.999
Cott687	Cott635	0.423
LCE29	Cott635	0.900
Cott100	Cott582	0.813
CottES19	Cott582	0.992
Cott127	Cott582	0.555
Cott687	Cott582	0.959
LCE29	Cott582	0.648
Cott635	Cott582	0.854
Cott100	Cott255	0.567
CottES19	Cott255	0.666
Cott127	Cott255	0.981
Cott687	Cott255	0.306
LCE29	Cott255	0.948
Cott635	Cott255	0.702
Cott582	Cott255	0.430

Table 8. F_{ST} values for eight populations, using eight loci for microsatellite analysis of various *Cottus* samples. Underlined values are not significantly different from 0. The population in boldface represents the Cultus Lake pygmy sculpin. Samples in italics are found within the Cultus Lake drainage. Cheak = Cheakamus river, Mam = Mamquam river, Squam = Squamish river, Cultus = samples from fish caught by trawl and minnow trap in Cultus Lake, Wash = Lake Washington, Tributaries = Watt and Windfall creeks

Population	Cheak	Mam	Squam	*Cultus*	*Frosst*	Wash	*Tributaries*
Mamquam	<u>0.0037</u>						
Squamish	<u>0.0010</u>	<u>0.0030</u>					
Cultus	0.0384	0.0277	0.0445				
Frosst Creek	0.0475	0.0438	0.0577	0.0111			
Lake Washington	0.0425	0.0314	0.0459	0.0405	0.0552		
Tributaries	0.0511	0.0466	0.0536	0.0078	<u>0.0069</u>	0.0655	
Norrish	0.0113	0.0062	0.0106	0.0222	0.0346	0.0367	0.0414

Table 9. Assignment test results for individual *C. aleuticus* caught by minnow trap in Cultus Lake. Tribs=Tributaries (Watt and Windfall Creeks), Cultus=Cultus Lake pygmy population. "Percentage" refers to the relative level of confidence of assignment to the most likely population for each individual fish.

Most likely population	Percentage	Month captured
Cultus	55.7	May
Cultus	51.2	May
Cultus	85.2	May
Cultus	62.3	May
Cultus	86.4	June
Cultus	55.3	July
Cultus	65.5	July
Cultus	91.0	July
Cultus	92.4	July
Cultus	74.6	July
Cultus	69.5	July
Cultus	57.6	July
Cultus	83.2	July
Cultus	59.3	July
Tribs	90.9	July
Tribs	89.9	July
Tribs	98.2	July
Tribs	95.6	July
Tribs	76.4	July
Tribs	96.7	July
Tribs	61.4	July
Tribs	94.0	July
Tribs	94.8	July
Tribs	99.2	July
Tribs	76.7	July
Tribs	68.1	July
Tribs	96.0	July
Tribs	86.1	July
Tribs	91.5	July
Tribs	59.0	July

Table 10. Structure results for microsatellite analysis across the eight original sample localities (Cheakamus, Mamquam and Squamish Rivers; Cultus Lake and its three tributary streams: Frosst Creek and combined Watt and Windfall creeks; Lake Washington; and Norrish Creek). The *K* values are an average of ten separate runs. The value in bold and underlined represents the most likely number of populations.

K	Log likelihood
1	-10597.9
2	-10282.6
3	**-10167.1**
4	-10204.3
5	-10291.8
6	-10357.8
7	-10393.0
8	-10444.2
9	-10424.5
10	-10559.8

Table 11. Structure results for microsatellite analysis, across the three localities within the Cultus Lake drainage (Cultus Lake; Frosst Creek; and combined Watt and Windfall creeks). The *K* values are an average of ten separate runs. The value in bold and underlined represents the most likely number of populations.

K	Log likelihood
1	-3611.67
2	**-3585.12**
3	-3622.12
4	-3641.41
5	-3673.02
6	-3655.73
7	-3633.07
8	-3631.91
9	-3655.49
10	-3662.75

Table 12. Analysis of molecular variance of microsatellite DNA allele frequencies. The first analysis (Squamish vs. Cultus vs. others) compared the three Squamish drainage rivers (Cheakamus, Squamish and Mamquam) with Cultus Lake and its three tributaries (Frosst, Watt and Windfall creeks) and also with the other two systems (Norrish Creek and Lake Washington). The second analysis compared Cultus pygmy sculpin (caught by trawl and minnow trap in Cultus Lake) with its three tributaries (Frosst, Watt and Windfall creeks).

Grouping hypothesis	Variance component	Sum of squares	Percent variation	P value
Squamish vs. Cultus vs. other	Among groups	25.625	2.10	0.0029
	Among populations within groups	19.576	0.46	0.0000
	Within populations	1462.821	97.45	0.0000
Cultus lake vs. streams	Between groups	4.602	1.24	0.3402
	Among populations within groups	3.313	0.00	0.0166
	Within populations	477.133	98.77	0.0098
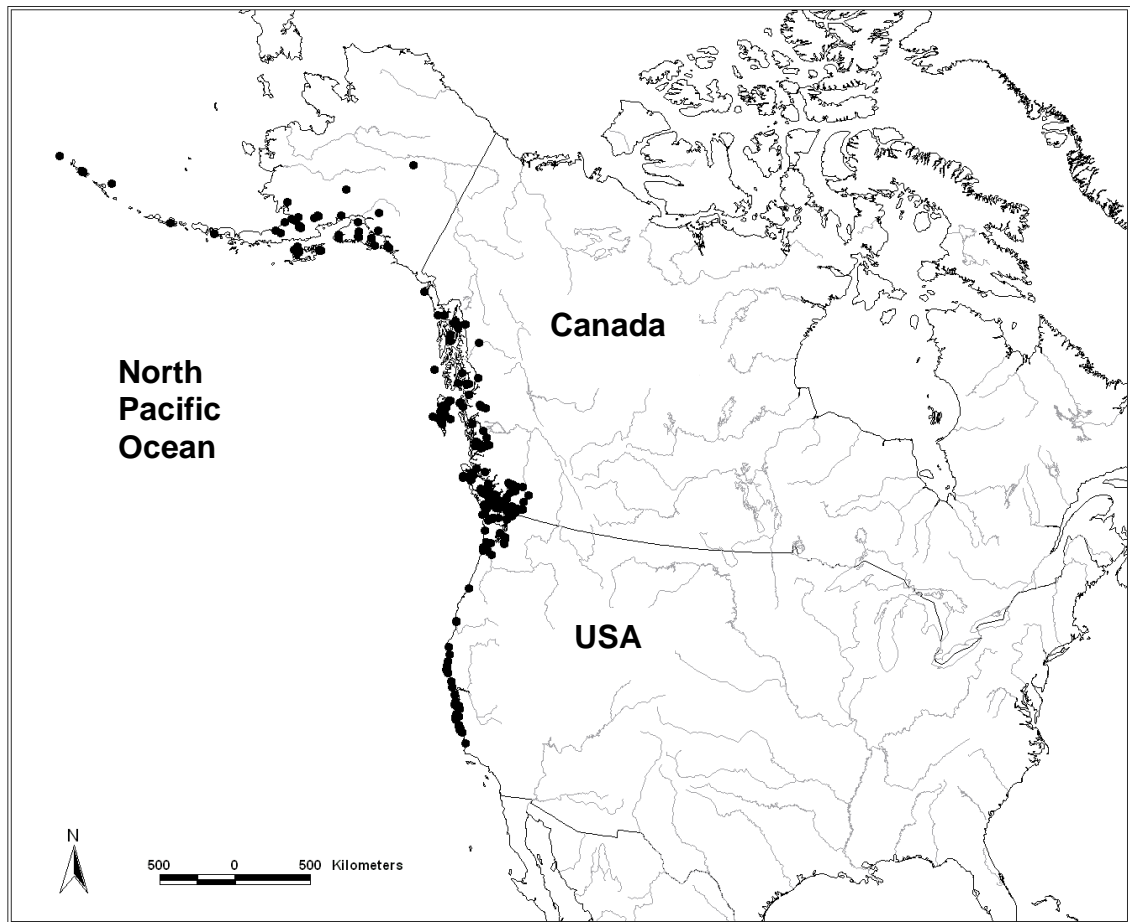
Figure 1. Global distribution of the coastrange sculpin based on 262 occurrence records obtained from the WFC (2006) and GBIF (2006) (from COSEWIC 2010).

Figure 2. A stream ecotype *C. aleuticus* (top), from Little Campbell River, compared with a Cultus pygmy sculpin (bottom). Both were identified as mature females by the presence of eggs.
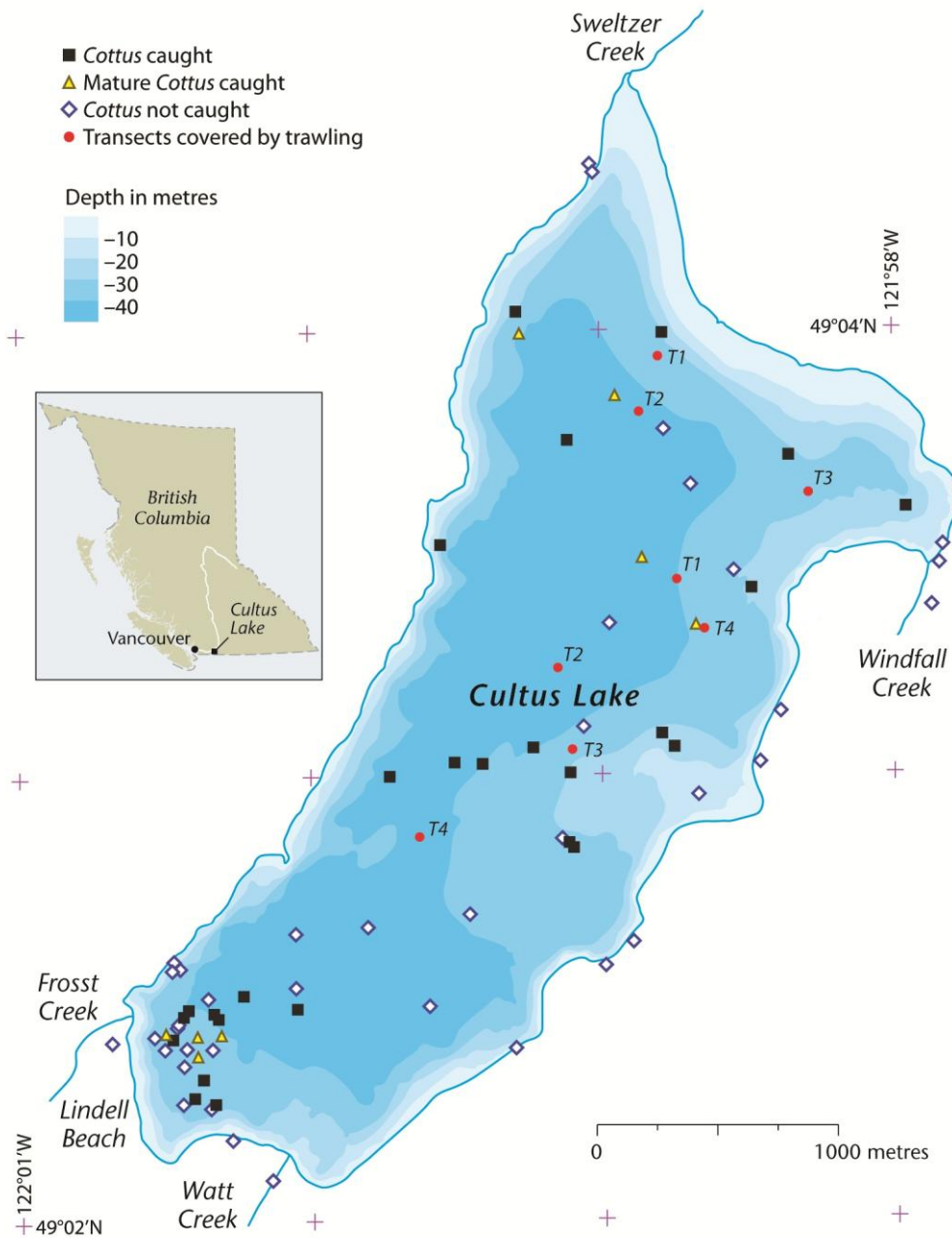
Figure 3. Cultus Lake bathymetric map (10 m intervals), modified from Shortreed (2007). Inset shows location of Cultus Lake within the boxed area in British Columbia. Mature *Cottus* were identified by the presence of eggs or testes. Trawl transects are denoted by T1-T4 (i.e., Trawls 1-4).
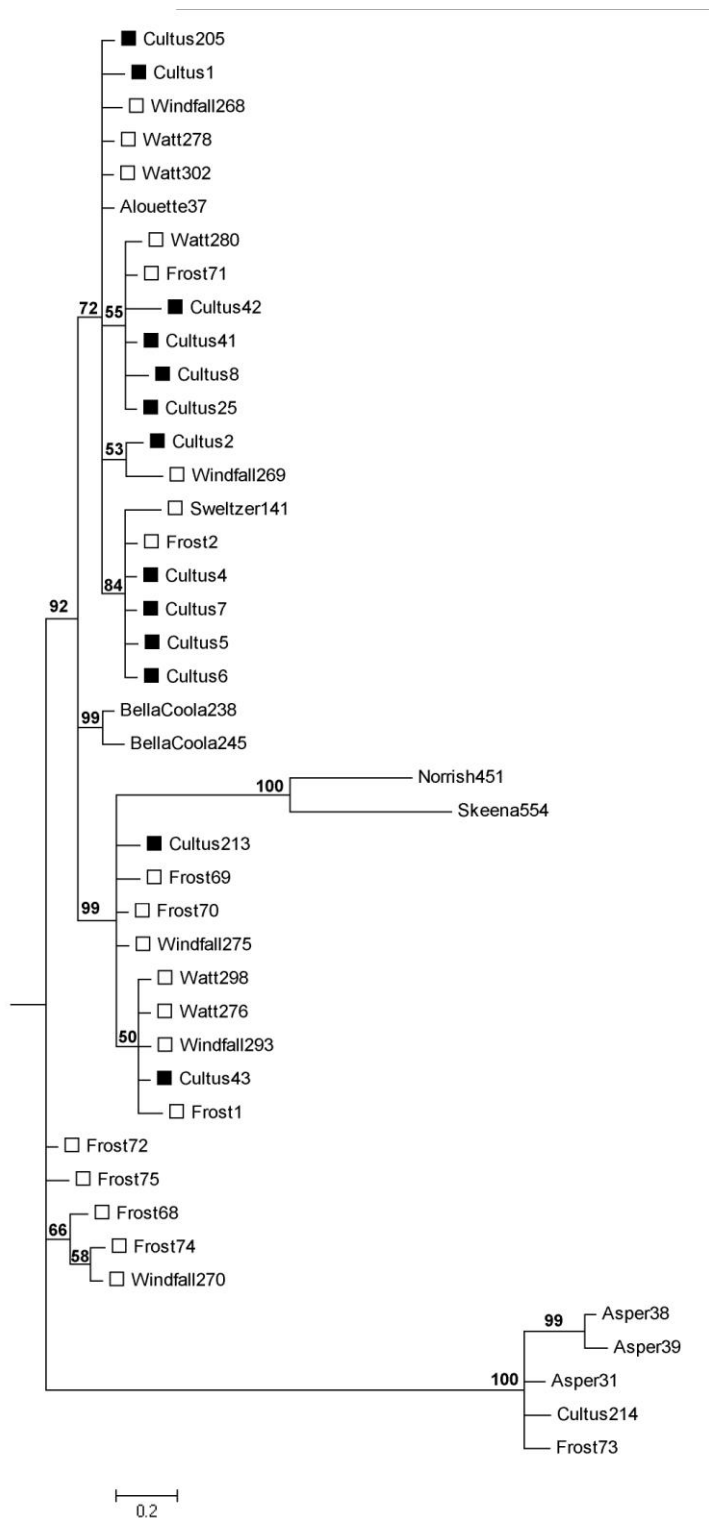
Figure 4. Phylogenetic analysis of mtDNA D-loop across 775 nucleotides under a GTR+I+G model of sequence evolution. Numbers represent the Bayesian posterior probability (%, 10,000 trees). Asper = *Cottus asper*. Sequences annotated with a filled square are Cultus pygmy sculpin, those with an open square are *C. aleuticus* from Cultus Lake tributary streams.
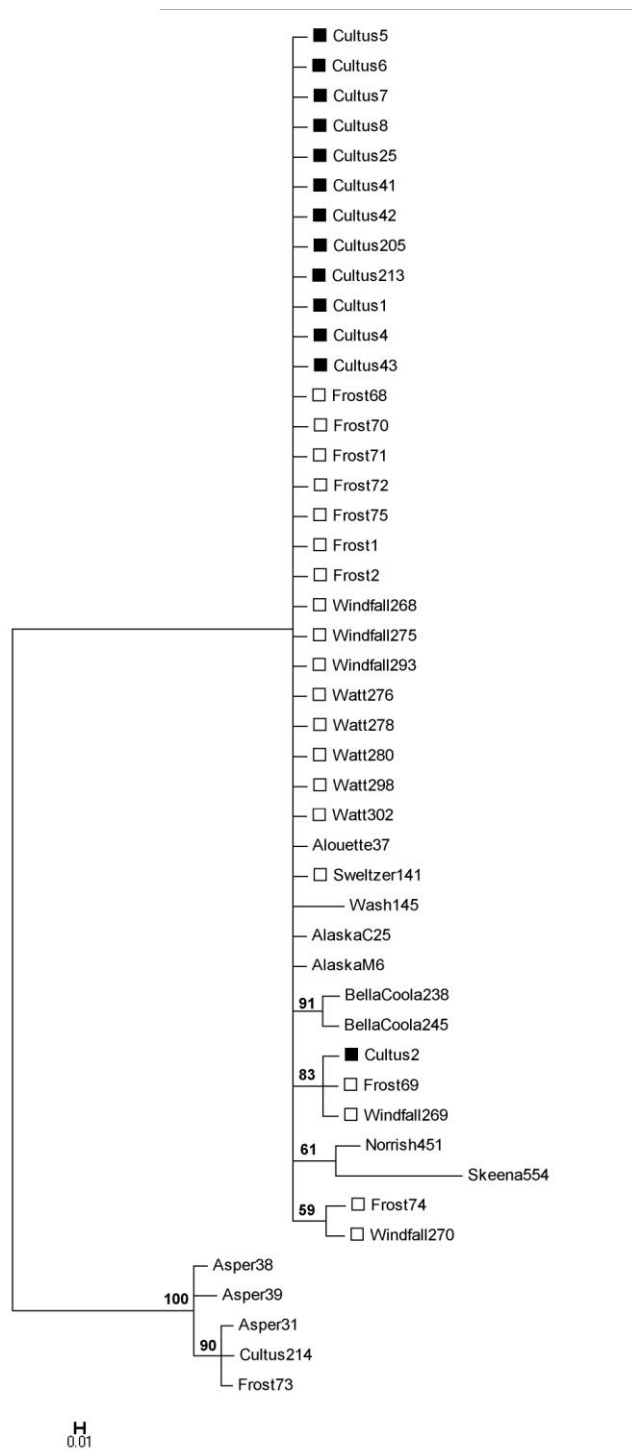
Figure 5. Phylogenetic analysis of mtDNA D-loop across 340 nucleotides under a GTR+I+G model of sequence evolution. Numbers represent the Bayesian posterior probability (%, 10,000 trees). Asper = *Cottus asper*. Sequences annotated with a filled square are Cultus pygmy sculpin, those with an open square are *C. aleuticus* from Cultus Lake tributary streams.
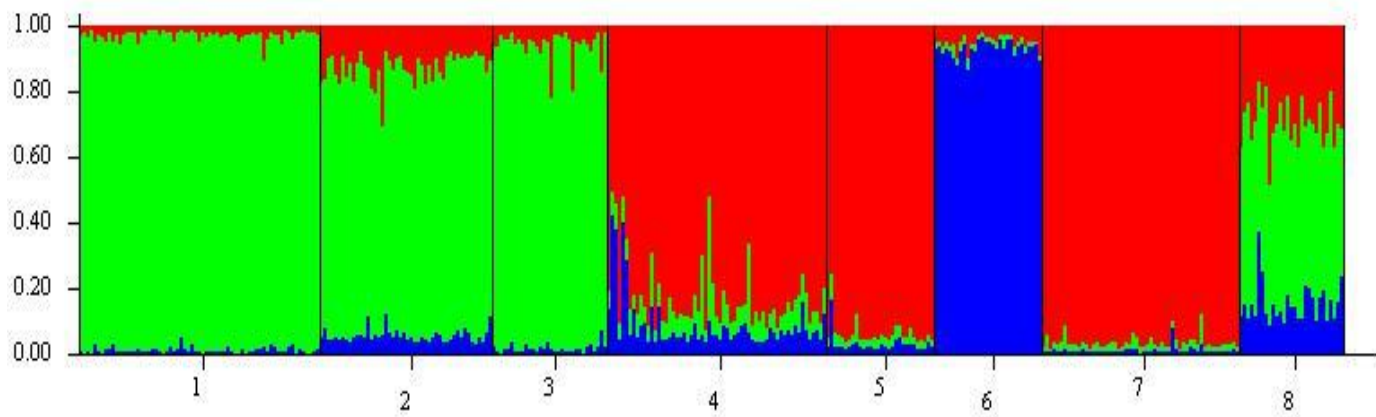
Figure 6. *Structure* results across eight localities and *K* = 3 genetic groups: green, blue, and red. Each fish is represented by a thin vertical line composed of one to three colours each of which represents the proportional contribution of each genetic group to a fish's genetic composition across eight microsatellite loci. In order from 1-8: 1 = Cheakamus River, 2 = Mamquam River, 3 = Squamish River, 4 = Cultus pygmy sculpin (trawl and minnow trap fish combined), 5 = Frosst Creek, 6 = Lake Washington, 7 = the two tributary streams (Watt and Windfall creeks), and 8 = Norrish Creek.
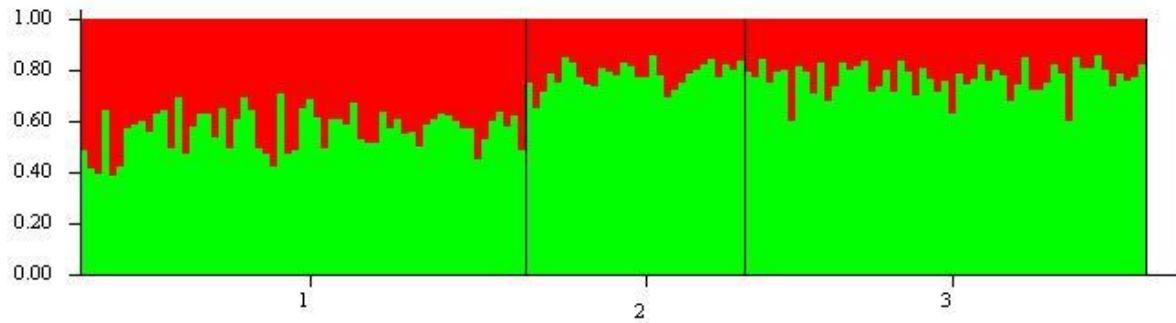
Figure 7. *Structure* results for Cultus Lake drainage samples and *K* = 2 genetic groups: red and green shading. Each fish is represented by a thin vertical line composed of one to two colours each of which represents the proportional contribution of each genetic group to a fish's genetic composition across eight microsatellite loci. In order from 1-3: 1 = Cultus Lake pygmy sculpin (trawl and minnow trap fish combined), 2 = Frosst Creek, and 3 = the two tributary streams (Watt and Windfall creeks).
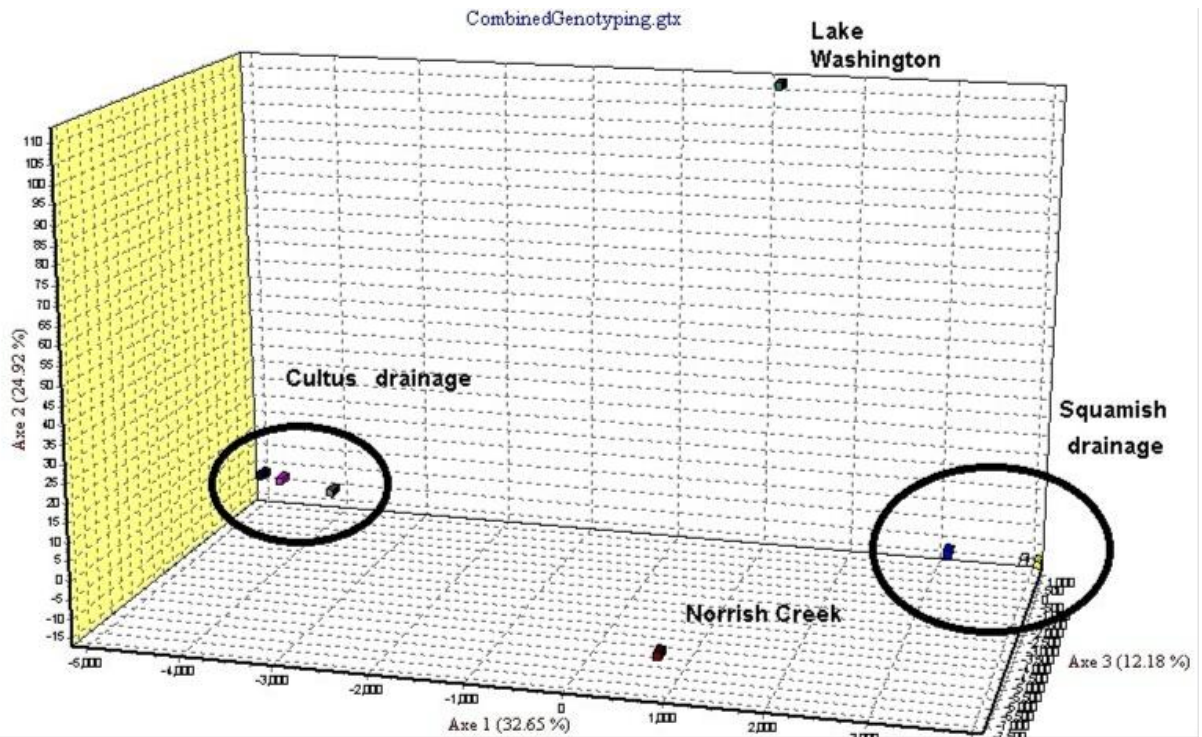
Figure 8. Factorial Correspondence Analysis (FCA) of variation across eight microsatellite loci among 350 *Cottus* samples (including *C. aleuticus* and the Cultus pygmy sculpin) collected from nine locations in 2006-2008. The three Squamish drainage populations, from left to right in the Squamish circle, include the Mamquam River (blue), the Squamish River (white), and the Cheakamus River (yellow). The three Cultus drainage populations, from left to right in the Cultus circle, include the two tributary streams (Watt and Windfall creeks) (brown), Frosst Creek (pink), and the Cultus pygmy sculpin (grey).

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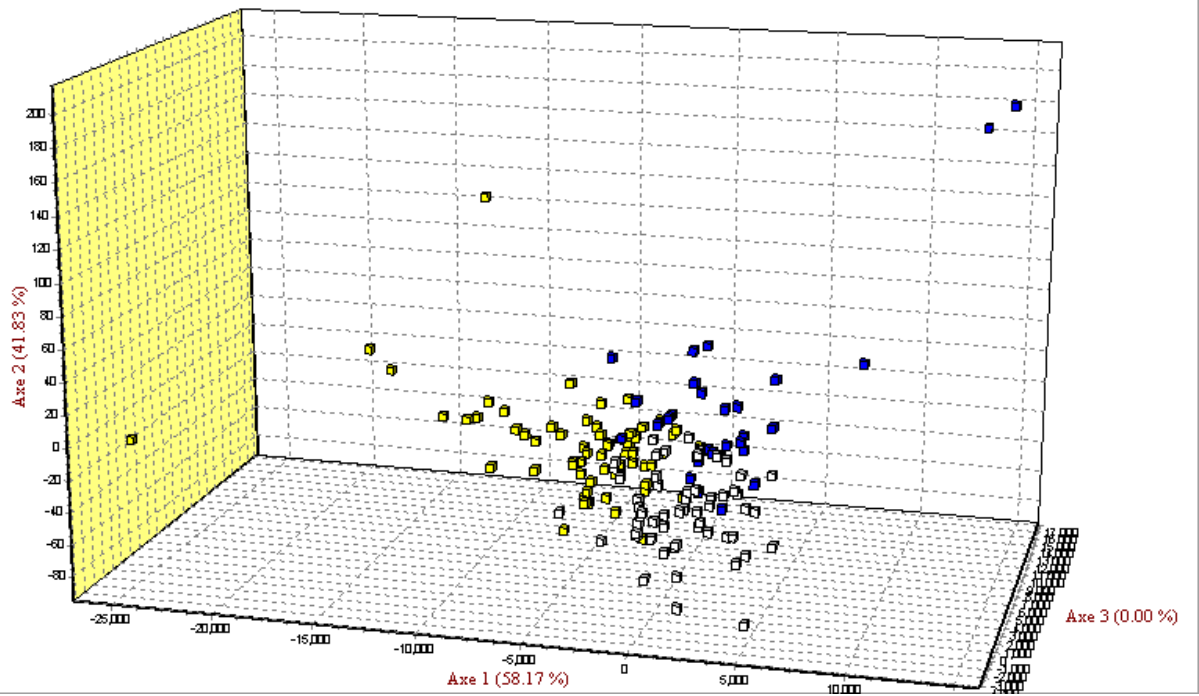
Figure 9. Factorial Correspondence Analysis (FCA) of variation across eight microsatellite loci between Cultus pygmy sculpin (N=61; yellow); Frosst Creek *C. aleuticus* (N=30; blue); and the two tributary streams (Watt and Windfall Creeks) (N=55; white).

Chapter 3: Habitat use and evidence for behavioural distinctiveness of the Cultus pygmy sculpin

Introduction

For a population to be a designatable unit (DU) under Canada's *Species-at-Risk Act* (*SARA*), it needs to have attributes that make it "discrete" and "evolutionarily significant" relative to other populations (COSEWIC 2009). One of the main criteria used to assess a DU is evidence of genetic distinctiveness, including inherited quantitative traits and neutral genetic markers (COSEWIC 2009). Quantitative traits include differences in morphology, life history and behaviour. These are important to assay when possible because molecular markers do not measure all potential genetic differences (e.g., behaviour was used to show reproductive isolation in cichlid fishes, Stauffer et al. 1995). Once a population is considered discrete, the significance of such discreteness to the evolutionary legacy of the species is then assessed. One way to assess the significance is if the discrete population persists in "an ecological setting that is unusual or unique to the species, such that it is likely to have given rise to local adaptations" (COSEWIC 2009). For my study, I compared habitat data from Cultus Lake with other lakes within the *C. aleuticus* range, and also conducted depth selection experiments in the lab to test the hypothesis that the limnetic life history of Cultus pygmy sculpin is associated with distinct habitat features of Cultus Lake and with differences in behaviour that distinguish Cultus pygmy sculpins from typical stream-dwelling forms.

Habitat

Sculpins (*Cottus* spp., or "cottids") are an important component of freshwater ecosystems in northwestern North America (Tabor et al. 2007), and in some habitats can be the most abundant species of fish (Bond 1963). For example, 73% of the fish biomass in Lake Washington has been estimated to be comprised of cottids (Eggers et al. 1978). When more than one species is present, sculpins tend to be spatially segregated; this segregation can occur both in different stream habitats (i.e., Taylor 1963) and at different depths throughout a lake (Madenjian and Bunnell 2008).

Coastrange sculpin (*C. aleuticus*) are most commonly found in riffle habitat in streams (Taylor 1963; Mason and Machidori 1976; Ringstad 1982; White and Harvey 1999), but have also been captured along the shores of lakes (Tabor et al. 2007). In streams, prickly sculpin (*C. asper*) tend to predominate in pool habitats, but are also found in the deep benthic habitats of lakes (Tabor et al. 2007). It is believed that both species have a planktonic larval stage; however, previous studies on both species have been limited to a few streams in coastal BC, and much remains unknown (McPhail 2007). The range of *C. aleuticus* extends along the mainland coast of BC, where the lakes tend to have a low dissolved solid content, which suggests that they are oligotrophic (Northcote and Larkin 1963). Total dissolved solid content of the water appears to be the most important single factor determining the general level of productivity in lakes throughout BC (Northcote and Larkin 1963). Pelagic sculpin, however, have been found in the deep offshore areas of productive lakes (i.e., Lake Washington and Cultus Lake; Arhonditsis et al. 2003; Shortreed 2007), in the presence of large numbers of *C. asper* (Tabor et al. 2007).

Behaviour

An important goal of evolutionary biology is to investigate the processes that lead to the divergence of natural populations along differential axes of resource exploitation (Schluter 1993; Rogers et al. 2002). Adaptive radiation (phenotypic divergence often leading to speciation) is the outcome of divergent natural selection, which can be promoted by resource heterogeneity and competitive interactions (Schluter 1993; Taylor and McPhail 1999; Rogers et al. 2002). The development and maintenance of specializations in water-column use and diet are important in the understanding of the initial stages of population divergence in freshwater fishes (Schluter 1993; Rogers et al. 2002). Adaptive behavioural differences have been observed between populations in terms of habitat isolation and predator avoidance (e.g., Rogers and Bernatchez 2007).

In contrast to *C. aleuticus*, the Cultus pygmy sculpin has only been found in the deep, offshore areas of Cultus Lake, similar to the pelagic *C. aleuticus* found in Lake Washington (Ikusemiju 1975; Larson and Brown 1975; Tabor et al. 2007). Ikusemiju (1975) suggested that the Lake Washington pelagic sculpins use the water column for feeding on planktonic invertebrates and predator avoidance. Cultus Lake has the highest productivity of any sockeye salmon nursery lake in B.C., with an unusually high proportion of *Daphnia* that are present throughout the year and occur below the thermocline (Shortreed 2007). *Daphnia* spp. are planktonic cladocerans and are the main food item of the Cultus pygmy sculpin (Ricker 1960). The deep benthic areas of Cultus Lake also support *C. asper*, a bottom-dwelling ambush feeder (Broadway and Moyle 1978) that feeds on smaller fish (Gadomski and Parsley 2005; McPhail 2007). The morphology of the Cultus pygmy sculpin includes larger cephalic pore sizes,

shorter pelvic fins, and higher pectoral fin ray counts, which could be adaptations for

sensory perception and for maintaining position in the water column during a

planktivorous lifestyle (COSEWIC 2010). Similar morphological characteristics are

exhibited by pelagic *C. aleuticus* in Lake Washington (Larson and Brown 1975) and by

the highly specialized limnetic sculpins of Lake Baikal (genera *Comephorus* and

Cottocomephorus, Kontula et al. 2003).

Behavioural differentiation can be influenced by the niches occupied by different

ecotypes (i.e., limnetic vs. benthic, stream vs. lake) and the difference in diet that can

result (Rogers et al. 2002). The limnetic ecotype present in many freshwater fishes is

generally smaller, feeds on pelagic prey, and should therefore occupy higher positions

in a water column (e.g., McLaughlin et al. 1994, Rogers et al. 2002). The Cultus pygmy

sculpin is smaller and consumes *Daphnia*, a pelagic invertebrate (Ricker 1960), and

thus I predicted that it should show behavioural differences that are associated with

greater use of the water column in Cultus Lake relative to typical stream-dwelling

forms of *C. aleuticus*.

The Cultus pygmy sculpin is listed as threatened under *SARA*, but its

qualification as a valid DU has been more inferred than demonstrated. Behavioural

studies have been used to distinguish between species, and populations that exhibit

distinctive behaviours have qualified as Evolutionarily Significant Units (ESU, analogous

to DU; e.g., Stauffer et al. 1995). My study used data collected from the field and

behavioural assays in the lab to determine whether the Cultus pygmy sculpin does

inhabit a unique ecological setting, with different behaviour than *C. aleuticus* found in

streams. The data in this chapter, therefore, complement the molecular genetic assessments of distinctiveness that I conducted in Chapter 2.

My first objective was to determine the general habitat distribution of the Cultus pygmy sculpin in Cultus Lake. There have been no studies conducted on finding the habitat of the Cultus pygmy sculpin; Ricker (1960) believed the pygmy sculpin to be found offshore because it was only found in the stomachs of a deepwater, offshore fish (bull trout). The Cultus pygmy sculpin has been captured as incidental bycatch in DFO trawl surveys; these surveys were focussed on catching sockeye salmon and also swept a distance of approximately 1.5 km, so the precise location of capture would be unknown.

My second objective was to determine whether there were any behavioural differences between the lacustrine Cultus pygmy sculpin and the fluvial coastrange sculpin. I examined DFO trawl survey records, to determine whether there were any other examples of pelagic sculpin in other sockeye lakes in BC. I also conducted a behavioural study of depth selection in the laboratory, to determine whether any behavioural differences observed in the field could be quantified.

Materials and methods

Fish samples

Fish were collected from the offshore area of Cultus Lake, and from Frosst Creek and the Little Campbell River (Table 13, Figure 3, Appendix 1). Frosst Creek is the main inlet to Cultus Lake (Figure 3) and the Little Campbell River flows into Semiahmoo Bay, just south of the Fraser River estuary. The streams were sampled using an

electrofisher (Smith-Root Model 12-B). The shoreline was sampled using minnow traps

deployed from shore, while the pelagic area was sampled using minnow traps

suspended off the bottom of the lake by a float, 10 m apart, and at depths that ranged

from 10-40 m. The minnow traps were baited with sockeye salmon (as sockeye salmon

carcasses are present in the lake at spawning time) and Cyalume yellow 12 hour "glow

sticks" which have been shown to help attract deepwater sculpin, *Myoxocephalus*

thompsonii (Sheldon et al. 2008). The collection localities were determined using a

GPS unit (Garmin 12 GPS) and mapped using ArcMap in ArcGIS (ESRI) (Figure 3).

Mature female fish were identified by the presence of eggs. One mature male was

identified by the presence of testes. The Little Campbell River was chosen as a

population outside Cultus Lake that represents the typical stream-dwelling form of *C.*

aleuticus; as the river flows directly into the Pacific Ocean, it is unlikely there is any

gene flow between sculpins from Little Campbell River and the Cultus Lake drainage.

To sample fish for the behavioural assays, the minnow traps in Cultus Lake were

deployed in the same area as that swept by the trawl net on the Fisheries and Oceans

Canada (DFO) juvenile sockeye salmon trawl surveys (Figure 3), and the fish were

captured in May, September and October. I did not sample fish during low water periods

in the summer in order to minimize the capture of tributary fish that appear to take

refuge in the lake when their streams dry-up (Chapter 2). Live fish were brought back

to an environmental chamber in the Biological Studies building at the University of

British Columbia (UBC), and were kept in 136 litre aquaria, with approximately 10-15

fish per tank. Fish from Cultus Lake, Frosst Creek, and the Little Campbell River were

maintained in separate aquaria at 14-16 degrees C for about one month before the

behavioural experiments began.

Behavioural assays

Two "drop tanks" 1.8 m in height and 0.3 m square were used for the depth selection experiments. For all experiments, the tanks were placed in an environmental chamber with white blinds on three sides (Houtman and Dill 1994). The temperature in the environment chamber was maintained at 14-16 degrees C. In the first experiment, each trial consisted of one fish being placed in each of the two drop tanks (the "focal" fish). These fish were allowed to acclimate to the tanks for approximately 30 minutes while the camera was set up, and were then filmed with a web cam (Logitech QuickCam) attached to a laptop (Toshiba Windows XP) for 30 minutes. In the second experiment, one sculpin from one of the three populations and one *C. asper* were placed in a tank together. The prickly sculpin (*C. asper*) will predate on smaller sculpins and can out-compete them for territory (McPhail 2007). The average size (total length) of the Cultus pygmy sculpins used in the experiments when they were alone was 55 mm (SD = 4.2) and their average size when studied with *C. asper* was 62 mm (SD = 8.3) (the increase in size was due to growth while in the laboratory aquaria, and due to mortality of the smaller fish). The average size of the Frosst Creek sculpins used in the experiments when they were alone was 62 mm (SD = 8.4) and their average size when studied with *C. asper* was 74 mm (SD = 5.5). The average size of the Little Campbell River sculpins used in the experiments when they were alone was 86 mm (SD = 11.8) and their average size when studied with *C. asper* was 85 mm (SD = 10.0) (the smaller average size was due to mortality). The average size of *C. asper* used in the experiments with the three sculpin populations was 112 mm (SD = 2.1). In all experiments and trials, the depth at which each focal fish was observed was recorded

every minute, and then averaged for each fish across 30 measurements per trial (Rogers and Bernatchez 2007). For each observation, I also recorded the number of times a focal fish was observed to be off the bottom of the tank, regardless of the actual depth selected.

The paired data (without predator – with predator) for depth selected and counts off the bottom for each population from the two experiments were compared using the paired t-test (Whitlock and Schluter 2009) in R v2.8.1 (R Development Core Team 2008). A single factor ANOVA comparing the differences between treatments among the three populations was used to determine whether the depth selection and number of times off the bottom were significantly different among the treatment groups of fish (Whitlock and Schluter 2009). The Tukey-Kramer test was used to determine which pairs of populations had behaviours significantly different from the others (Whitlock and Schluter 2009).

Results

Habitats used by sculpins in Cultus Lake

Cultus pygmy sculpin were caught in minnow traps set offshore in the benthic habitat rather than in the littoral habitat of the lake (Figure 3, Appendix 1). Most fish were captured either on the bottom or within 10 m of the bottom, with some fish being caught up to 30 m above the bottom (Appendix 2). Cultus pygmy sculpin were captured every month from May-October, 2008 (Table 14, Appendix 2). The highest catches occurred in July and August, most likely due to recruitment from young-of-the-year as the average total length of the fish was lowest during this period (Table 14). Prickly

sculpin (*C. asper)* were also captured in minnow traps, along with the Cultus pygmy sculpin and, in two cases, large prickly sculpin had pygmy sculpin protruding from their mouths. Mature Cultus Lake pygmy sculpin, including eight females and one male, were captured from May-July, 2008 (Figure 3).

Midwater trawl surveys for sockeye were conducted by DFO in 62 lakes in BC (eg., Hume and MacLellan 2000 for methods). These data indicated that in only three of these lakes were mature-sized *C. aleuticus* fish (i.e., > 40 mm; Ruzycki et al. 1998) caught in the water column (Table 15). Sculpins were caught more often in Cultus Lake, and the catches consisted of a range of size and age classes, compared to the other two lakes. The species identification of sculpin in Red Bluff Lake has not been confirmed, however, this lake is within the *C. aleuticus* range. Both Red Bluff and Pitt Lakes are deeper than Cultus Lake (80 m and 143 m maximum depths, respectively) and are also less productive (Slaney 1988; Bodtker et al. 2007), making them less likely to support a pelagic population. The number of mature-sized fish in Cultus Lake is a conservative estimate, as only an average and range of sizes were included, due to the large number of sculpin caught in each trawl; the lengths of all fish were recorded for the other systems, due to the small number of sculpins caught per survey, and so these numbers are an exact count. Large numbers of mature-sized sculpin have only been caught in the water column of Cultus Lake, compared with the other sockeye lakes surveyed by DFO.

Depth selection of sculpins

When the fish were first introduced to the tank, their behaviour was variable, ranging from immediately heading to the bottom and staying there, to swimming around

at different depths in the tank. No trends in initial behaviour were observed for any population (i.e., each individual fish seemed to have its own behavioural pattern). There was no apparent difference in behaviour between 0-30 minutes (after camera set-up) and 30-90 minutes, so the filming took place in the first thirty minutes (Rogers et al. 2002; Rogers and Bernatchez 2007).

In comparisons within each population, the Cultus pygmy sculpin population did not show a significant difference in its average depth selection (mean difference =-10.76cm, degrees of freedom (df) = 22, sample size (n) = 23, p = 0.3020) or the number of times off the bottom (mean difference = 0.78, df = 22, n = 23, p = 0.75) when the predator was present or absent (Table 16, Figures 10-13). However, Frosst Creek and Little Campbell River fish exhibited significantly lower depth selection in the presence of the predator and also went off the bottom a significantly fewer number of times (Table 16, Figures 10-13). Frosst Creek fish had a more significant change in behaviour (mean difference in depth = 24.35, df = 30, n = 31, p = 0.0067; mean difference in times off the bottom = 7.52, df = 30, n = 31, p = 0.00011) than did the fish from Little Campbell River (mean difference in depth = 2.63, df = 11, n = 12, p = 0.038; mean difference in times off the bottom = 1.92, df = 11, n = 12, p = 0.043).

The differences in depth distribution and number of times fish were observed off the bottom were significantly different between the three populations (p = 0.0049 and p = 0.029, respectively). Cultus pygmy sculpin and fish from Frosst Creek had the most significant differences in their depths (p = 0.0038) and the time spent off the bottom (p = 0.033). Fish from Little Campbell River also differed from Cultus pygmy sculpin in depth (p = 0.024) and number of times off the bottom (p = 0.038). However, the behavioural

changes between Frosst Creek and Little Campbell River fish were not significantly different for their depth and time off the bottom (p = 0.22 and p = 0.20, respectively).

Discussion

Pelagic coastrange sculpin have been found in two productive lakes. Northcote and Larkin (1956) found a positive relationship between abundances of plankton and fish with the amount of total dissolved solid content in lake waters, with the relationship strongest in lakes of low mean depth (Northcote and Larkin 1963). The majority of coastal lakes in BC have low dissolved solids and a low level of oxygen depletion, which characterizes oligotrophic lakes (Northcote and Larkin 1963). Cultus Lake, however, has the highest amount of total dissolved solids recorded of any BC sockeye salmon nursery lake (Shortreed 2007) and is classified as mesotrophic. By comparing Lake Washington and Cultus Lake with other lakes found within the range of *C. aleuticus*, it appears that pelagic sculpins tend to be found in shallow (< 70 m maximum depth, ~ 30 m mean depth), productive (mesotrophic) lakes. The low number of such lakes along the BC coastline that overlap with the distribution of *C.aleuticus* perhaps explains why pelagic sculpins are found only in Cultus Lake in BC.

Studies comparing different ecotypes have found differences in depth selection or habitat in correlation with preferred prey (Schluter 1993; Skulason et al. 1996; Rogers et al. 2002). Rogers et al. (2002) found that dwarf whitefish (*Coregonus clupeaformis*) stayed higher off the bottom in laboratory experiments than the normal form; this result was expected, as the dwarf ecotype was smaller and had a diet of limnetic zooplankton prey. When a benthic predator (*C. asper*) was introduced to the tank, the smaller Cultus Lake sculpin did have a higher average depth selection than

the other two populations of fish and remained up in the water column instead of decreasing its depth and movement. Smaller benthic fish in streams will leave deeper pools and head to shallower water in the presence of predators (Magoulick 2004); smaller fish also tend to get displaced from refuges by larger species (Rahel and Stein 1988; McNeely et al. 1990). In comparison to the stream populations, the different behaviour of the Cultus pygmy sculpin could, therefore, be a result of a response to move to shallow (closer to the surface) water to escape predation, or a response to being forced off the bottom by the larger and perhaps more aggressive prickly sculpin. Larger competitors and predators are both capable of forcing smaller fish out of deep water habitats (Schlosser 1987; Lonzarich and Quinn 1995); fish are found to occupy deeper habitats as they increase in size (Lonzarich and Quinn 1995), which could explain why the Cultus pygmy sculpin, which were the smallest fish tested, were found more often in the water column than fish from the other, larger populations.

Adaptation to local environments is a major factor promoting divergence among populations (Taylor 1991; Cousyn et al. 2001; Kawecki and Ebert 2004). For example, changes in fish predation levels have been shown to increase genetic differentiation in a predator-avoidance trait in *Daphnia magna* (e.g., Cousyn et al. 2001). Predator presence has also induced a change in morphology in Crucian carp (*Carassius carassius*), resulting in an increased locomotor response (e.g., Domenici et al. 2007). Differences between benthic-limnetic morphs in a variety of species complexes have been shown to be genetically based (Schluter 1993; Skulason et al. 1996; Rogers et al. 2002). The morphological differences in the Cultus pygmy sculpin (COSEWIC 2010), combined with the predation pressures unique to their lacustrine life history, could result

in the observed difference in escape response when confronted with a larger, benthic predator.

Behavioural characters can be used to identify different taxa (Trewavas 1983) and to estimate the phylogenetic relationships amongst taxa (Esteve and McLennan 2007). In some cases, behaviour has been shown to be as useful as morphology in estimating phylogeny (de Queiroz and Wimberger 1993) and can be used to resolve ambiguities in molecular data (Esteve and McLennan 2007). Ecologically significant traits, such as changes in behaviour, can lead to divergence through natural selection (Schluter 2001), and can be present even when there is no difference in allele or haplotype frequency at neutral loci between populations (e.g., Legge et al. 1996; Fraser and Bernatchez 2001). Individual genetic strains of paradise fish (*Macropodus opercularis*) have been characterized by stable, heritable differences in behaviour (Gervai and Csanyi 1985). The Cultus pygmy sculpin displayed a different behaviour in the presence of a predator than two different stream populations of *C. aleuticus*, which could indicate an adaptive difference to its environment and associated genetic change.

Fish such as sculpins that have cryptic colouration typically respond to predators by reducing their movements (McNeely et al. 1990). The saddle patches on freshwater sculpins (Armbruster and Page 1996), combined with their ability to change colour to match their substrate (Houtman and Dill 1994; Whiteley et al. 2009), suggest that sculpins rely on disruptive colouration and crypsis to reduce predation pressure. The coastrange sculpins from the two stream populations that I tested had low average depth and stayed on the bottom when *C. asper* was present, as would be expected from a fish that relies on cryptic behaviour to escape predation. In contrast to this response, Cultus pygmy sculpin went up into the water column when a predator (*C.*

asper) was present. As *C. asper* is a larger sculpin, it would likely be able to outcompete the Cultus pygmy sculpin for any refuge on the bottom and is also a known predator on Cultus Lake sculpin (McNeely et al. 1990). In fact, it is unusual for coastrange sculpin (*C. aleuticus*) to be found in large numbers in habitat occupied by prickly sculpin (*C. asper*); in streams, the two species tend to spatially segregate, with prickly sculpin favouring areas such as pools with slower flow, while coastrange sculpin are more commonly found in the faster flowing, riffle habitat (Taylor 1963). In lakes, *C. aleuticus* can be found along the shoreline; however, smaller pelagic sculpin have been found in the deep benthic habitat of Lake Washington (Tabor et al. 2007). Similar to the Lake Washington pelagic sculpin, Cultus pygmy sculpin are also found in the offshore benthic habitat, and may be captured in minnow traps along with prickly sculpin. My data and these observations suggest, therefore, that in addition to lake productivity an important factor in the evolution of a limnetic life history of the Cultus pygmy sculpin could be predation/competition from sympatric prickly sculpin.

Future studies

Several additional lines of investigation could provide more precise information on behaviour and habitat use. For instance, the behavioural depth selection experiment tested for possible differences between stream- and lake-dwelling fish and the results were quite variable within treatment groups. One way to eliminate the large amount of variance would be to take measures at thirty second (or possibly even smaller) intervals, as this method would provide more measurements of depth to reduce variance. Increasing the number of individuals per population would also reduce the standard error (Whitlock and Schluter 2009). Previous work on escape response in sculpin involved changing the colour of the substrate to something that sculpin were unable to match (Houtman and Dill 1994); the only substrate used in my experiment was grey, the colour of sand or gravel, which would promote cryptic behaviour by sculpin and would have constrained their willingness to leave the bottom (Houtman and Dill 1994).

In addition, during my surveys Cultus pygmy sculpin were only captured in the offshore areas of Cultus Lake, which agreed with the findings of Ricker (1960). Mature fish were also captured, and appeared to be mainly found closer to the shore, and at shallower depths (similar to the findings of Ruzycki et al. 1998); the dates and locations of these captures can assist in the search for spawning locations which, along with precise spawning timing, is still unknown. My work focussed on determining the status of the Cultus pygmy sculpin as a designatable unit; critical habitat still needs to be identified to allow recovery planning. It is also unknown whether larval Cultus pygmy sculpins have a pelagic stage in the water column (as many stream-dwelling *Cottus*

aleuticus do), or are more benthically-oriented as juveniles; this information is needed to determine the habitats used by all life stages, so all stages can be protected.

In conclusion, the Cultus pygmy sculpin has only been found in one lake in BC, which has a high level of productivity. The Cultus pygmy sculpin uses the water column both for feeding on *Daphnia*, a pelagic invertebrate, and probably as a refuge from the benthic and predacious *C. asper*, instead of relying only on its cryptic colouration. The Cultus pygmy sculpin is found in an unusual ecological setting, and displays a distinctive behavioural response, different from that of the stream *C. aleuticus*, to a common predator (the prickly sculpin); the data from this chapter strongly suggests that the Cultus pygmy sculpin qualifies as a DU under *SARA*.

Table 13. Locations and sample size of the populations of *Cottus aleuticus* used for the depth selection study.

Location	Watershed code	Drainage	N
Cultus Lake	100-065700-09700-13300	Chilliwack	25
Frosst Creek	100-065700-09700-13300-9760	Chilliwack	32
Little Campbell River	900-000500	Semiahmoo Bay	27

Table 14. Number of Cultus pygmy sculpins captured by month using stratified minnow traps in 2008, and the average length of the fish captured.

Month	Number of sculpins caught	Average total length (mm, range)
May	22	50.73 (43-65)
June	28	47.21 (30-59)
July	53	33.74 (18-58)
August	44	39.05 (15-63)
September	13	56.08 (44-62)
October	7	52.29 (47-58)

Table 15. The number of mature-sized (> 40 mm total length) sculpin caught in DFO mid-water sockeye trawls throughout the province of BC. Of the 62 total lakes surveyed, only four caught sculpin that were the size of mature fish. Due to the large number of sculpin caught in Cultus Lake in some of the trawls, lengths were not recorded for all fish, and so the number of mature fish caught in Cultus Lake is a conservative estimate. The sculpin caught in Pitt and Red Bluff lakes were not all identified to species, so it is possible that other species (i.e., *C. asper*) are included, and this number is likely an overestimate of mature *C. aleuticus* sampled from those lakes.

Lake name	Total number of surveys	Number of surveys where sculpin caught	Number of mature-sized sculpin caught
Cultus	89	64	48
Pitt	41	16	3
Red Bluff	1	1	4

Table 16. The average depth and number of times off bottom, with standard deviation for each mean in brackets, shown by the Cultus pygmy sculpin (Cultus) and the *C. aleuticus* from the streams (Frosst = Frosst Creek; LCR = Little Campbell River). Experiment one used a "focal fish", or one fish per tank; experiment two used a "focal fish" with a *C. asper* in the tank.

Population	N	Average depth (focal fish; cm off bottom)	Average number of times off bottom (focal fish)	Average depth (focal fish with *C. asper;* cm off bottom)	Average number of times off bottom (focal fish with *C. asper*)
Cultus	25	16.0 (20.5)	7.8 (7.6)	23.1 (41.4)	6.3 (7.6)
Frosst	32	28.9 (34.1)	9.8 (8.9)	5.5 (10.4)	2.6 (3.4)
LCR	27	5.4 (7.4)	4.6 (5.8)	0.5 (1.3)	0.8 (2.1)

Without C.asper
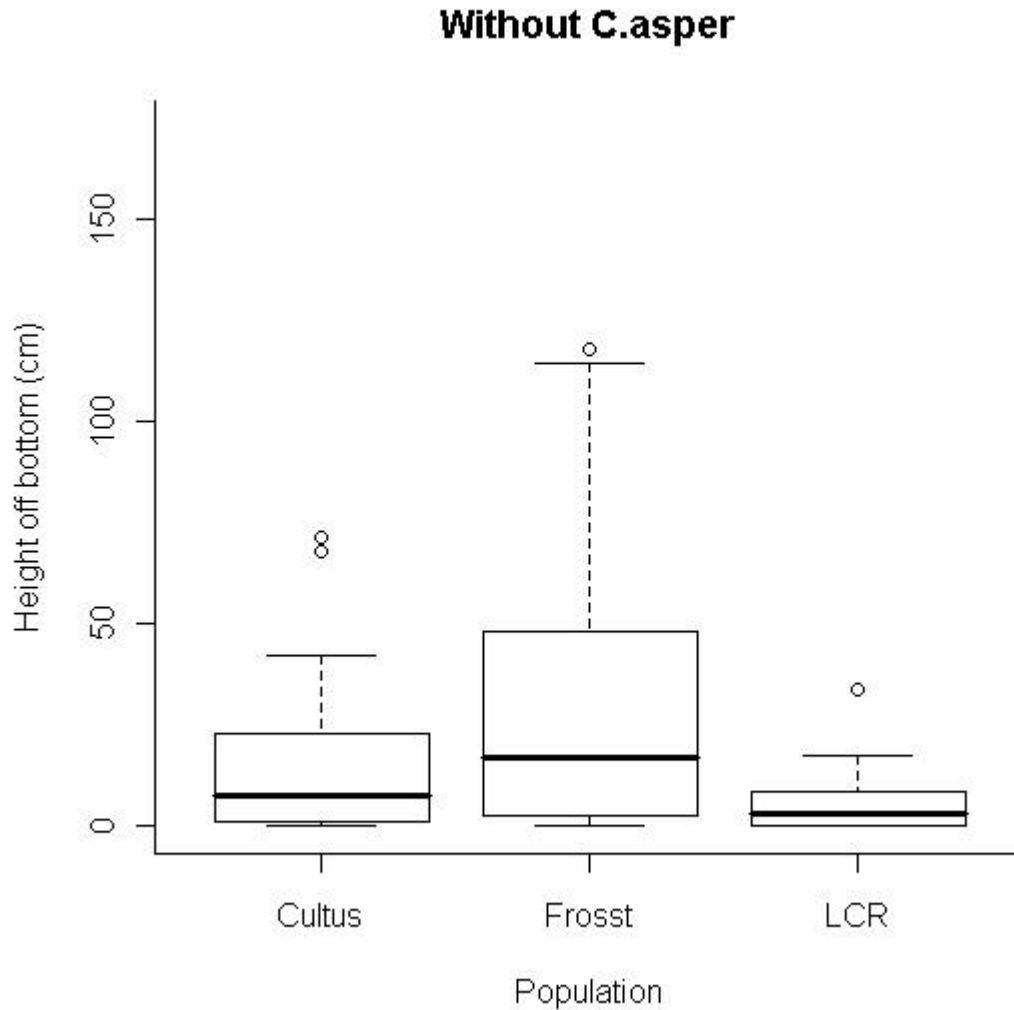
Figure 10. Box plots of the distribution of the average depths by population of *Cottus aleuticus*, with no *C. asper* present. Cultus = Cultus pygmy sculpin (n=25); Frosst = Frosst Creek (n=32); LCR = Little Campbell River (n=27). The thick horizontal line shows the median depth, with the top and bottom of the box showing, respectively, the 75th and 25th percentiles (Crawley 2007). The dashed lines represent approximately two standard deviations, with the circles representing outliers (Crawley 2007).
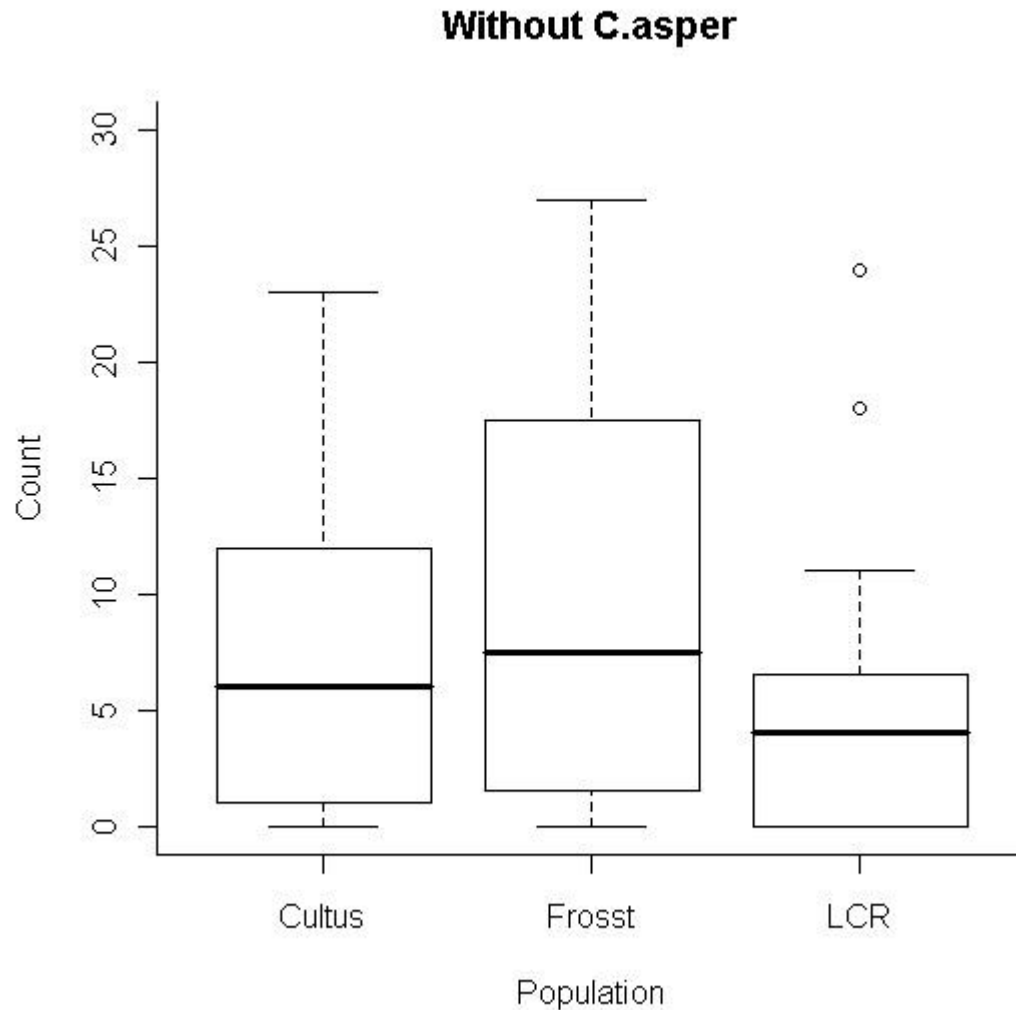
Figure 11. Box plots of the distribution of the number of times *Cottus aleuticus* were off the bottom, by population, with no *C. asper* present. Cultus = Cultus pygmy sculpin (n=25); Frosst = Frosst Creek (n=32); LCR = Little Campbell River (n=27). The thick horizontal line shows the median count, with the top and bottom of the box showing, respectively, the 75th and 25th percentiles (Crawley 2007). The dashed lines represent approximately two standard deviations, with the circles representing outliers (Crawley 2007).
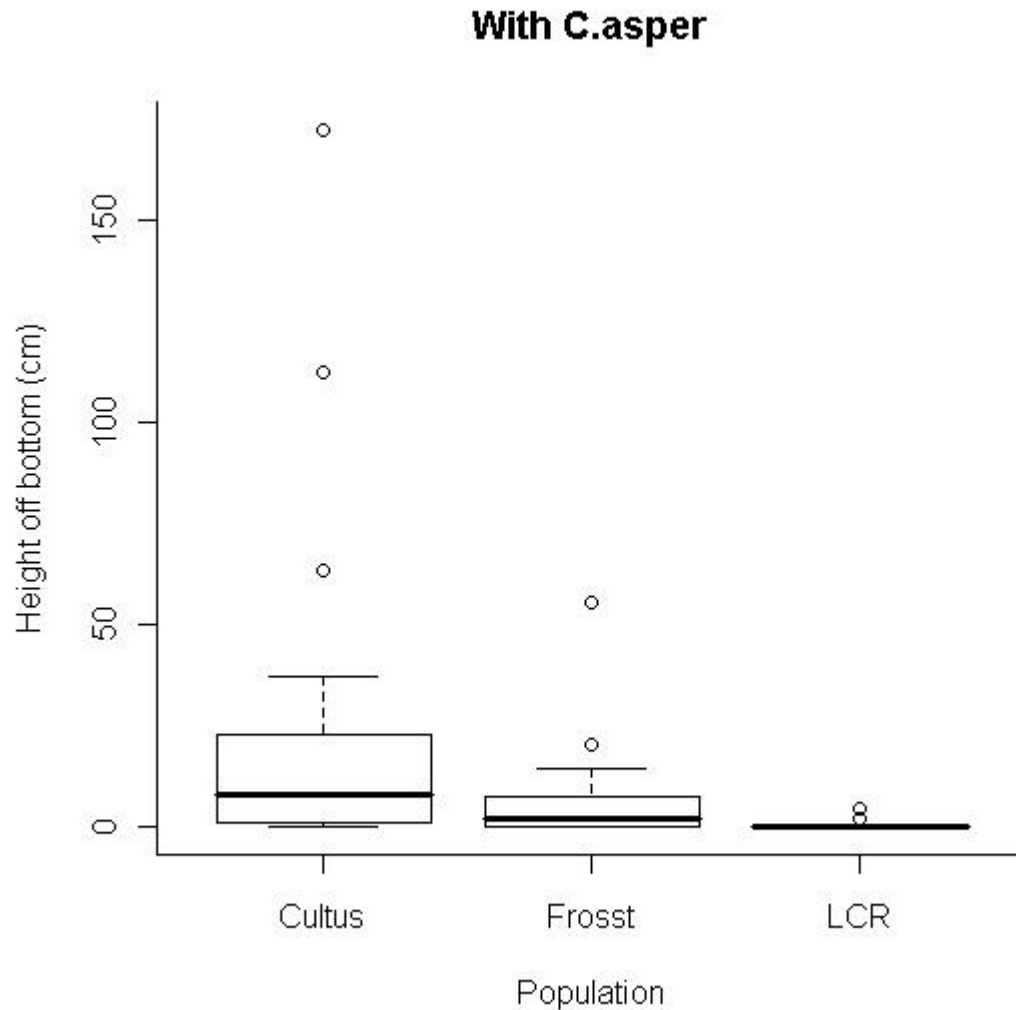
With C.asper

Figure 12. Box plots of the distribution of the average depths of *Cottus aleuticus* by population, with *C. asper* present. Cultus = Cultus pygmy sculpin (n=23); Frosst = Frosst Creek (n=31); LCR = Little Campbell River (n=12). The thick horizontal line shows the median depth, with the top and bottom of the box showing, respectively, the 75th and 25th percentiles (Crawley 2007). The dashed lines represent approximately two standard deviations, with the circles representing outliers (Crawley 2007).
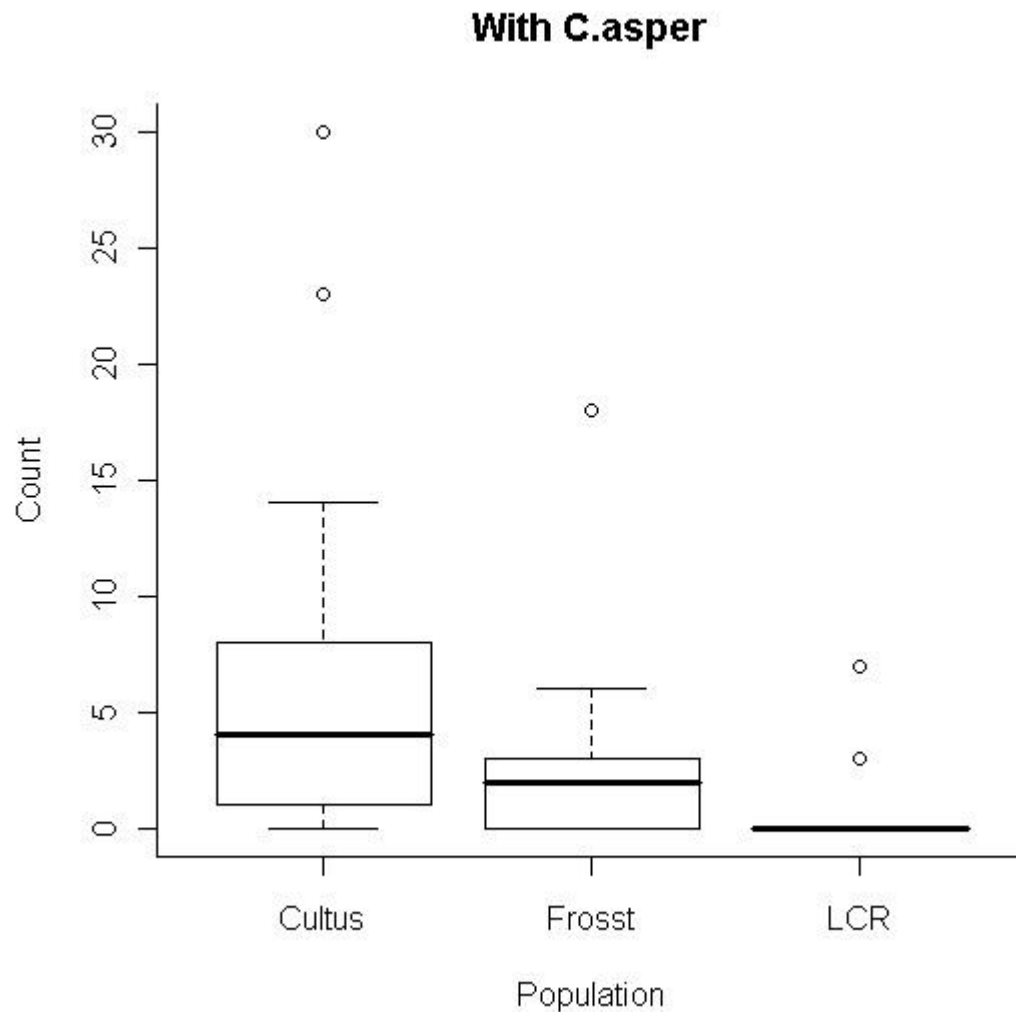
Figure 13. Box plots of the distribution of the average number of times *Cottus aleuticus* were off the bottom, by population, with *C. asper present.* Cultus = Cultus pygmy sculpin (n=23); Frosst = Frosst Creek (n=31); LCR = Little Campbell River (n=12). The thick horizontal line shows the median count, with the top and bottom of the box showing, respectively, the 75th and 25th percentiles (Crawley 2007). The dashed lines represent approximately two standard deviations, with the circles representing outliers (Crawley 2007).

Chapter 4: Summary and general discussion

Due to the repeated glacial advances and retreats during the Pleistocene era, BC has very low taxonomic diversity in its freshwater fish fauna; however, there are many examples of highly divergent "ecotypes" and intraspecific phylogroups (e.g., McPhail and Taylor 1999; McPhail 2007). Fish that recolonized habitat following deglaciation typically have lower levels of molecular neutral genetic diversity than populations in unglaciated portions of their geographic ranges (Bernatchez and Wilson 1998; Hewitt 2000). The low sequence divergence estimates between Cultus pygmy sculpin and coastrange sculpin, combined with the paraphyletic mtDNA haplotypes, suggest a recent postglacial divergence. Differentiation at the microsatellite level is common in species that have diverged since the last glaciations (i.e., Icelandic Arctic char, *Salvelinus alpinus,* Gislason et al. 1999; *Salvelinus* spp., Salmenkova et al. 2005), while other species show some divergence at the mtDNA level (i.e., whitefish, *Coregonus clupeaformis*, Pigeon et al. 1997; sticklebacks, *Gasterosteus aculeatus* in both BC, Taylor and McPhail 1999; and Iceland, Olafsdottir et al. 2007). The F_{ST} values of the Cultus pygmy sculpin in comparison with other *C. aleuticus*, were low, but statistically significant, and demonstrated that the Cultus pygmy sculpin is genetically distinct at the population level. This result could also be visualized with the differentiation observed in the factorial correspondence analysis (Figure 9).

The microsatellite analyses showed that the pelagic sculpin found in Lake Washington were highly distinct, which suggests that the similar adaptations to pelagic lifestyles (i.e., larger cephalic pores, shorter pelvic fins, higher numbers of pectoral fin rays) found in both Lake Washington and Cultus Lake fish are a result of parallel

evolution. Similar morphological and ecological shifts have evolved multiple times under similar circumstances (Schluter and McPhail 1993); in fish evolving in postglacial lakes, the resources are partitioned such that one form is limnetic, feeding on plankton in open water, while the other form is benthic, feeding on macroinvertebrates in the benthos (Schluter and McPhail 1993; Robinson and Wilson 1994; Taylor 1999). Similar to previous studies with other fish species (i.e., Schluter 1993; Olafsdottir et al. 2007; Bernatchez et al. 2010), *C. aleuticus* has evolved into a dwarf pelagic form at least twice, if not three times (Ricker 1960; Ikusemiju 1975; Larson and Brown 1975). This diversification is common in novel or newly emergent habitats such as in the Holarctic, which suggests that species can expand their niche in the absence of competitors (Robinson and Wilson 1994). Similar to the limnetic Arctic char (*Salvelinus alpinus*) morphs of Lake Thingvallavatn, the Cultus pygmy sculpin uses the benthos but feeds primarily on zooplankton in the open water (Malmquist et al. 1992; Robinson and Wilson 1994). The interspecific competition with the larger *C. asper* could contribute to ecological character displacement observed in the Cultus pygmy sculpin (Schluter and McPhail 1993).

Sculpin species tend to be spatially segregated: for example, *C. aleuticus* is found in riffle habitat in streams and along the shorelines of lakes, while *C. asper* is found in stream pools and the deep benthic habitat of lakes (Tabor et al. 2007); it is only in the absence of *C. asper* that coastrange sculpin are found in pools (McPhail 2007). The Cultus pygmy sculpin, however, has only been found in the offshore benthic habitat of Cultus Lake, similar to the pelagic sculpin found in Lake Washington (Ikusemiju 1975, Larson and Brown 1975). Both lakes are highly productive, in comparison to most other coastal lakes in BC, with Cultus Lake also having large numbers of *Daphnia* (an

invertebrate food source) available year-round (Shortreed 2007). This constant food source, that also occurs below the thermocline within the colder water preferred by fish (Shortreed 2007), coupled with the large number of a potential competitor/predator on the bottom, could be an important factor in the adaptation and persistence of the Cultus pygmy sculpin. The lower productivity and greater depths of most coastal lakes in the *C. aleuticus* range could mean that a similar niche for a pelagic sculpin is not available in other lakes, although there is always the possibility that other as yet unsampled lakes contain pelagic sculpins (e.g., Gow et al. 2008). As the history of Lake Washington has shown, however, increasing amounts of anthropogenic nutrients can result in eutrophication and a plankton community dominated by inedible cyanobacteria (Edmondson 1994; Arhonditsis et al. 2003). This increase in productivity causes a decrease in water quality (Arhonditsis et al. 2003) and a decline in fish diversity (Lopez-Rojas and Bonilla-Rivero 2000). A similar process could threaten the persistence of the Cultus pygmy sculpin (see below).

As fishes recolonized habitats after glaciations, new and empty niches may have provided novel ecological opportunities and promoted divergence among populations in resource exploitation (Schluter 1993; Rogers et al. 2002). This process could explain how pelagic sculpin, such as the Cultus pygmy sculpin, developed specializations in water-column use for feeding and predator avoidance (Ikusemiju 1975; Rogers and Bernatchez 2007). One difference in behaviour was observed when Cultus pygmy sculpin were placed in a tank with a benthic predator commonly found in Cultus Lake (*C. asper*): while stream-dwelling *C. aleuticus* decreased their movement and may have relied more heavily on cryptic colouration for protection, Cultus pygmy sculpin swam up

into the water column, exhibiting a different escape response, even though they are also cryptically coloured.

In summary, my thesis has combined information on molecular genetic variation, habitat use, and behaviour to compare the Cultus pygmy sculpin to its presumed ancestral form of typical stream-dwelling *Cottus aleuticus*. My data has contributed to our understanding of the unusual environment that the Cultus pygmy sculpin inhabits, the pelagic areas of Cultus Lake, in contrast to the flowing, riffle habitat in streams where *C. aleuticus* are most commonly found. I also demonstrated that the Cultus pygmy sculpin is genetically distinct from the coastrange sculpins found in the lake's tributary streams. Although the colouration of the Cultus pygmy sculpin allows it to take advantage of cryptic behaviour to reduce predation pressure, the Cultus pygmy sculpin also appears to make greater use of the water column, a presumably adaptive response to the large numbers of predaceous *C. asper* found in the benthic areas of Cultus Lake. The genetic distinction of the Cultus pygmy sculpin and the differences in its habitat and behaviour, coupled with its morphological traits that are plausibly interpreted as adaptations to a pelagic lifestyle (COSEWIC 2010), strongly substantiate and support the Designatable Unit status for this population under Canada's *Species at Risk Act*.

The status of the Cultus pygmy sculpin was recently (2010) assessed by COSEWIC as "threatened", a designation that was also applied in 2000. The COSEWIC status report describes several potential threats to the Cultus pygmy sculpin (COSEWIC 2010). It is unknown how the declining numbers of sockeye salmon in Cultus Lake (*O. nerka*) could impact the Cultus pygmy sculpin. It is believed that sockeye salmon fry and Cultus pygmy sculpin could compete for zooplankton prey;

however, returning sockeye adults can contribute marine nutrients such as phosphorus and nitrogen to nursery lakes (Gende et al. 2002), which can increase zooplankton abundance (COSEWIC 2010). Shortreed (2007) found an increase in lake productivity since the 1930's, despite the declining number of sockeye salmon adults and the marine derived nutrients that they add to the system. In years of low sockeye salmon abundance, predators shift from consuming sockeye salmon towards other prey species, including sculpin species (Ricker 1941). Consequently, the dramatic declines of sockeye salmon could result in an increase in predation on the Cultus pygmy sculpin. Alternatively, if the major predator on Cultus pygmy sculpin is *C. asper*, and if the other piscivorous fish target the larger and more abundant prickly sculpin then predation on the Cultus pygmy sculpin could decrease.

The effect of northern pikeminnow (*Ptychocheilus oregonensis*) on the persistence of Cultus pygmy sculpin is unknown. Ricker (1960) did not find any Cultus pygmy sculpin in the stomachs of northern pikeminnow, but since his study in the 1930s, there has been an increase in northern pikeminnow and a decrease in sockeye salmon. The main predator of Cultus pygmy sculpin was bull trout (*Salvelinus confluentus*; Ricker 1960), and the current population size of this piscivore is unknown. Fishing pressure on its spawning grounds may have reduced the bull trout numbers in Cultus Lake (J.D. McPhail, pers. comm.), but this decrease has not been substantiated.

At this time, the only documented exotic species in Cultus Lake is Eurasian milfoil (*Myriophyllum spicatum*) and its only suspected effect has been to provide increased cover, possibly allowing northern pikeminnow numbers to increase. The lower Fraser Valley, however, has large numbers of invasive species (Nowosad 2010), including brown bullhead (*Ameiurus nebulosus*), which was responsible for the

extinction of the threespine stickleback species pair in Hadley Lake, BC (COSEWIC 2001). It is possible that these invasive species could expand their range into the Cultus Lake watershed, or that species such as yellow perch (*Perca flavescens*) and bass (*Micropterus* spp.), favoured by anglers, could be illegally introduced into Cultus Lake (see Dunphy 2006 and Koopmans 2006 for examples of other instances of illegal introductions), where they could prey upon or compete with Cultus pygmy sculpin.

The large number of visitors to Cultus Lake, and the increasing development of the area, have the potential to increase eutrophication, another potential threat to the Cultus pygmy sculpin. The productivity of the lake has increased, suggesting that anthropogenic factors are contributing nutrients (average lake temperature has also increased). This is in spite of a possible reduction in the annual influx of marine nutrients due to a decline in the number of sockeye salmon returning to Cultus Lake. Poor sewage control and additions from agricultural fertilizers can increase nutrients (Schubert et al. 2002), which can result in algal blooms and the eutrophication of the lake, resulting in declining water quality and a decrease in plankton and fish communities (Edmondson 1994).

Further research

At least two critical questions remain regarding the biology of Cultus Lake sculpin with relevance to its conservation. First, the abundance of Cultus pygmy sculpin and how it varies within and between years is unknown. The only available "guessestimate", of 3,000 - 10,000 individuals, comes from Cannings (1993) and has no apparent quantitative basis. I found the numbers from trawl UTM coordinates from DFO surveys

to be too low and too variable for use in estimating the Cultus pygmy sculpin population.

The trawls are designed to target sockeye salmon. The pygmy sculpin is only

opportunistic bycatch and the potential habitat is underrepresented in the sampling.

Better methods to estimate the population could include catch per unit effort on minnow

trap sampling and mark-recapture studies. The Cultus pygmy sculpin has shown up on

the echosounding equipment of the DFO researchers, suggesting that it could be

detected with hydroacoustic surveys (J. Hume, pers. comm.). The Cultus pygmy

sculpin numbers show seasonal variation (Chapter 3, COSEWIC 2010), possibly due to

pulses of reproduction from different parts of the lake, as well as sculpin from seasonal

streams taking refuge in the lake. It would therefore be useful to conduct a multi-year

study, in order to capture the variation over time, and to incorporate ageing and

population assignment studies.

Second, because the Cultus pygmy sculpin is listed under *SARA* as a Schedule

1 species, its critical habitat must be defined and protected. As defined in the *Species*

at Risk Act (2001), "critical habitat" is "the habitat that is necessary for the survival or

recovery of a listed wildlife species, and that is identified as the species' critical habitat

in the recovery strategy or in an action plan for the species". This habitat would include

spawning and rearing areas of the lake, as well as foraging and refuge areas. While I

have captured mature fish in the lake, the actual spawning sites have not been

determined. More intensive sampling of the sites where I captured mature fish, at the

same time of year, could help determine whether those sites are spawning areas (i.e., if

large numbers of mature fish are caught there, both males and females, or if any of the

fish captured appear to have recently spawned). To help determine fertilized egg

incubation areas, it would be necessary to continue to sample the spawning areas, in

an attempt to capture the earliest developmental stages and to determine whether

Cultus pygmy sculpin fry remain on the benthos as juveniles or are pelagic as larvae.

Confirming that the main prey of Cultus pygmy sculpin is *Daphnia*, and determining

areas of the lake with the highest *Daphnia* concentrations and whether those areas are

correlated with large numbers of Cultus pygmy sculpin would help determine feeding

areas. Finally, mapping the benthic composition of the lake bottom and comparing that

to locations where Cultus pygmy sculpin are found could help define the type of habitat

required (i.e., do Cultus pygmy sculpin prefer rocky areas, or sandy areas with

vegetation for cover, spawning, etc.). The easiest way to protect these areas would be

to protect the whole lake, including the shoreline and benthic areas, possibly by adding

them to the already existing Cultus Lake Provincial Park. It is important to protect the

inlet streams to ensure the water quality remains high and to monitor the shoreline

developments to minimize any sedimentation and improper sewage treatment or

storage. Protecting the water quality of the lake and the integrity of the benthos would

ensure that the habitat continues to support the persistence of the Cultus pygmy

sculpin.

Although sculpin are an integral part of the freshwater fish fauna of BC, few

studies have examined possible local adaptations within a sculpin species. My

research used field, molecular and behavioural studies to contribute to our knowledge

of the postglacial divergences of freshwater fishes in Canada.

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Appendix 1. UTM coordinates of minnow trap sites in Cultus Lake.

Date (YYYYMMDD)	Trap number	Bottom depth (m)	Zone	Easting	Northing
20070507	1	n/a	10	572500	5432754
20070507	2	n/a	10	572499	5432732
20070507	3	n/a	10	572506	5432721
20070507	4	n/a	10	572492	5432710
20070507	5	n/a	10	572509	5432712
20070507	6	n/a	10	572505	5432729
20070507	7	n/a	10	572523	5432721
20070507	8	n/a	10	573730	5432939
20070705	1	n/a	10	574297	5432726
20070705	2	n/a	10	572742	5432015
20070705	3	n/a	10	573918	5432395
20070705	4	n/a	10	575009	5433803
20070705	5	n/a	10	574236	5436031
20070705	6	n/a	10	575667	5434402
20070705	7	n/a	10	572246	5432421
20070705	8	n/a	10	574403	5432832
20070705	9	n/a	10	574923	5433580
20070705	10	n/a	10	575004	5433794
20070705	11	n/a	10	575670	5434478
20071105	1	n/a	10	572642	5432603
20071105	2	n/a	10	572517	5432493
20071105	3	n/a	10	572589	5432181
20071105	4	n/a	10	572938	5432052
20071105	5	n/a	10	573915	5435434
20071105	6	n/a	10	57361	5434362
20071105	7	n/a	10	574664	5433437
20071105	8	n/a	10	574625	5434723
20080513	1	n/a	10	573004	5432643
20080513	2	n/a	10	573306	5432893
20080513	3	n/a	10	573996	5433632
20080513	4	n/a	10	574106	5433259
20080513	5	n/a	10	574299	5434138
20080513	6	n/a	10	574435	5434426
20080513	7	n/a	10	574520	5434957
20080514	1	n/a	10	572672	5432531
20080514	2	n/a	10	572784	5432606
20080514	3	n/a	10	574133	5433243
20080514	4	n/a	10	572707	5432448
20080514	6	n/a	10	574446	5434418
20080514	5	n/a	10	572609	5432443
20080514	7	n/a	10	572611	5432363

Appendix 1 cont. UTM coordinates of minnow trap sites in Cultus Lake.

Date (YYYYMMDD)	Trap number	Bottom depth	Zone	Easting	Northing
20080614	1		10	572479	5432456
20080614	2	32	10	572678	5432164
20080614	3	33	10	573604	5434472
20080614	4	38	10	573604	5434472
20080614	5	40	10	573932	5435344
20080614	6	38	10	575049	5434840
20080614	7	38	10	574662	5434143
20080726	1	40	10	572543	5432522
20080726	2	43	10	573009	5432550
20080726	3	42	10	573393	5433506
20080726	4	25	10	574135	5433528
20080726	5	41	10	574123	5434907
20080726	6	36	10	574823	5434362
20080726	7	40	10	574323	5435086
20080821	1	37	10	572499	5432430
20080821	2	43	10	573008	5432852
20080821	3	40	10	573656	5433574
20080821	4	28	10	575530	5434628
20080821	5	23	10	574563	5433642
20080821	6	41.5	10	573555	5432561
20080821	7	30.3	10	572542	5432158
20080927	1	38	10	572663	5432390
20080927	2	40	10	572683	5432514
20080927	3	35	10	572547	5432320
20080927	4	32.4	10	573774	5433564
20080927	5	26	10	574507	5433698
20080927	6	32	10	574518	5435357
20080927	7	39	10	572562	5432551
20080927	8	30	10	572660	5432149
20081024	1	35	10	572463	5432389
20081024	2	33	10	572422	5432440
20081024	3	35	10	572507	5432482
20081024	4	37	10	572555	5432392
20081024	5	36	10	572628	5432263
20081024	6	34	10	574149	5433217
20081024	7	38.4	10	574191	5433720
20081024	8	34	10	574891	5434293

Appendix 2. List of lengths of Cultus pygmy sculpin captured in minnow traps in Cultus Lake, May-October 2008.

Date of capture	Trap number	Depth of capture (m)	Depth from bottom (m)	Total length (mm)
May 15	4	40	n/a	54
May 15	1	30	n/a	45
May 15	7	10	n/a	48
May 15	2	30	n/a	53
May 15	1	40	n/a	45
May 15	1	40	n/a	45
May 15	3	40	n/a	43
May 15	6	40	n/a	47
May 15	2	40	n/a	51
May 15	2	40	n/a	45
May 15	2	40	n/a	48
May 15	2	40	n/a	46
May 15	2	40	n/a	49
May 15	5	40	n/a	50
May 15	5	40	n/a	65
May 15	5	40	n/a	45
May 15	5	40	n/a	60
May 15	5	40	n/a	54
May 15	7	40	n/a	53
May 15	7	40	n/a	59
May 15	7	40	n/a	58
May 15	7	40	n/a	53
June 15	1	40	n/a	35
June 15	6	20	18	50
June 15	7	20	18	55
June 15	3	30	3	30
June 15	3	30	3	50
June 15	3	30	3	52
June 15	3	30	3	48
June 15	7	40	0	54
June 15	7	40	0	47
June 15	6	40	0	53
June 15	6	40	0	46
June 15	6	40	0	47
June 15	6	40	0	45
June 15	6	30	8	47
June 15	6	30	8	58
June 15	6	30	8	45
June 15	6	30	8	30
June 15	6	30	8	49
June 15	1	40	n/a	56
June 15	1	40	n/a	46

Appendix 2 cont. List of lengths of Cultus pygmy sculpin captured in minnow traps in Cultus Lake, May-October 2008.

Date of capture	Trap number	Depth of capture (m)	Depth from bottom (m)	Total length (mm)
June 15	1	40	n/a	50
June 15	1	40	n/a	54
June 15	1	40	n/a	35
June 15	3	40	0	48
June 15	3	40	0	45
June 15	3	40	0	54
June 15	2	30	2	34
June 15	5	40	0	59
July 26	7	30	0	40
July 26	2	20	13	25
July 26	5	40	1	35
July 26	4	40	0	23
July 26	3	30	12	28
July 26	4	30	0	50
July 26	4	30	0	37
July 26	4	30	0	30
July 26	4	30	0	34
July 26	4	30	0	35
July 26	4	30	0	21
July 26	4	30	0	21
July 26	4	30	0	20
July 26	4	30	0	20
July 26	4	30	0	20
July 26	4	30	0	18
July 26	4	30	0	20
July 26	1	40	0	50
July 26	1	40	0	43
July 26	1	40	0	25
July 26	1	40	0	21
July 26	7	30	10	47
July 26	7	30	10	45
July 26	7	30	10	42
July 26	7	30	10	53
July 26	7	30	10	58
July 26	7	30	10	35
July 26	7	30	10	40
July 26	7	30	10	35
July 26	7	30	10	31
July 26	7	30	10	34
July 26	1	30	10	43
July 26	1	30	10	36
July 26	1	30	10	45
July 26	7	30	10	42

Appendix 2 cont. List of lengths of Cultus pygmy sculpin captured in minnow traps in Cultus Lake, May-October 2008.

Date of capture	Trap number	Depth of capture (m)	Depth from bottom (m)	Total length (mm)
July 26	7	30	10	41
July 26	7	30	10	37
July 26	7	30	10	35
July 26	7	30	10	35
July 26	7	30	10	34
July 26	7	30	10	35
July 26	7	30	10	36
July 26	7	30	10	29
July 26	7	30	10	34
July 26	7	30	10	30
July 26	7	30	10	20
July 26	7	30	10	20
July 26	1	30	10	26
July 26	1	30	10	38
July 26	1	30	10	32
July 26	1	30	10	35
July 26	1	30	10	33
July 26	1	30	10	36
August 22	4	40	0	24
August 22	3	10	30	58
August 22	4	30	0	39
August 22	3	40	0	54
August 22	3	40	0	57
August 22	3	40	0	25
August 22	5	30	0	29
August 22	5	30	0	18
August 22	5	30	0	30
August 22	5	30	0	23
August 22	5	30	0	21
August 22	5	30	0	16
August 22	5	30	0	20
August 22	5	30	0	25
August 22	5	30	0	15
August 22	3	30	10	38
August 22	3	30	10	50
August 22	3	30	10	63
August 22	3	30	10	35
August 22	3	30	10	45
August 22	3	30	10	57
August 22	3	30	10	35

Appendix 2 cont. List of lengths of Cultus pygmy sculpin captured in minnow traps in Cultus Lake, May-October 2008.

Date of capture	Trap number	Depth of capture (m)	Depth from bottom (m)	Total length (mm)
August 22	3	30	10	58
August 22	3	30	10	50
August 22	3	30	10	35
August 22	3	30	10	62
August 22	3	30	10	47
August 22	3	30	10	52
August 22	3	30	10	36
August 22	3	30	10	30
August 22	3	30	10	31
August 22	1	40	0	25
August 22	1	40	0	30
August 22	1	40	0	60
August 22	1	40	0	21
August 22	1	40	0	35
August 22	1	40	0	45
August 22	1	40	0	56
August 22	1	40	0	47
August 22	1	40	0	25
August 22	1	40	0	35
August 22	1	40	0	51
August 22	1	40	0	54
August 22	1	40	0	56
September 28	6	30	2	58
September 28	6	40	0	62
September 28	6	40	0	59
September 28	4	40	0	58
September 28	7	20	19	55
September 28	2	40	0	56
September 28	2	40	0	44
September 28	5	30	0	53
September 28	5	30	0	59
September 28	5	30	0	57
September 28	5	40	0	55
September 28	5	40	0	55
September 28	5	40	0	58
October 25	5	40	0	52
October 25	6	30	4	53
October 25	6	30	4	47
October 25	6	40	0	52
October 25	6	40	0	49
October 25	8	30	4	58
October 25	8	40	0	55

Appendix 3. Animal care certificate used in conducting field sampling and laboratory experiments.

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A08-0654

Investigator or Course Director: Eric B. Taylor

Department: Zoology

Animals:

> Minnows brassy minnow 100
> Sticklebacks threespine stickleback 200
> Trout Arctic char 150
> Minnows Finescale and redbelly dace 80

Start Date:	April 1, 2008	Approval Date:	October 6, 2008

Funding Sources:

Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Molecular phylogeny and speciation of a regional fauna

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental

Appendix 3 cont.

project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

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