Dave Pflug
Personal Copy

FINAL REPORT TO SKAGIT ENVIRONMENTAL ENDOWMENT COMMISSION

SKAGIT CHAR PROJECT (PROJECT 94-1)

BY

J. D. McPHAIL AND E. B. TAYLOR

DEPARTMENT OF ZOOLOGY,
6270 UNIVERSITY BOULEVARD,
UNIVERSITY OF BRITISH COLUMBIA,
VANCOUVER, BC,
V6T 1Z4

JULY 1995

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF FIGURES

1.0 SUMMARY

One hundred and fifty char were examined from the upper Skagit system (i.e., above Ross Reservoir). We used morphology, mitochondrial DNA, ribosomal DNA, and two growth hormone loci to investigate the genetic relationships of native char in the upper Skagit River and its tributaries. All of these measures suggest the presence of two native char, and by comparing the Skagit data to similar data from geographic areas containing only Dolly Varden or only bull trout, we established that the two native Skagit char are Dolly Varden and bull trout. Although both species occur in the system, it is also clear that they hybridize. Over 50% of the individuals that appear to be morphologically Dolly Varden, and also carry the growth hormone restriction fragment pattern (RFLP) diagnostic for Dolly Varden, have a mitochondrial DNA pattern that is normally diagnostic for bull trout. Since mitochondrial DNA is inherited exclusively through the female parent, this observation implies crossing between Dolly Varden males and bull trout females. The mitochondrial DNA data establish that Dolly Varden and bull trout have hybridized in the upper Skagit, but it gives no estimate of current rate of hybridization. The growth hormone and rDNA loci, however, are inherited from both parents, and there are a RFLP patterns diagnostic for current hybrids. About 12% of the char examined in the upper Skagit display the hybrid growth hormone RFLP. Based on our molecular data, we estimate that about 20% of the char examined were "pure" Dolly Varden and about 8% "pure" bull trout.

Despite this remarkable level of hybridization, the two native char in the upper Skagit appear to maintain themselves as distinct and separate entities. Ecologically, the Dolly Varden phenotype predominates in tributary streams (e.g., the Klesilkwa and Sumallo rivers, and Nepopekum, Shawatum, St. Alice, and McNaught creeks). It is a small char (sexually mature individuals rarely exceed 200 mm), and a stream resident. It forages primarily on drift (nymphs of aquatic insects) and spawns mostly in the tributary streams. There is, however, a small population in the main river. In contrast, the bull trout phenotype predominates in the main river (and, presumably, in the reservoir). Adult bull trout are large (they do not mature until over 400 mm in length) and have a complex life-history that involves size-related movements between the main river, tributary streams and, eventually, the reservoir. Bull trout are piscivores (fish-eaters) and take up this foraging mode at a remarkably small size (<100 mm). Their main prey are Dolly Varden and rainbow trout. The only bull trout spawning site we located was a two kilometer stretch of the main river between Twenty-six Mile Bridge and the mouth of Shawatum Creek.

Given the level of hybridization between the two char, they should collapse into a single gene pool (introgress). Yet, they remain recognizable, and separate, entities. This implies strong selection against hybrids. Given their different life-histories and foraging modes, this selection could be ecological. Another possibility is that hybrids are at a reproductive disadvantage relative to the parental species. Whatever the cause, we postulate that the two native char are maintained by a balance between gene flow and selection against hybrids. If true, this balance could easily be disturbed by a decline in the numbers of one of the species, and the species at risk is the bull trout.

To protect bull trout, we recommend the early closure (from mid-September on) to all angling in the main river from Twenty-six Mile Bridge downstream to the mouth of Shawatum Creek. In addition, we suggest that an aerial survey of the main river in late October may be a cost effective way of assessing the bull trout spawning population. In terms of further research, a formal life-history study of both species in the upper Skagit is a necessary first step towards a rational management plan. If such a study is undertaken, it is imperative that it include the entire Skagit system above Ross Dam (i.e., Ross Reservoir and its tributaries, as well as the Canadian portion of the Skagit) as a single research venue.

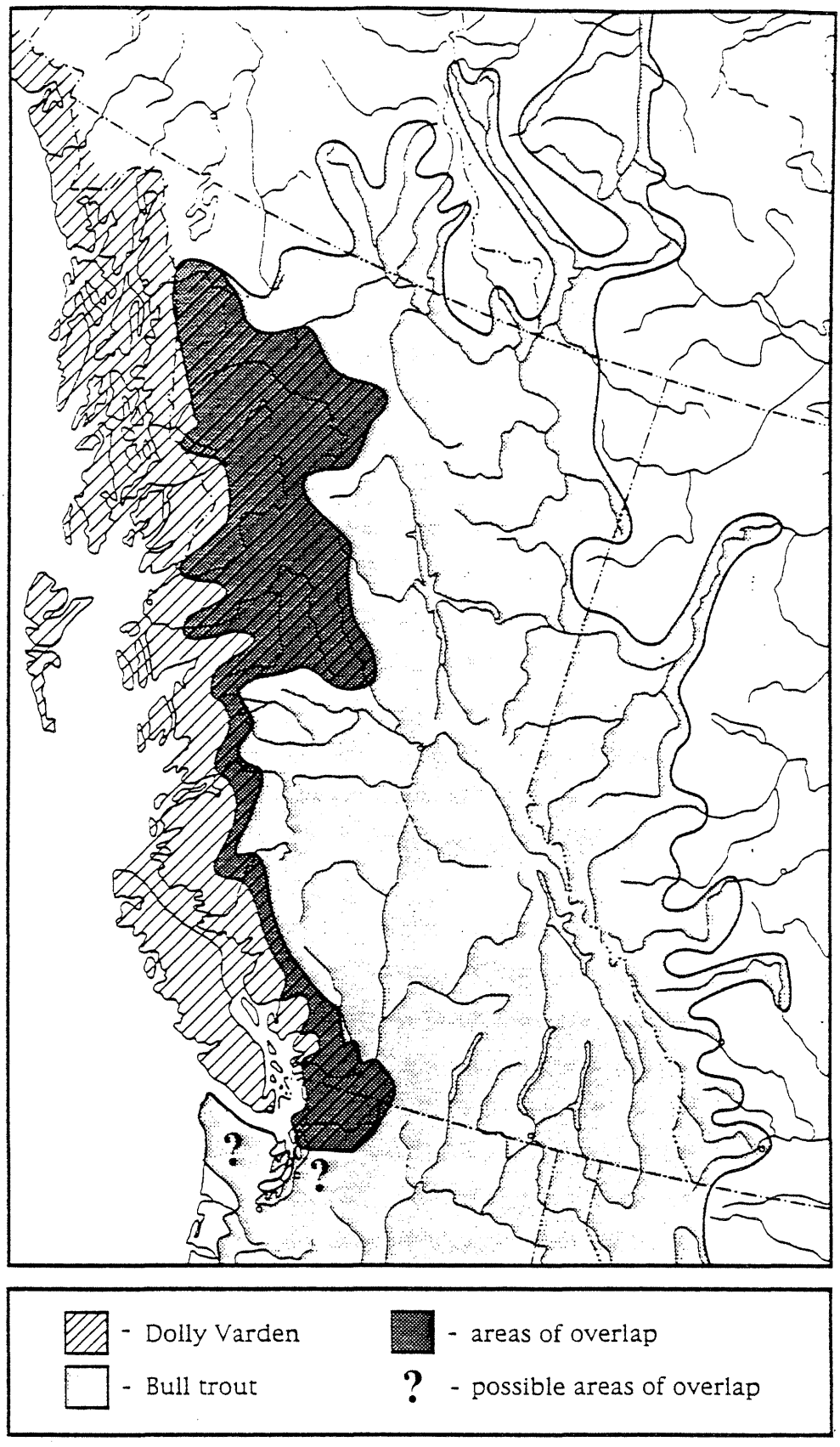


FIGURE 1. Approximate British Columbia distributions of bull trout (*Salvelinus confluentus*) and Dolly Varden (*Salvelinus malma*) showing areas of range overlap.

2.0 INTRODUCTION

A fundamental in the management of any species is an ability to recognize that species. Obviously, when there is only one in a region, species identification is not a problem; however, if two related species are present, proper identification can have serious management implications. This is because information on habitats and life-histories are basic components of any rational management plan, and even closely related species are unlikely to have precisely the same suite of habitat and life-history requirements. This is the problem facing fisheries managers in the Skagit system. Chars (genus *Salvelinus*) are an important component in the recreational fishery on both sides of the border but it is not clear if one, or two, native species inhabit the system.

Unfortunately, species identification in chars is not a trivial problem. The taxonomy of western North American chars is confused, and for over a decade taxonomists have debated whether the bull trout (*S. confluentus*) and the Dolly Varden (*S. malma*) are the same, or different, species (Cavender 1978, Haas and McPhail 1991). At first glance, the evidence is clear. In British Columbia, Haas and McPhail (1991) were able to distinguish morphologically between bull trout and Dolly Varden, although it required a multivariate statistical analysis. This was because the differences are subtle and depend on traits like head shape and mouth size; traits that are known to be plastic and subject to selection by both the physical and foraging environments.

In most of British Columbia the geographic distributions of the two species are completely separate: the Dolly Varden is a coastal species and is often anadromous (migrates to the sea), while the bull trout is an interior species and does not normally migrate to the sea (Figure 1). The northern regions where the species overlap are in the Coast Range, and in the upper Fraser and upper Peace rivers where the headwaters of these systems abut on the headwaters of the Skeena system, and in the B.C: headwaters of the Liard system. In these areas, although there is clear evidence of hybridization (Baxter et al. 1995), the two char coexist and the majority of individuals are recognizable as either one species or the other. This ability to coexist, and remain as separate entities, constitutes a powerful argument for the existence of two species.

At the southern end of the range of the Dolly Varden the picture is less clear. For example, the normal migratory behaviour of the two forms appears to reverse in southwestern British Columbia and western Washington (McPhail and Baxter 1995). Along the north coast of British Columbia many Dolly Varden populations are anadromous, but bull trout usually are

confined to fresh water. However, no extant anadromous populations of Dolly Varden are known from south of Campbell River on Vancouver Island, and the anadromous char in the lower Fraser and Squamish rivers clearly are bull trout! This apparent reversal in anadromous behaviour is confusing and, in conjunction with the morphological difficulty of distinguishing the species, has led some researchers to question whether there really are two species (Washington Dept. of Wildlife, Draft Rept. #92-22).

Aside from the management implications of the taxonomic status of the native chars, there also is a serious conservation issue. In the conterminous United States, the bull trout is on the US category 2 list (Federal Register 50: 37958-37967) and the American Fisheries Society list of species of special concern (Williams et al. 1990). This means they are a candidate species for protection under the US Endangered Species Act. If they are granted threatened status, this will have major management implications. Thus, if the bull trout occurs in the Skagit system it may eventually require special protection but, if the Skagit char are Dolly Varden, they can be managed outside the constraints applied to threatened species. If both species occur in the Skagit system, then some reliable method of identification is a necessity. It is a fundamental ecological principle that no two species can coexist indefinitely if they share exactly the same habitat requirements. Thus, if both Dolly Varden and bull trout are present in the Skagit system, there probably are differences in their use of habitats and in their life-histories. If so, treating them as a single species (the proposed management policy for native chars in Washington State; Washington Dept. of Wildlife, Draft Rept. #92-22) is an untenable strategy in the Skagit system. There is also the special problem that the Skagit chars are a trans-boundary resource, and the proposed management strategy for Washington State (treating native char as a single species) is in direct opposition to the B.C. policy which is to manage and conserve wild fish stocks as separate entities (BC Environment, Fisheries Program Strategic Plan 90/95). Clearly, for the Skagit chars, an answer to the species question is not simply an academic exercise — it is the necessary foundation for any management or conservation program.

Thus, the primary goal of this research was to determine if Dolly Varden and bull trout coexist in the Skagit system and, if so, to estimate the extent of hybridization (if any). A secondary goal was to provide reliable methods for identifying fry, juveniles, and adults in the field. During the course of the study we acquired some information on the life-histories and habitat use by Skagit char. Although this information was collected incidentally to the main project, much of it is new and potentially of interest to managers. Consequently, we have

included the observations even though the sample sizes often are too small to warrant firm conclusions.

3.0 MATERIALS AND METHODS

3.1 COLLECTION SITES

Char were collected from the Skagit River and its tributaries from April to mid-November, 1994. Sampling in the main river was restricted to the spawning season: September, October, and early November. Tributary streams were sampled periodically throughout the spring, summer and autumn of 1994. The tributaries sampled were the Klesilkwa River, the Sumallo River, Nepopekum Creek, Shawatum Creek, St. Alice Creek, and McNaught Creek. The fry, juvenile and adult sampling sites are shown in Figure 2.

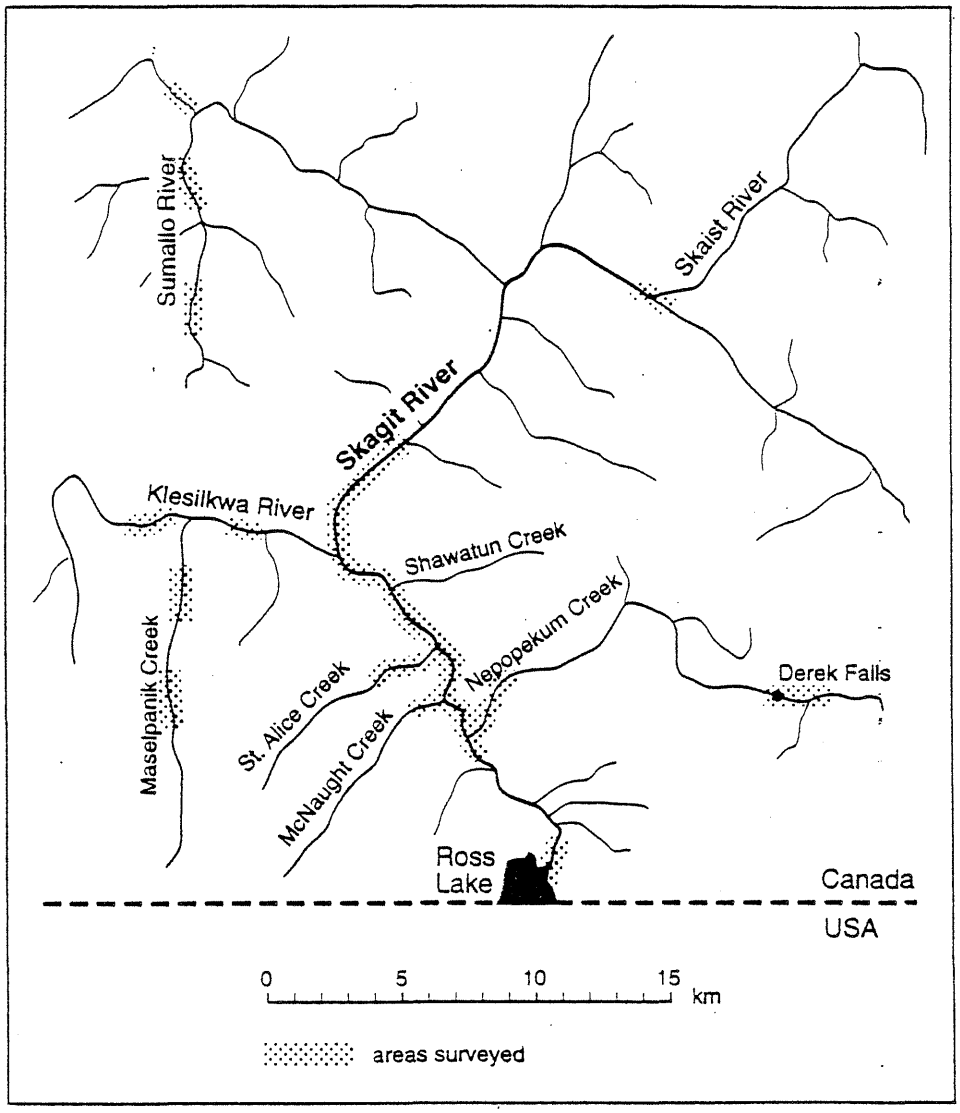


FIGURE 2. Upper Skagit collection and observation sites.

Early in the season newly-emerged fry were collected with dip-nets. The fry sampling sites are shown in Figure 3. Because they were too delicate to examine in the field, and we needed to confirm identifications, a small subsample of fry (23) was killed and preserved. The information obtained from these fry then was used to field-identify fry from the other sites. Juveniles were collected in minnow traps and with an electro-shocker. Again, to confirm field identifications, a small subsample of juveniles (20) was killed and preserved and the data from these animals used to field-identify juveniles from other sites. Reproductive adults were caught in large (0.8 m in diameter and 1.5 m long) wire mesh traps. Adults, and most juveniles, were anesthetized (with CO_2), measured and their meristics counted. A fin clip was taken for a tissue sample. The fish were allowed to recover from the anesthetic and then released back into the stream. In total, 142 fry, 70 juveniles, and 80 reproductive adults were sampled.

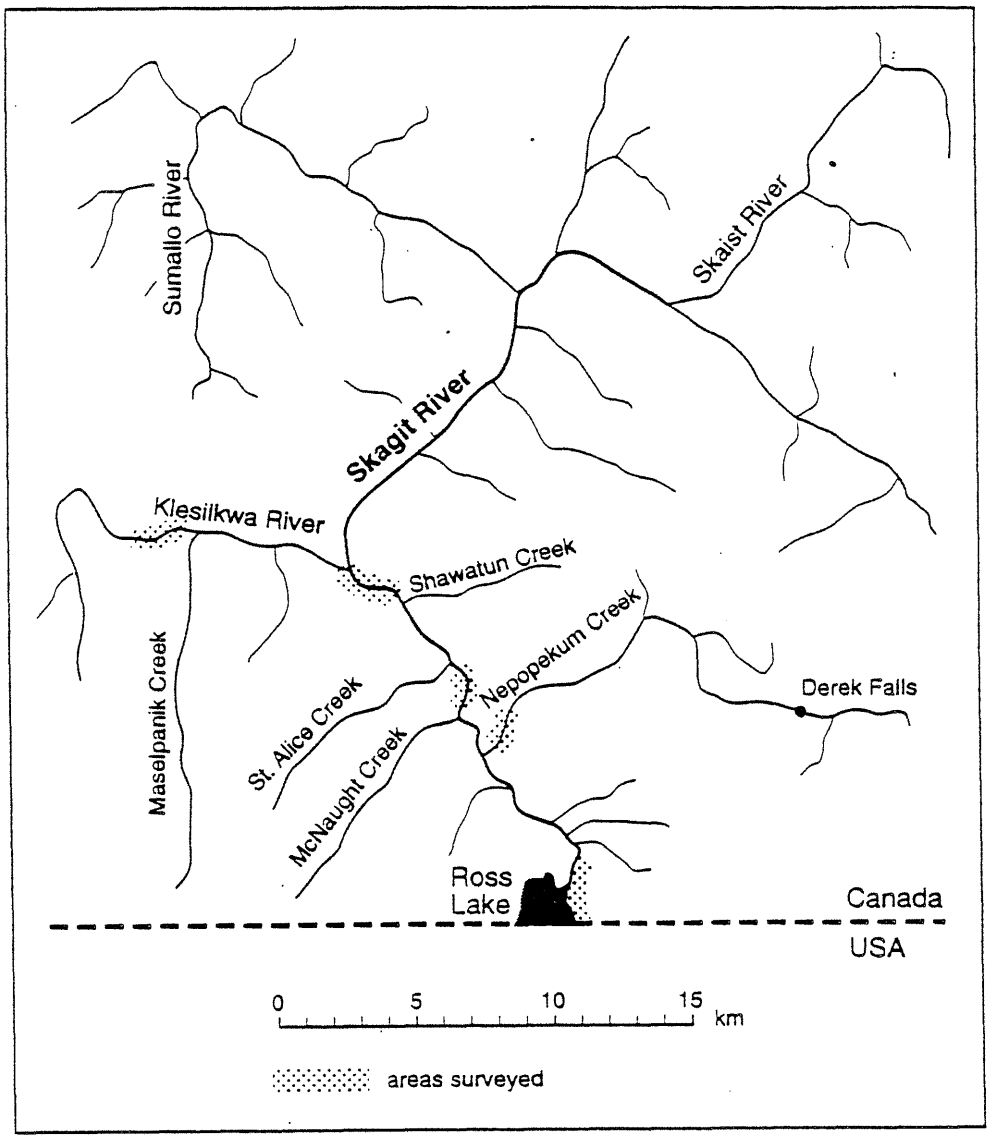


FIGURE 3. Fry collection sites.

3.2 MOLECULAR

We used three DNA assays to identify Dolly Varden, bull trout, and their hybrids. These assays involved restriction fragment length polymorphisms (RFLPs) in: (i) mitochondrial DNA (mtDNA), (ii) ribosomal DNA (rDNA), and (iii) growth hormone (GH) genes. The chief advantage of this combination of molecular markers is their different modes of inheritance. These can be exploited to identify not only hybrids but also the direction of hybridization (i.e., who was the maternal parent and who was the paternal parent). For example, mtDNA is almost universally inherited through the female parent (maternal inheritance, Avise and Lansman 1983; Taylor, unpublished data for *Salvelinus*). In contrast, both rDNA and the growth hormone loci are classes of DNA contained within the nucleus and, therefore, they are inherited in equal proportions from both parents (biparental inheritance). Consequently, hybrids between Dolly Varden and bull trout will possess rDNA and growth hormone RFLPs characteristics of both parental species, but hybrids identified by rDNA and GH analyses will possess the mtDNA RFLP of only the female parent (thus the identity of the male parent can also be deduced). Many studies of interspecific hybridization use the complementary nature of mtDNA and nuclear DNA molecular markers to both identify hybrids, and to identify the direction of hybridization (e.g., Baxter et al. 1995; Baker et al. 1989; Wilson and Hebert 1993).

3.2.1 Mitochondrial DNA: We used the enzyme *Hin*d III to distinguish Dolly Varden and bull trout mtDNAs. The *Hin*d III dimorphism results from a single restriction site change between the mtDNA genomes of the two species (five sites in Dolly Varden and four sites in bull trout, Grewe et al. 1990). This dimorphism is visualized as an RFLP with five mtDNA fragments characteristic of Dolly Varden and four fragments characteristic of bull trout. Extraction of DNA from ethanol-stored tissue, enzyme restriction, Southern blotting, and hybridization follow procedures outlined in Taylor et al. (1995) and Baxter et al. (1995). Restriction site variation was assayed using hybridization of membrane-bound char DNA with digoxigenin-labelled smelt (*Osmerus mordax*) mitochondrial DNA.

3.2.2 Nuclear DNA analysis:

3.2.2.1 *Ribosomal DNA* - One of our nuclear markers involves restriction site analysis of the first internal transcribed spacer region of the ribosomal DNA (rDNA) gene complex. Ribosomal DNA is a class of tandemly repetitive nuclear DNA consisting of coding and non-coding (spacer regions) DNA sequences. The coding regions produce rRNAs that combine with ribosomal proteins to produce ribosomes (reviewed in Hillis and Dixon 1991).

Comparison of the published ITS 1 sequences of Dolly Varden and bull trout indicated several restriction enzymes that would reveal differences between the species. To exploit these differences, we used the polymerase chain reaction (PCR) to amplify the ITS 1 region from fish using primers found in the flanking 18S and 5.8S coding regions. Procedures for PCR amplification and restriction enzyme analysis of char ITS 1 are described in Baxter et al. (1995). As predicted from published sequences (Pleyte et al. 1992), when the ITS 1 PCR products were incubated with a number of restriction enzymes, there were site differences between Dolly Varden and bull trout. One of these enzymes, *Sma* I, cuts the 825 bp ITS 1 of bull trout into two smaller fragments (500 base pairs and 325 bp), but the 825 bp ITS 1 PCR product is not cut in Dolly Varden. As rDNA is biparentally inherited, first generation hybrids between Dolly Varden and bull trout produce a composite *Sma* I restriction fragment length polymorphism. Thus, pure Dolly Varden have a single 825 bp fragment, pure bull trout have two fragments (500 and 325 bp), and hybrids have three-bands (800, 500, and 325 bp fragments).

3.2.2.2 ***Growth Hormone Loci*** - The growth hormone (GH) loci studied represent a "duplicated" locus resulting from a doubling of the salmonid nuclear genome some 50-100 million years ago (Allendorf and Thorgaard 1984; Devlin 1993). The two growth hormone loci (GH1 and GH2) share enough DNA sequence similarity between and within species that they can be assayed by Southern-hybridization analyses using a DNA probe designed from one of the GH loci (pers. comm., B. Devlin, Dept. of Fisheries and Oceans, West Vancouver Laboratory). Our assay involved using a DNA probe developed from a sockeye salmon genomic "library" that reveals polymorphism at both loci in a wide variety of salmonids. In all species were segregation of alleles at the two loci has been studied in pedigrees, the loci are passed from parents to offspring with no apparent linkage between the loci (the latter has yet to be confirmed for char). The sockeye DNA probe is effectively a bi-locus probe; it resolves either one (homozygote) or two fragments (heterozygote) for each of the GH1 and GH2 loci simultaneously (a total of 2-4 fragments per individual).

For char, variation at GH1 and GH2 is resolved by RFLP analysis and produces species-specific alleles at both loci for a number of restriction enzymes. Our analysis uses the restriction enzyme *Hinc* II because it produces RFLPs in char of a convenient molecular size range (2.0-4.0 kilobase pairs, kbp). All molecular-based analyses used to identify species, and interspecific hybrids, assume no within species variation for the markers employed. To verify that our molecular markers are diagnostic, we obtained samples of "pure" Dolly Varden from outside the range of bull trout (allopatric populations), and bull trout from outside the range

of Dolly Varden. We used a wide range of sample localities to confirm the absence of within species variation for our molecular markers. These allopatric samples consisted of ten Dolly Varden populations from southern Vancouver Island and the Queen Charlotte Islands, and eleven bull trout populations distributed from the upper Fraser River to the Metolius River (a Columbia River tributary in Oregon). In the almost 100 allopatric char assayed, there was no evidence of within species variation in our mtDNA and rDNA species markers. Thus, we conclude that they are diagnostic for each of the species (Baxter et al. 1995). At the GH loci, all assayed Dolly Varden and bull trout were homozygous for alternative alleles. In Dolly Varden, the allele resolved at GH1 was a 2.9 kbp fragment, and the only allele resolved at GH2 was a 2.5 kbp fragment. For bull trout, the corresponding alleles were 3.5 and 2.2 kbp fragments at GH1 and GH2, respectively. We also confirmed the maternal inheritance of the mtDNA *Hind* III RFLPs and the biparental inheritance of rDNA (ITS 1 *Sma* I) and *Hinc* II GH RFLPs) in a small sample of artificial, laboratory-raised hybrids produced by Haas and McPhail (1991).

3.3 MORPHOLOGY

In the field, fish were lightly anesthetized and measured with a pair of Helios calipers accurate to 0.1 mm. All fish were measured for standard length, upper jaw length, head length, and length of the anal fin base. In addition, we counted the number of branchiostegal rays The counts and measurements are illustrated in Figure 4. We also noted sex, state of maturity, and general colour pattern of all adult fish.

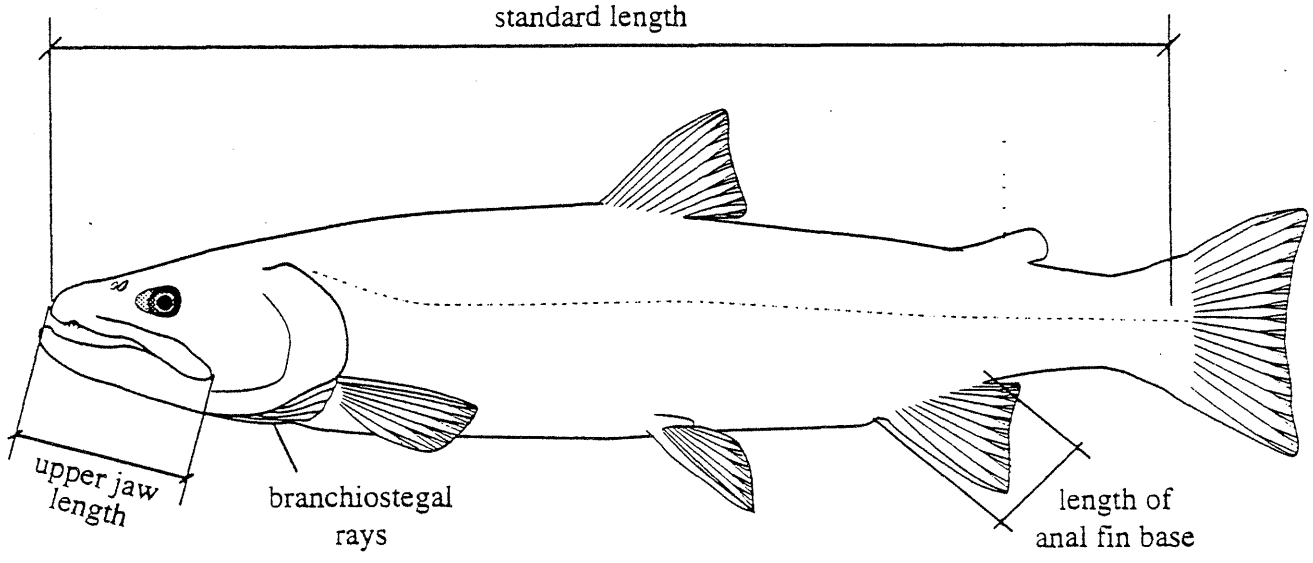


FIGURE 4. Counts and measurements mentioned in text.

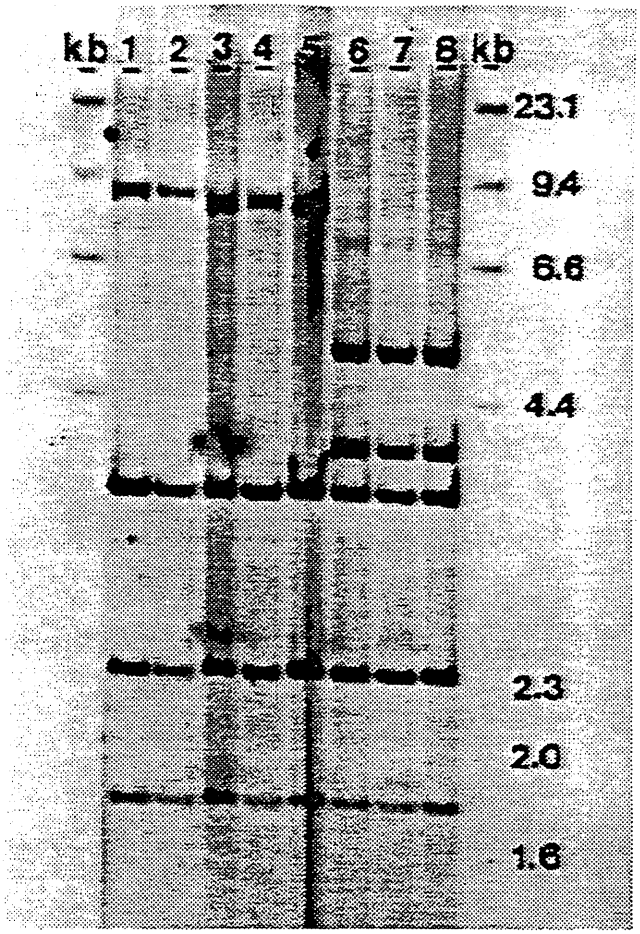


FIGURE 5. Mitochondrial DNA (mtDNA) restriction fragment haplotypes used to distinguish bull trout and Dolly Varden. Haplotypes were resolved by restricting genomic DNA with *Hind* III followed by hybridization with digoxigenin-labeled *Osmerus mordax* mtDNA at 58° C. Bull trout: lane 1 = Metolius River (Oregon), lane 2 = North Thompson River, (upper Fraser River, B.C.), lane 3 = Attycelley Creek (Thutade Lake, B.C.), lane 4 = South Pass Creek (Thutade Lake, B.C.), lane 5 = North Kemess Creek (Thutade Lake, B.C.). Dolly Varden: lane 6 = Attycelley Creek (Thutade Lake, B.C.), lane 7 = Cowichan Lake (Vancouver Island, B.C.), lane 8 = Honna River (Queen Charlotte Islands). KB = molecular weight size marker (in kilobase pairs).

3.4 HABITAT AND LIFE-HISTORY OBSERVATIONS

The locality where each fish was collected was recorded and notes taken on substrate, cover, water velocity, and water depth. For reproductive adults, spawning sites and spawning times were recorded.

3.5 DATA ANALYSES

3.5.1: Morphology: Although multivariate statistical techniques (e.g., Linear Discriminant Function Analysis, Principal Components Analysis) are powerful tools for separating closely related species, our experience suggests that attempts to apply these techniques in the field create more problems than they solve. Since one aim of this study was to provide a means of field identification, we opted for simple bivariate plots. Thus, morphometric data were graphed and compared by analysis of covariance, and branchiostegal ray counts presented as histograms to emphasize the bimodal nature of the data.

4.0 RESULTS

4.1 EVIDENCE FOR TWO FORMS OF NATIVE CHAR

4.1.1 Molecular:

4.1.1.1 *Mitochondrial DNA* - Two *Hin*d III RFLPs were resolved in the mtDNA genomes of char collected from the Skagit River: one with four restriction fragments and one with five fragments (Figure 5). These two RFLPs correspond to patterns documented for Dolly Varden (five fragment pattern) and bull trout (four fragment pattern) in our survey of allopatric char populations (Baxter et al., 1995). In total, 150 char were assayed for the *Hin*d III RFLP in the upper Skagit River: 116 carried the bull trout RFLP, and 34 carried the five fragment Dolly Varden RFLP. In addition, we observed two fish that were characterized by a *Hin*d III RFLP diagnostic of brook trout (Taylor and McPhail, unpublished data). Thus, the mtDNA data indicate the presence of both Dolly Varden and bull trout in the upper Skagit system.

4.1.1.2 *Ribosomal DNA* - A total of nine char, selected by their morphology to represent "good" bull trout and Dolly Varden, were assayed for the *Sma* I RFLP with the ITS 1 fragment of rDNA. From this sample, five displayed the single fragment pattern diagnostic of Dolly Varden and four showed the two fragment RFLP diagnostic of bull trout (Figure 6). Again, the evidence from rDNA suggests the presence of two native char.

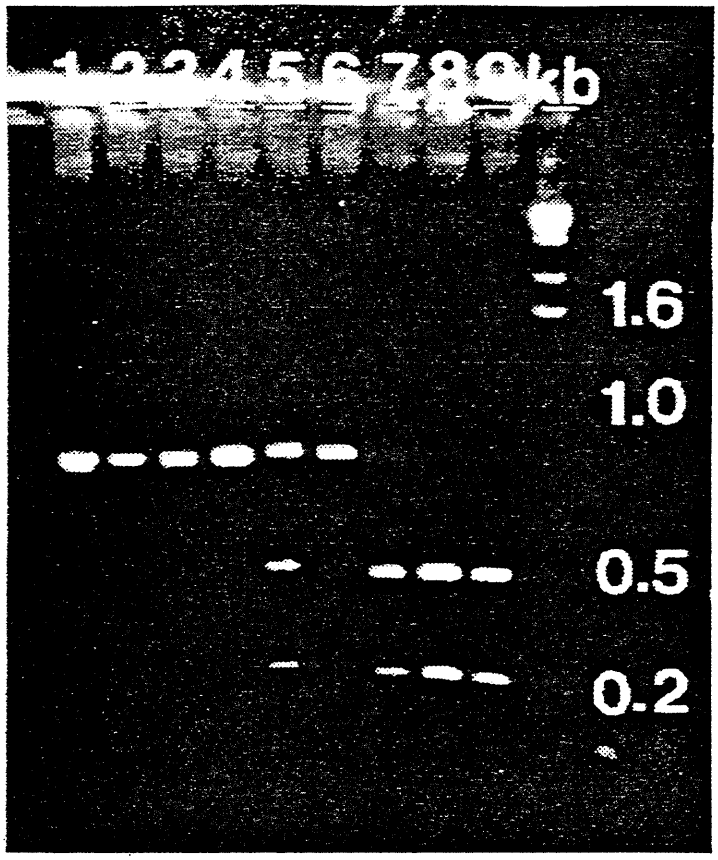


FIGURE 6. Ribosomal DNA genotypes used to distinguish bull trout, Dolly Varden, and their hybrids. Genotypes were resolved by cutting PCR-amplified char ITS 1 rDNA with *Sma* I and electrophoresing samples in 2.0% agarose gels. Dolly Varden: lane 1 = Cowichan Lake (Vancouver Island), lane 2 = Honna River (Queen Charlotte Islands), lane 3 = Kumealon Lake (Central B.C. Coast), lane 4 = Attycelley Creek (Thutade Lake, B.C.). Hybrid char: lane 5 = South Pass Creek, (Thutade Lake, B.C.), lan e 6 = North Kemess Creek (Thutade Lake, B.C.). Bull trout: lane 7 = Attycelley Creek (Thutade Lake, B.C.), lane 8 = Metolius River (Oregon), lane 9 = North Thompson River (Upper Fraser River, B.C.). Thutade Lake individuals in lanes 4 - 7 are the same as those in lanes 6, 5, 4, and 3, respectively in Figure 5. KB = molecular weight size marker (in kilobase pairs).

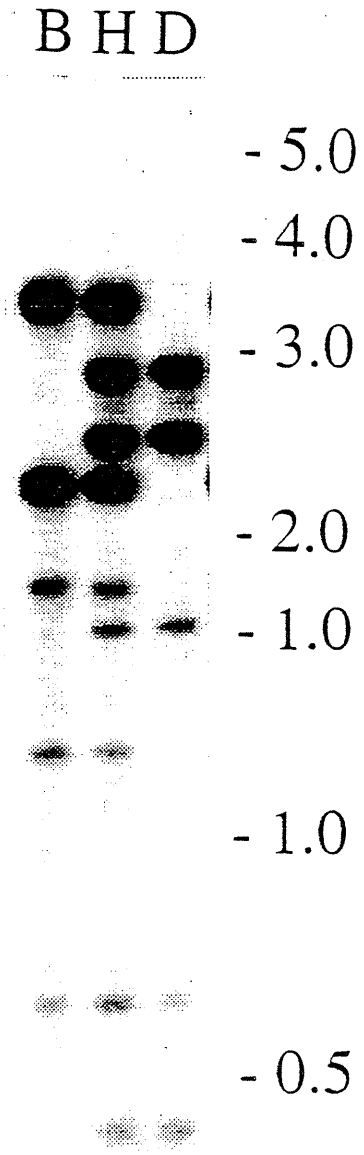


FIGURE 7. Growth Hormone Loci
B = bull trout
H = hybrid
D = Dolly Varden

4.1.1.3 *Growth Hormone Loci* - In our survey of RFLP variation at the two growth hormone (GH) loci, RFLP patterns diagnostic for Dolly Varden and for bull trout were found in 108 char from the upper Skagit River. Individual char exhibiting the Dolly Varden RFLPs (N = 100) were all homozygous for the GH1 2.9 kilobase pair (kbp) allele and for the GH2 2.5 kbp allele (Figure 7). Individuals bearing the RFLPs diagnostic of bull trout (N = 8) were all homozygous for the 3.5 kbp allele at GH1 and the 2.2 kbp allele at GH2 (Figure 7). Consequently, the growth hormone loci data also indicate the presence of both Dolly Varden and bull trout in the upper Skagit system.

4.1.2 Morphological:

4.1.2.1 *Fry* - On May 18, 1994, a sample of newly-emerged char was preserved from exposed side-channels in the delta where the Skagit River enters Ross Reservoir near International Point (Figure 8). These fry ranged from 17-27 mm in standard length and their size distribution was bimodal (Figure 9). Also, based on colour pattern, there appeared to be two kinds of fry at the site. In the laboratory, pigment patterns (Figure 10) were used to separate the preserved fry into two groups. Jaw lengths in the two groups of fry were compared (Figure 11), and even at this small size there is a significant difference between the two groups ($P < 0.001$). Presumably, the long-jawed group are bull trout fry and the short-jawed group are Dolly Varden. To test this assumption, four individuals from each group were reared in adjacent flow-through aquaria until they reached 50 mm in standard length. These animals were then sacrificed and identified. The results confirmed that the two pigment patterns found in Skagit char fry represent Dolly Varden and bull trout, respectively.

4.1.2.2 *Juveniles* - As in the fry, there were two forms of juvenile char (35-80 mm in standard length) in the upper Skagit system. The two forms differed in colour pattern, head shape, jaw length, and the length of the anal fin base (Figure 12 and Figure 13). The two forms also differed in distribution, and in abundance, within the main river and its tributaries (see section 5.2). Although the majority of juveniles fell into one or the other group, some individuals could not be placed unequivocally in either group. Thus, although the morphology of juveniles argues strongly for the presence of two forms, the existence of intermediate individuals implies the possibility of hybridization.

4.1.2.3 *Adults* - During the breeding season (September to November) two non-overlapping size classes of mature char were present in the Skagit system. Ripe males in the small char group averaged 128 mm in standard length and ranged in size from 82-245 mm; while ripe males in the large char group averaged 500 mm in standard length and ranged from 425-680 mm. The two size classes also differed in colour pattern, head shape, jaw length, and

the length of the anal fin base (Figure 14 and Figure 15). Thus, the morphology of adults again strongly supports the contention that there are two native char in the upper Skagit system. Nevertheless, some adults did not fit into either category and, again, this suggests the possibility of hybrids.

In addition to Dolly Varden and bull trout, two adult male brook trout (*Salvelinus fontinalis*), in breeding condition, were encountered: one in Nepopekum Creek at a site where Dolly Varden were breeding, and the other in the mainstem Skagit. Some hybridization between this introduced species and Dolly Varden is indicated by the presence of two (out of 150 fish sampled) juvenile Dolly Varden-Brook trout hybrids (identified by a combination of their morphology and mitochondrial DNA markers).

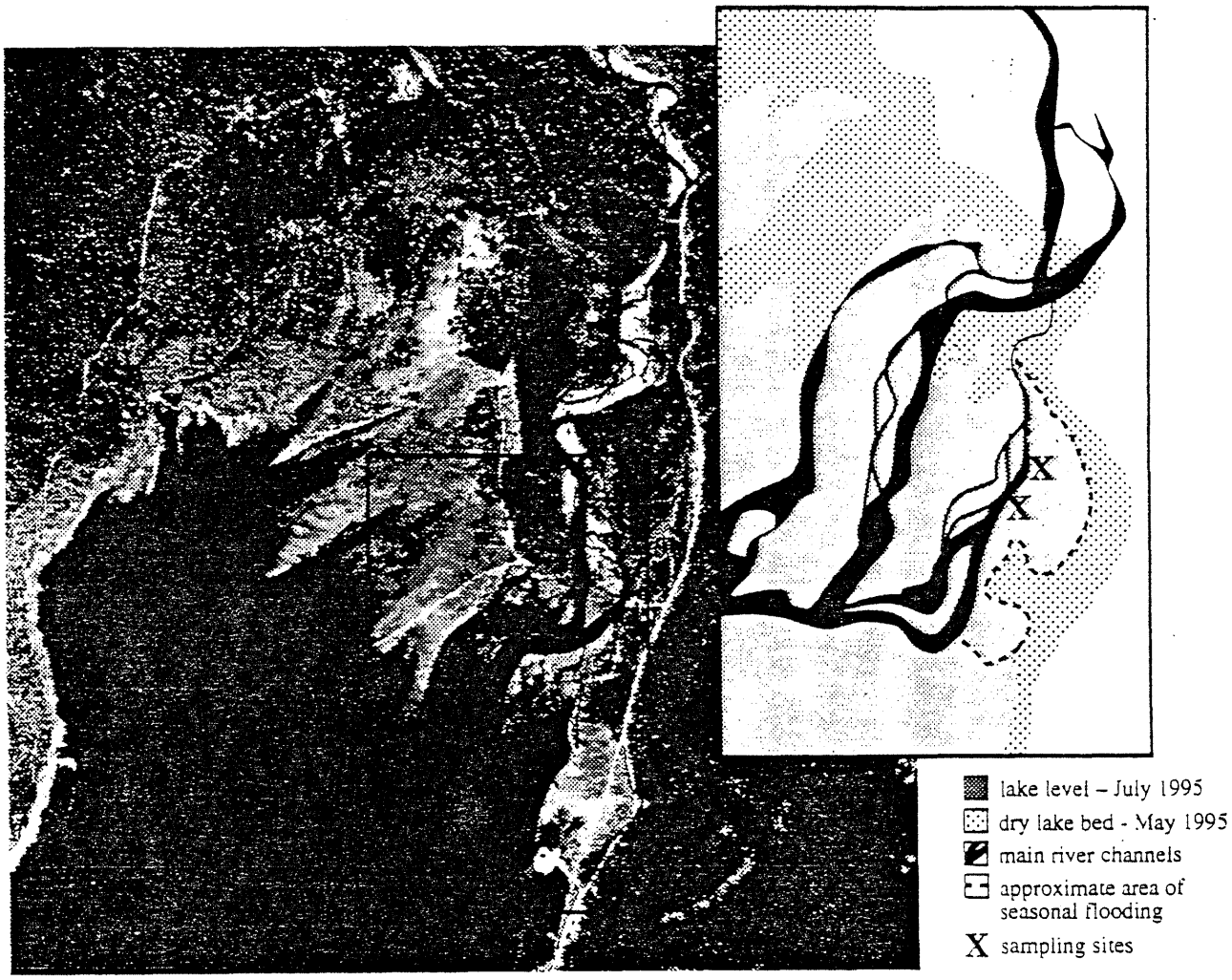


FIGURE 8. Fry sampling site in seasonally flooded side channels where Skagit River enters Ross Reservoir.

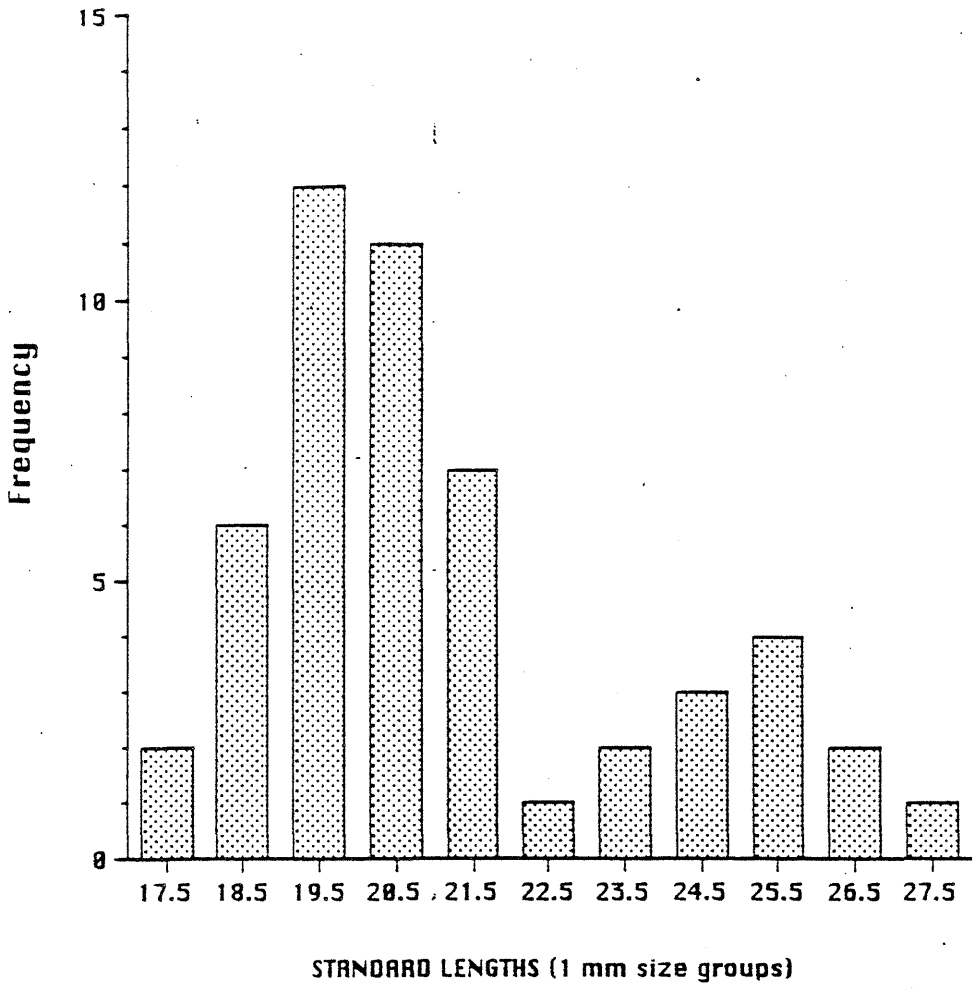


FIGURE 9. Length - frquency of fry sampled at Ross Reservoir site (Fig. 8).

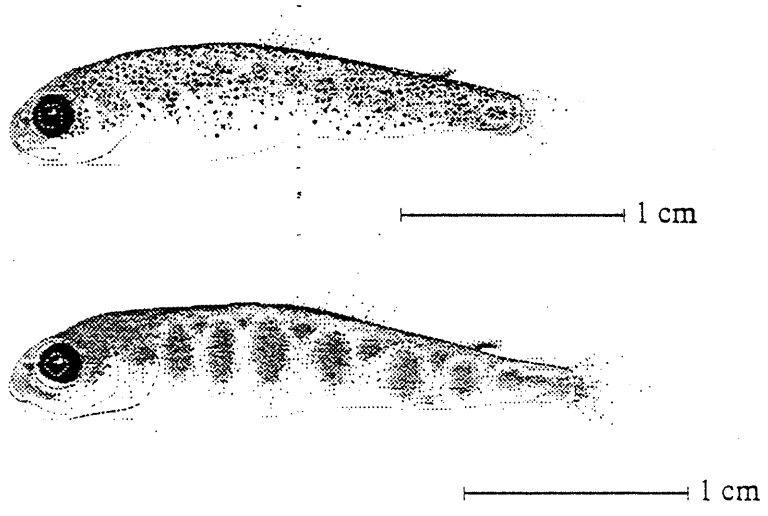


FIGURE 10. Newly emerged char fry: top Dolly Varden, *Salvelinus malma*; bottom bull trout *Salvelinus confluentus*. Note difference in development of parr marks.

4.2 EVIDENCE OF HYBRIDIZATION BETWEEN NATIVE CHARS:

4.2.1 Molecular:

4.2.1.1 *Growth Hormone Loci and Ribosomal DNA* - A total of 108 Skagit char when assayed for growth hormone RFLPs. Of these fish, 12 were heterozygous for the species-specific alleles at both the GH1 and GH2 loci (i.e., each animal possessed four *Hinc* II fragments. The "upper" two fragments (see Figure 7) represent the 3.5 and 2.9 kbp alleles diagnostic for bull trout and Dolly Varden, respectively, at the GH1 locus. Fragments of approximate molecular weight 2.5 and 2.2 kbp represent the alleles diagnostic for Dolly Varden and bull trout, respectively, at the GH2 locus (Figure 7). Since the growth hormone loci are inherited from both parents, these heterozygous fish must be hybrids.

Twelve of the char identified as hybrids by growth hormone RFLPs also were assayed for the *Sma* I RFLP in the rDNA ITS 1 fragment. They all possessed a three-banded genotype (i.e., they possessed the RFLPs diagnostic for both Dolly Varden and bull trout, Figure 6). This confirms their identity as hybrids between the two species.

4.2.1.2 *Identification of the direction of hybridization* - The 12 fish that were identified as hybrids by the two independent nuclear DNA markers (rDNA and growth hormone loci) had been characterized earlier by the *Hind* III mtDNA RFLP (see section 4.1.1.1.). All 12 hybrid char possessed the *Hind* III mtDNA RFLP diagnostic of bull trout (i.e., they had four restriction fragments, Figure 5). A total of 101 upper Skagit char were assayed for both mtDNA and GH loci. Most of these char possessed a different species marker for mtDNA than for the growth hormone loci. For example, 59 char had Dolly Varden growth hormone RFLPs and bull trout mtDNA, and 12 fish were hybrids at GH1 and GH2 (as well as for rDNA) but had bull trout mtDNA. Since mtDNA is maternally inherited, the high proportion (about 58%) of what appear to be Dolly Varden (as identified by morphology and GH RFLPs) carrying bull trout mtDNA could only be achieved by crossing between Dolly Varden males and bull trout females.

In contrast, only eight char had both bull trout mtDNA and bull trout GH RFLPs. This suggests that only about eight percent of the char examined were "pure" bull trout. Twenty-two of the char assayed possessed both Dolly Varden mtDNA and Dolly Varden GH1 and GH2 RFLPs suggesting that about 20% of the char in the upper Skagit are "pure" Dolly Varden.

4.2.2 Morphology:

4.2.2.1 *Branchiostegal rays* - Figure 16 compares the number of branchiostegal rays in upper Skagit char with the number in allopatric (populations where only a single species occurs) Dolly Varden and bull trout. In allopatry, this frequency

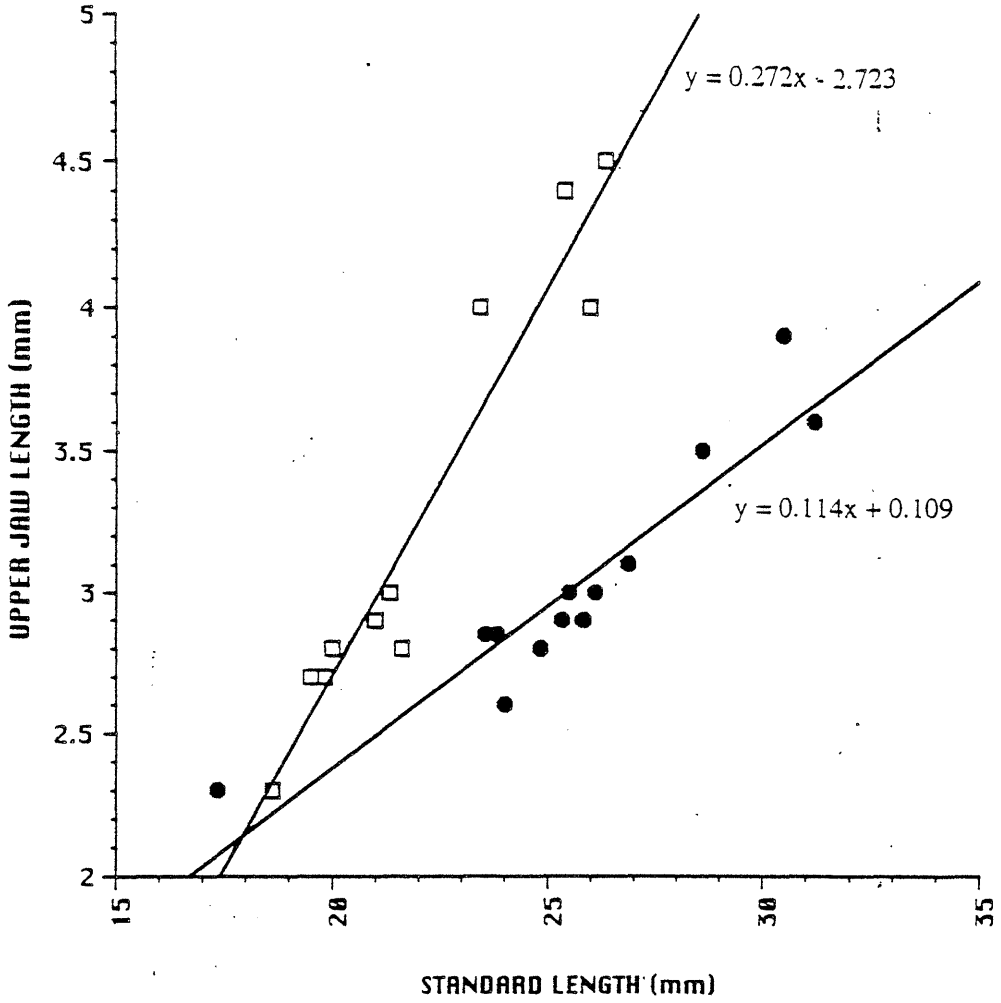


FIGURE 11. Plot of upper jaw length *vs.* standard length in newly-emerged fry (equations are for regression lines: ● = Dolly Varden, □ = bull trout.

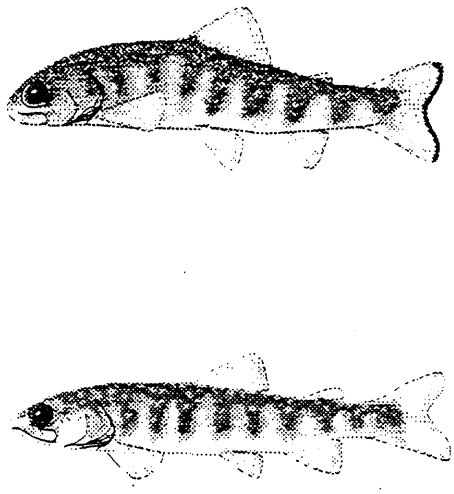


FIGURE 12. Yearling Skagit char: top Dolly Varden, *Salvelinus malma*; bottom, bull tout, *Salvelinus confluentus*.

distribution is strikingly bimodal, with only slight overlap at 24 or 25 rays. In contrast, in the upper Skagit, although the frequency distribution of branchiostegal rays is still bimodal, the much shallower valley between the two modes strongly suggests hybridization.

4.2.2.2 *Ratio of upper jaw length to anal fin base* - Figure 17 compares the ratio of upper jaw length to length of the anal fin base in adult (>100 mm) upper Skagit char with the same ratio in allopatric populations of Dolly Varden and bull trout. In allopatry, the frequency distribution of this ratio is bimodal, with no overlap; however, a larger sample probably would produce some overlap. In contrast, in the upper Skagit, although the frequency distribution of this ratio still hints at bimodality, many individuals have intermediate ratios. Again, this filling in of the valley between the two modes suggests hybridization.

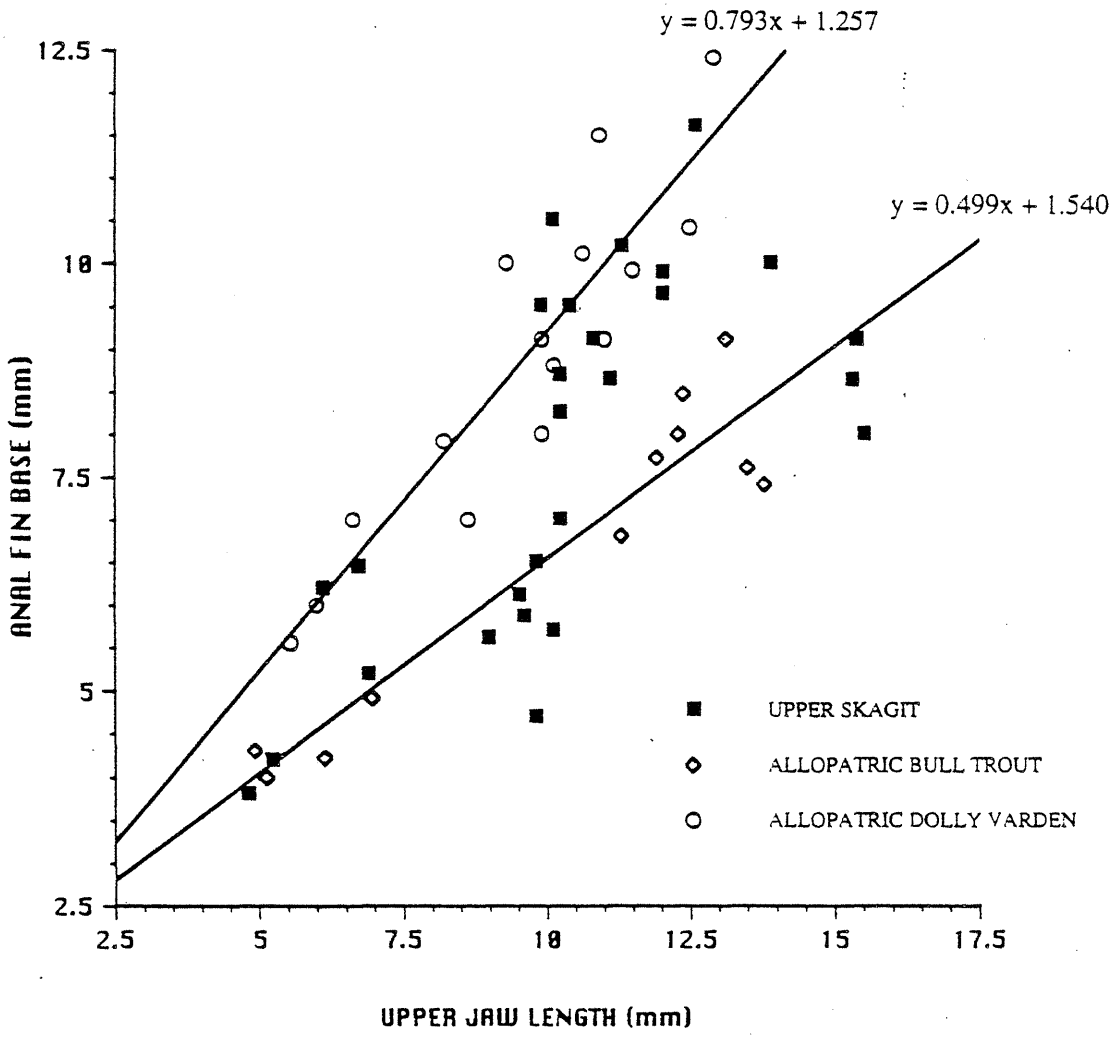


FIGURE 13. Plot of length of anal fin base *vs.* upper jaw length in juvenile char (< 100 mm). The two lines (and their equations) are for allopatric populations. Note that the points for the Skagit cluster around both lines but, also, that some points fall in between the lines.

5.0 HABITAT USE AND LIFE-HISTORIES

5.1 FRY

In the upper Skagit system, newly-emerged char fry were moderately abundant during April and May. For the first few weeks after emergence, they are nocturnal, solitary, and denser than water. This makes them difficult to sample. Qualitatively, bull trout fry (as assessed by colour pattern, Figure 10) were moderately abundant along the edges of the main river in the region below Twenty-six Mile Bridge. In the same area, Dolly Varden fry were relatively rare. In contrast, Dolly Varden fry, were moderately abundant in the lower portion of Nepopekum Creek and in the upper Klesilkwa and Sumallo rivers, while bull trout fry were rare in these areas. Interestingly, the areas of highest fry abundance were the slow channels that meander across the delta where the Skagit River enters the upper end of Ross Reservoir. Since there are no suitable spawning sites in these channels, the fry probably entered them from the main river. Whether movement to these delta channels is voluntary, or the fry are flushed out of the main river, or displaced by other fry, is unknown. The substrate in these channels is mainly fine sand and mud, and there is little cover. Consequently, the fry here are easier to locate than fry in the main river, and their abundance in these habitats may be more apparent than real. Both forms of fry were present, although the bull trout form out numbered the Dolly Varden form by about 10 to one. At sites where both forms of fry occurred, we could detect no obvious differences in their habitat use.

In the main river, newly-emerged fry are associated with the shallow (<5 cm deep) edges, especially in areas with low water velocities (< 20 cm^{-s}) such as side-channels and shallow bays. Here, they are found in and around gravel about 20-100 mm in diameter. They are secretive during the day and, if disturbed, they quickly seek shelter under rocks. They remained associated with this habitat for their entire first summer. The tributary streams, especially Nepopekum Creek, were dominated by Dolly Varden fry. They used the same shallow edge habitat as the fry in the main river. By the end of the summer, the young-of-the-year of both forms ranged in size from 35 to 80 mm.

5.2 JUVENILES

In Nepopekum Creek, yearling, and older Dolly Varden, remained in the stream. As they grew they shifted to deeper and faster water. In the day, juveniles were associated with cover, such as large rocks, woody debris, root wads, and undercut banks. These Dolly Varden appear to remain stream residents throughout their lives, although small numbers of adult Dolly Varden and, rarely, juveniles were collected in the main river.

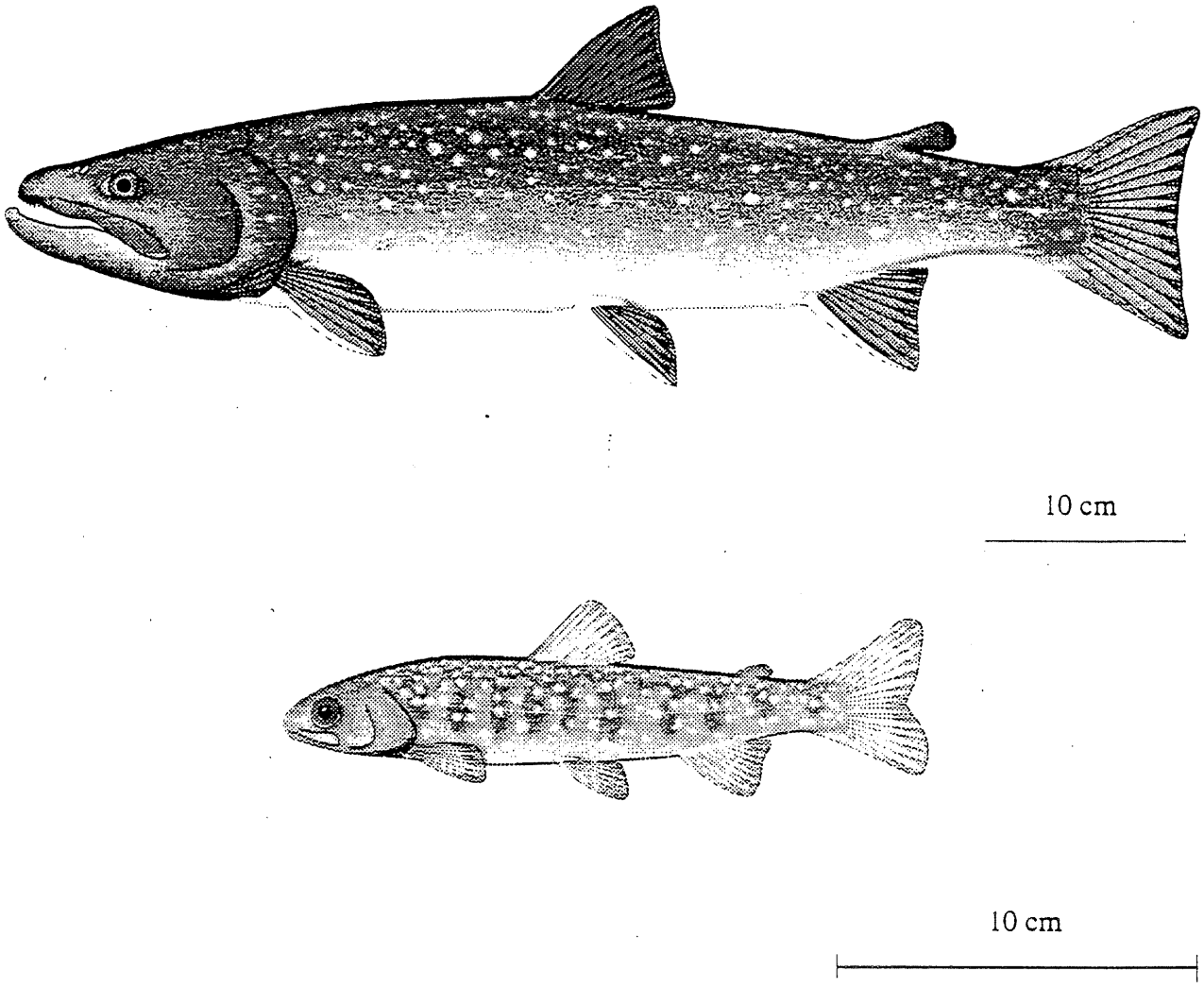


FIGURE 14. Mature male char (top,adfluvial male bull trout (*Salvelinus confluentus*) from the main river; bottom, sream-resident male Dolly Varden (*Salvelinus malma*) from the Klesilka River. Note difference in size.

In contrast, bull trout juveniles appear to have a more complex life-history. Sometime in their first winter, or during the next summer, they shift from edge habitats and begin to concentrate around the mouths of small tributaries. Here, they remain in quiet water areas such as backwaters, beaver ponds and blind channels. In their third spring, they begin to work their way up these small tributary streams. At this time they switch to a diet of fish (in the upper Skagit the prey are young-of-the-year rainbow trout and Dolly Varden). This diet switch can occur at sizes of less than 100 mm. Apparently, they spend several years in the tributary streams. We caught immature bull trout over 300 mm long at least two kilometers up Nepopekum Creek. At about 300 mm, bull trout disappear from tributary streams, and, presumably, move downstream into either the main river or the reservoir.

5.3 ADULTS

In the upper Skagit system, adult Dolly Varden are primarily stream residents. In tributary streams, they occupy the same habitats as juveniles (pools, under-cut banks, and areas with large woody debris) but occur in deeper water. The few stomachs we examined contained nothing but aquatic insect nymphs. This suggests that these stream-resident Dolly Varden are drift feeders. In the main river, adult Dolly Varden were rare, and associated with cover along the edges of deep glides. In contrast to Dolly Varden, large adult bull trout were relatively common in the main river and, except for the breeding season, they appear to be absent in tributary streams. Large bull trout also occur in Ross Reservoir but we did not collect in the reservoir. In the main river, adult bull trout are associated with cover (usually large woody debris) adjacent to relatively deep water. They appear to be primarily piscivores.

5.4 REPRODUCTION

The first spawning char were encountered in the Klesilkwa River on September 28. They were small: the smallest ripe male was 82 mm in standard length and the smallest female was 98 mm (average length for both sexes was about 120 mm), and males outnumbered females by a ratio of 2:1. Although the males were dripping milt and the females had ovulated, we did not locate any redds or observe any spawning activity. Morphologically, and by the growth hormone assay, all of the breeding Klesilkwa char appeared to be Dolly Varden; nonetheless, 80% of those examined (50 individuals) carried the bull trout mtDNA marker. Water temperature at this time was 9.0° C.

On October 8, redds and spawning activity were observed in Nepopekum Creek. Again, the spawning fish were small (average standard length about 120 mm), but a few individuals

were larger (up to 245 mm). In Nepopekum Creek, a total of 10 redds were observed; 9 of the 10 redds were sited on the upslope at the tail-end of pools just before the water broke into a new riffle. The other redd was sited in a slow glide between two riffles. The surface velocity over redds averaged 0.35 m^{-s} (SD = 0.13); the bottom velocity on the redds averaged 0.22 m^{-s} (SD = 0.05). Although the substrate in the redds contained a variable mix of rocks (50-100 mm), large gravel (20-50 mm), small gravel (5-20 mm), and fines (<5 mm), there was usually about 10% fines. The water temperature was 6.1° C, and spawning activity continued throughout the day. Morphologically, all of the spawning Nepopekum char appeared to be Dolly Varden, except for one ripe male brook trout. Again, however, over 70% of these putative Dolly Varden carried the bull trout mtDNA marker. New redds were being constructed in Nepopekum Creek on the last day of observation (November 3). At this time the water temperature was 3.1° C.

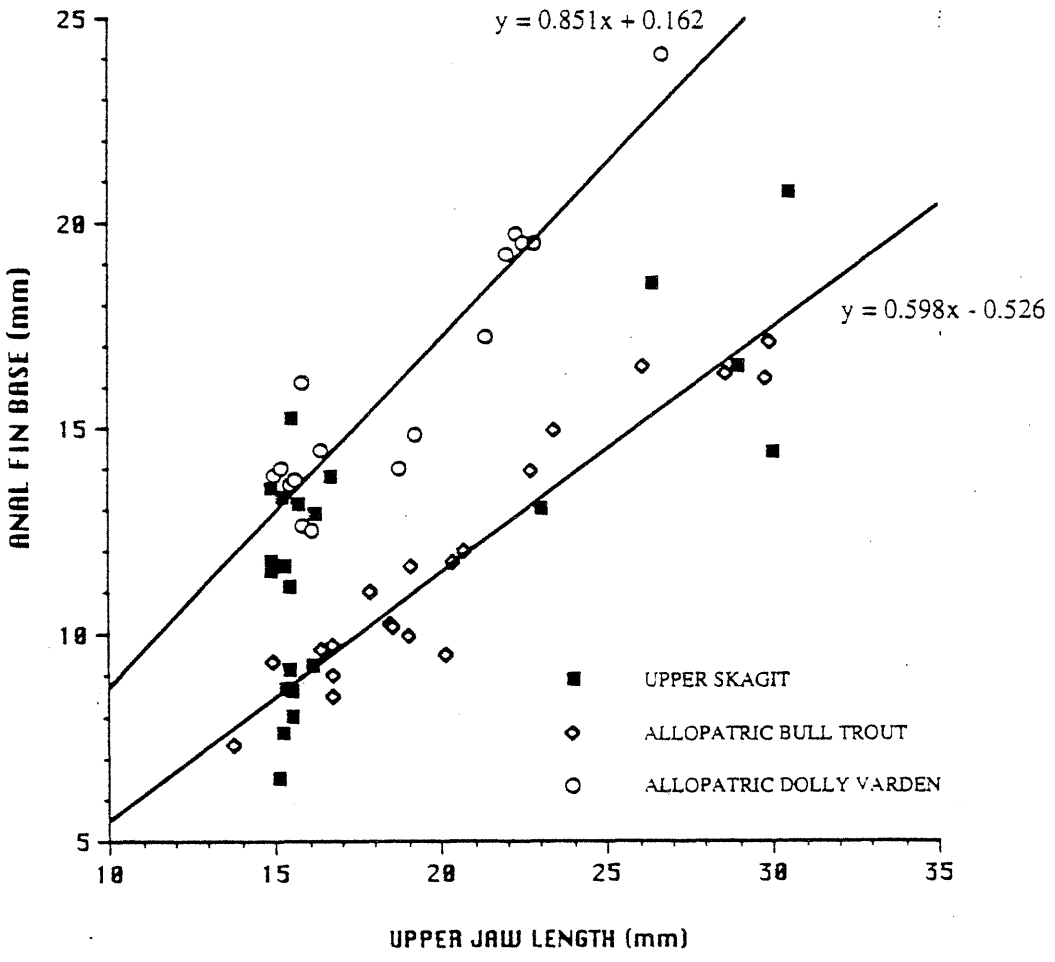


FIGURE 15. Plot of length of anal fin base *vs.* upper jaw length in adult char (> 100 mm). The two lines (and their equations) are for allopatric populations. Note the points for the Skagit cluster around both lines but, also, that some points fall between the lines.

The first redds in the mainstem Skagit were observed on October 19. At this time the water temperature was 5.8° C. These redds were much larger and deeper than the redds found in tributary streams, and were assumed to be bull trout redds. No spawning activity was observed in the day, but new redds appeared overnight. We set wire mesh traps in the vicinity and caught seven large, ripe male char. Morphologically, these males were bull trout, and they all displayed the diagnostic bull trout mtDNA marker; however, both their growth hormone and rDNA restriction fragment patterns indicated that five of the seven males were hybrids. All of the bull trout redds were found in glides or runs, usually in water over 0.5 m deep and with a surface velocity greater than 5 m^{-s}. The substrate contained a higher proportion of rocks and large gravel than the redds in Nepopekum Creek. Interestingly, although we surveyed over 16 km of the main river between Twenty-eight Mile Creek and Nepopekum Creek, all of the bull trout redds were concentrated in a 2 km stretch of river (Figure 18). No new redds were observed in the five days prior to stopping observations (November 4). At this time, the water temperature in the main river was 4.1° C.

6.0 DISCUSSION

6.1 IMPLICATIONS OF THE DATA

Both the molecular and morphological data indicate that there are two native chars in the upper Skagit system. All of the morphological traits (e.g., upper jaw length, length of anal fin base, and the number of branchiostegal rays) that distinguish Dolly Varden and bull trout in allopatry are bimodal in the upper Skagit system. In addition, the two forms appear to occupy different foraging niches and also display strikingly different sizes at sexual maturity. Together, this morphological, ecological and reproductive bimodality strongly suggest the presence of two species. Morphologically, however, there are intermediate individuals, and some of these are intermediate in all morphological traits while others resemble one form in one trait but the other form in another trait. The presence of both intermediate individuals, and individuals with a mosaic of morphological traits, not only suggests hybridization but also backcrossing (i.e., recombination). Although suggestive, the morphological data alone are not sufficient to warrant strong conclusions about the genetic relationships of the two forms of native char.

The molecular data are clearer than the morphological data. The value of both the mtDNA and growth hormone markers were established with samples of Dolly Varden and bull trout tissue from allopatric populations (27 Dolly Varden and 74 bull trout). In all cases, the two markers concurred and were diagnostic. In the upper Skagit, we have both mtDNA and

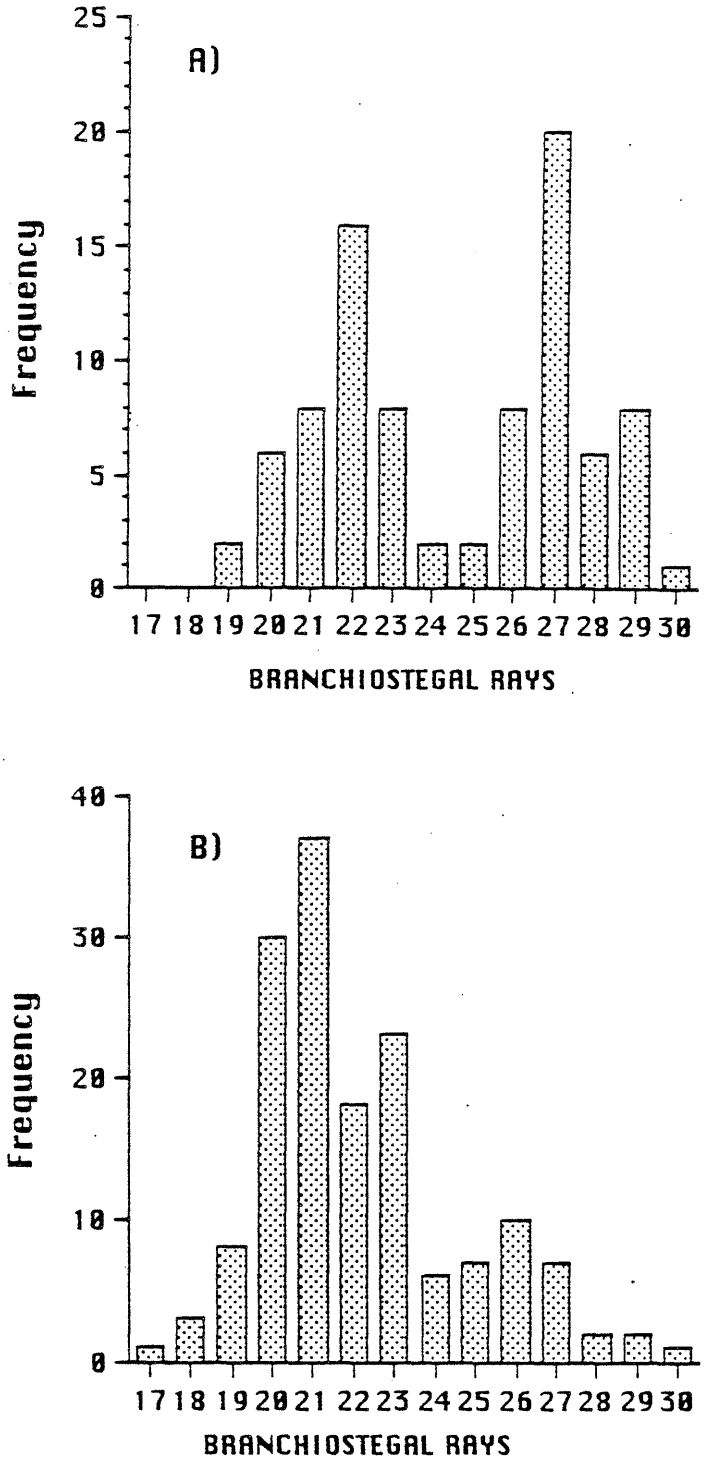


FIGURE 16. Distribution of branchiostegal rays in allopa[r]
and in the upper Skagit (B).

growth hormone data on 101 individuals. Of these, 8.0% were diagnosed as bull trout by both markers, 21.7% were diagnosed as Dolly Varden by both markers, 58.4% were diagnosed as Dolly Varden by the growth hormone marker but as bull trout by the mtDNA marker, and 11.8% were diagnosed as F_1 hybrids by the growth hormone and rDNA markers. Thus, the molecular data reinforce the morphological data, and unequivocally establish that both Dolly Varden and bull trout occur in the upper Skagit system, and that they hybridize.

The combined data imply three things:

1) That not only is there hybridization, but also substantial gene flow, between the native chars in the upper Skagit system. Backcrossing and, consequently, gene flow, is implied by the small number (12 out of 101) of F_1 hybrids and the large number (59 out of 101) of Dolly Varden carrying the bull trout mtDNA marker. At this frequency of F_1 hybrids, backcrossing is required to ratchet the bull trout mtDNA marker up to over 50% in the Dolly Varden population. In addition, the blurring of the morphological difference between the species implies backcrossing. We also have growth hormone data from another study of sympatric Dolly Varden and bull trout (Baxter et al. 1995) that unequivocally establish that some hybrids backcross.

2) That the gene flow is asymmetrical. Over half of the putative Dolly Varden in the system are carrying the bull trout mtDNA marker, but none of the putative bull trout carried the Dolly Varden mtDNA marker. Since the mtDNA markers are inherited exclusively through the mother, this pattern implies crossing between Dolly Varden males and bull trout females. Given the size difference between mature male of both forms in the upper Skagit, the simplest explanation for this pattern is that Dolly Varden males sometimes act as "sneakers" in bull trout spawnings.

3) That there is a substantial disadvantage (ecological, behavioural or reproductive) associated with hybrids. This is implied by the continued presence, in spite of hybridization and gene flow, of two forms of native char in the system. Given random mating, and no selection against hybrids, almost twelve percent hybridization should have fused the two gene pools in a few generations.

6.2 STATUS OF THE NATIVE CHARS

One of the original goals of this investigation was to determine if there are two species of char in the upper Skagit system. Our data clearly indicate that there are two native char in the system, and that they correspond morphologically to two commonly recognized species: the Dolly Varden (*S. malma*) and the bull trout (*S. confluentus*). Given our data set, however,

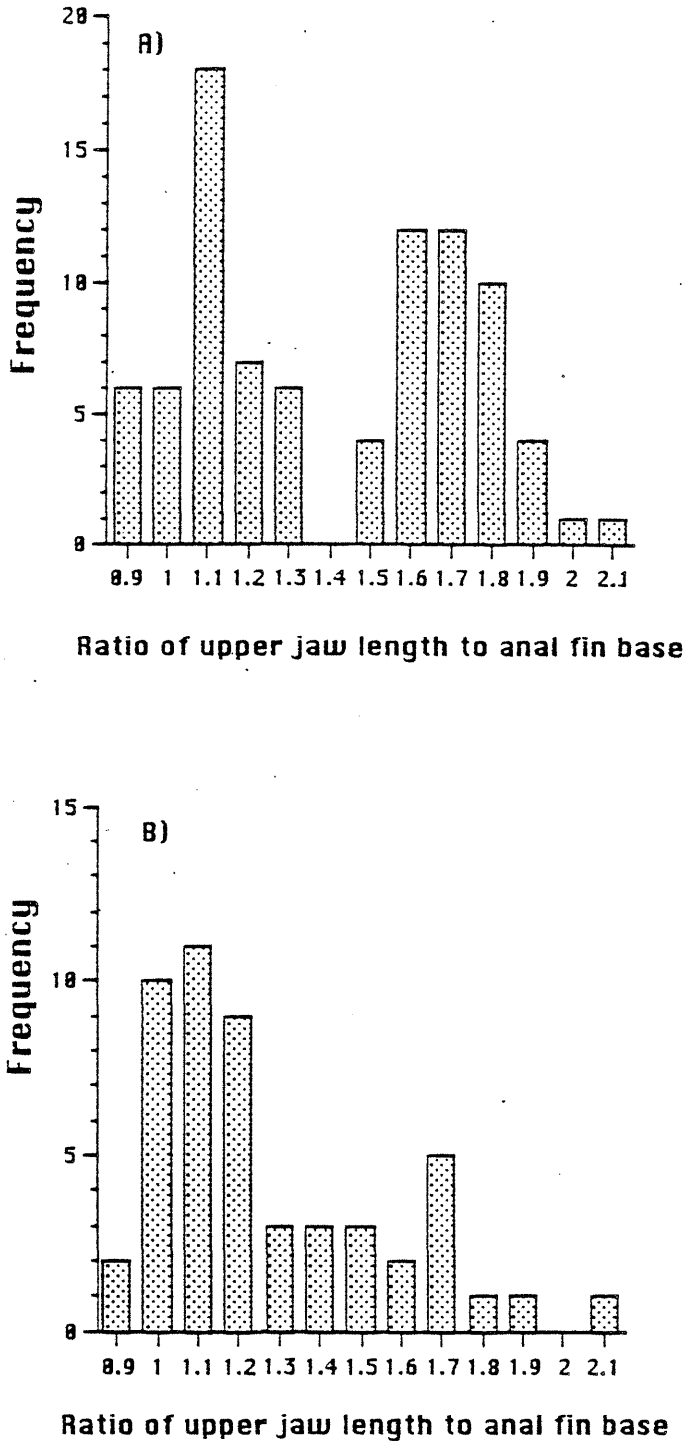


FIGURE 17. Distribution of the ratio of anal fin base to upper jaw length in allopatric Dolly Varden and bull trout (A), and in the upper Skagit (B).

many biologists would object to using the term "species" for entities that hybridize and exchange genes. This is because the definition of what a species is, is still a contentious issue in biology (see Endler 1989 for a recent review). For sexually reproducing animals like fish, species usually are defined as interbreeding genetic units. Thus, the members of a species will interbreed with each other, but not with members of another species. The mechanisms that prevent interbreeding are called reproductive isolating mechanisms and, in freshwater fish, reproductive isolation often is maintained by subtle differences in spawning time or in spawning sites. These mechanisms are especially vulnerable to environmental disturbances (both natural and man-made). Consequently, hybridization is remarkably common among freshwater fish (Hubbs 1955). Still, even in the face of persistent hybridization, most freshwater fish species maintain themselves as distinct genetic entities. This suggests that the critical test of species in freshwater fish is not coexistence without hybridization but, rather, coexistence without the collapse of two gene pools into a single gene pool (introgression).

Under this species definition, the fate of hybrids is all-important. For example, if hybrids are sterile there will be no gene flow and, thus, the gene pools will remain separate in spite of hybridization. Unfortunately, with freshwater fish, things are rarely simple, and hybrids between closely related species usually are not completely sterile. In such cases, what mechanisms prevent introgression? The usual answer is hybrid inferiority. Closely related species that coexist, do so through resource partitioning. That is, they either forage on a different array of food items (although some overlap is possible), or they forage in different places, or at different times. This partitioning of resources reduces competition and allows coexistence and, typically, natural selection fine-tunes the behaviour and morphology of coexisting species in a way that increases their efficiency in their respective niches.

In fish, the genetic mechanisms underlying most morphology and behaviour are polygenic (i.e., they involve a large number of independent genes). One natural consequence of polygenic inheritance is that interspecific hybrids are intermediate in many traits. Thus, hybrids often are not as efficient at exploiting resources as either of the parental species in their respective niches. Consequently, they usually are at a competitive disadvantage (both ecologically and reproductively) relative to the "pure" parental species. This may be the situation in the Skagit char. In this system, Dolly Varden and bull trout seem to be in some kind of balance between gene flow and natural selection. Our morphological and molecular data unequivocally establish hybridization between the two species. Moreover, both the mitochondrial and the growth hormone gene data imply some backcrossing. The significance of backcrossing is that it establishes the fertility of at least some hybrids. Consequently, gene

flow between the species is possible. Given the relatively high level of hybridization (in excess of 10% per generation), without some selection against hybrids, the two gene pools should have fused (introgressed). Yet, this has not happened and, therefore, some mechanism must select against hybrids.

In the upper Skagit, our ecological data suggest that the two species occupy different trophic niches: adult Dolly Varden are drift feeders, while adult bull trout are piscivores. To efficiently exploit such different trophic niches, the two species probably require different morphologies and different foraging behaviours. Although our morphological data indicates that hybrids are intermediate, there is no data on the behaviour of either the parental species or hybrids. Thus, at this stage, we can only speculate that an inability to compete with the parental species is the major mechanism maintaining the two species of Skagit char in a selection-gene flow balance.

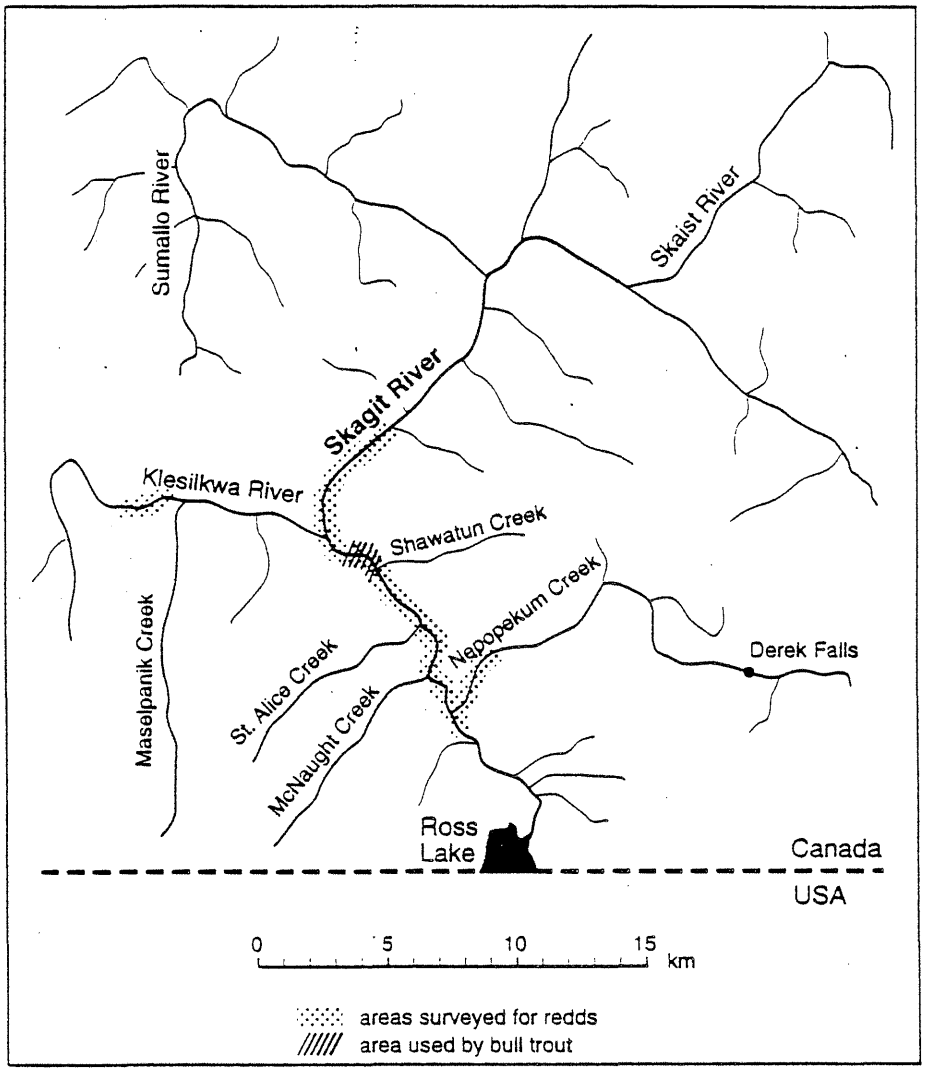


FIGURE 18. Areas surveyed for redds, and the main bull trout spawning area, in the upper Skagit system.

7.0 RECOMMENDATIONS

7.1 MANAGEMENT

Implicate in the speculation that upper Skagit Dolly Varden and bull trout are in a gene flow-selection balance, is the notion that the balance can be disturbed. The hypothesis is that hybrids are selected against because they are intermediate in their behaviour, ecology and morphology and, therefore, competitively inferior to both parental forms in their respective niches. Thus, if one of the parental forms declines in abundance, the survival of hybrids in that niche should increase and, in turn, this could increase gene flow between the species. Since gene flow in this system is asymmetrical, one possible outcome of increased gene flow is the genetic swamping of one of the species.

Given this scenario, our data suggest that the bull trout is the species at risk. Dolly Varden are abundant in tributaries, they spawn at a variety of sites, and are too small to be significant in the recreational fishery. In contrast, adult bull trout are confined to the main river and reservoir, they appear to spawn at only one restricted site, and they are vulnerable to the recreational fishery. Although the fishery in the upper Skagit is catch and release, it still inflicts some mortality through gill-hooking and poaching. In addition, the rainbow trout fishery continues through most of the bull trout breeding season and in the bull trout spawning area. In this area, angler's footprints are conspicuous around the redds, and trampling of redds can be an important source of egg mortality (Roberts and White 1992). This is probably also true for bull trout. To provide protection for both bull trout spawners and to their redds, we recommend an early closing (mid-September) of the river to angling between Twenty-six Mile Bridge and Shawatum Creek.

The full extent of bull trout spawning in the upper Skagit is an important piece of management information that, at present, is missing. Although our survey found bull trout redds concentrated in one small area, there may be other spawning sites. For example, we saw one large adult bull trout in the upper Sumallo River and, although we checked the area for redds on two occasions and found none, we may have missed a spawning site. Since the redds are large, and the cleaned areas are conspicuous against an undisturbed background, they should be easy to see from the air. The whole river, from the border to the upper Sumallo, could be flown in less than an hour. Consequently, we recommend an annual aerial survey as a cost effective method of assessing the extent of bull trout spawning activity.

7.2 RESEARCH

Relative to other bull trout populations (McPhail and Baxter 1995), the life-history of those in the upper Skagit appear to have some unique attributes. For example, the size at which they become piscivores is unusually small, and their use of tributary streams as rearing areas until they reach about 300 mm is unusual. Finally, because the upper Skagit is one of the few accessible areas where Dolly Varden and bull trout coexist, a close look at the relative survival rates of the two species and their hybrids is possible. All of the above could be accomplished by a formal life-history study of Dolly Varden and bull trout in the upper Skagit. The resulting data on habitat use, movements, growth, age and survival would provide a solid basis for a management plan.

Obviously, the native char do not respect the international boundary. Consequently, it is imperative that any management plan for the Skagit char be based on data from both sides of the border. For example, the restricted spawning site used by bull trout in the main river may be an illusion, since some of the major tributaries on the U.S. side may support spawners. In the same vein, the relative importance of the main river and the reservoir as foraging and nursery environments can only be ascertained with comparable data from both areas. Thus, our main recommendation regarding future research is that the entire Skagit system above Ross Dam be treated as a single research venue. This will require cooperation on both sides of the border, but without a unified approach to data collection any management strategy cooked-up on one side of the border will necessarily be "half-baked".

8.0 ACKNOWLEDGEMENTS

This study would not have been as comprehensive without the molecular expertise of Bob Devlin and Carlo Biagi, Department of Fisheries and Oceans (West Vancouver Laboratory). We are in their debt. Ross Neuman and Marvin Rosenau (Ministry of Environment, Lands and Parks, Fisheries Branch, Surrey Office) suggested the project and provided permits and encouragement. In Victoria, Al Martin and Jim Walker provided support, while Jeff Burrows provided pull at a critical moment. Mr. E. B. Kelly (Ministry of Environment, Lands and Parks, Parks Branch) kindly gave permission to work in the Skagit Recreational Area. Peter Mylechreest provided his usual keen observations, solid information and clear insights. Gordon Haas shared his char lore, and James Baxter, Ernest Keeley, Ian Kusabs, and Peter Troffe helped in the field. To all of these people we are grateful and extend genuine thanks. This research was funded by a grant (Project 94-1) from the Skagit Environmental Endowment Commission. Without their financial aid this research would not have been done.

9.0 REFERENCES

Allendorf, F. W., and G. H. Thorgaard. 1984. Tetraploidy and the evolution of salmon fishes, p. 1-53. *In* B. Turner (ed.), Evolutionary genetics of fishes. Plenum Press, New York, New York.

Avise, J. C., and R. A. Lansman. 1983. Polymorphism of mitochondrial DNA in populations of higher animals, p. 147-164. *In* M. Nei and R. K. Koehn (eds.), Evolution of genes and proteins. Sinauer Associates Inc., Sunderland, Massachusetts.

Baker, R. J., S. K. Davis, R. D. Bradley, M. J., Hamilton, and R. A. Van den Bussche. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies of a contract zone in the pocket gopher, *Geomys*. Evolution 43: 63-75.

Baxter, J. S., E. B. Taylor, J. D. McPhail, and J. Hagen. 1995. Evidence for natural hybridization between bull trout and Dolly Varden char in a north-central British Columbia watershed. In press.

Cavender, T. M. 1978. Taxonomy and distribution of the bull trout, *Salvelinus confluentus* (Suckley) from the American Northwest. Calif. Fish and Game 64: 139-174.

Devlin, R. H. 1993. Sequence of sockeye salmon type 1 and 2 growth hormone genes and the relationship of rainbow trout with Atlantic and Pacific salmon. Can. J. Fish. and Aquat. Sci. 50: 1738-1748.

Endler, J. A. 1989. Conceptual and other problems in speciation, p. 625-648. *In* D. Otte and J. A. Endler (eds.), Speciation and its consequences. Sinauer Associates Inc., Sunderland, Massachusetts.

Grewe, P. M., N. Billington, and P. D. N. Hebert. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. Can. J. Fish. and Aquat. Sci. 47: 984-991.

Haas, G. R., and J. D. McPhail. 1991. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. Can. J. Fish. Aquat. Sci. 48: 2191-2211.

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic reconstruction. Quart. Rev. Biology 66: 411-452.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1-20.

McPhail, J. D., and J. S. Baxter. 1995. A review of bull trout (*Salvelinus confluentus*) life-history and habitat use in relation to compensation and improvement opportunities. Report prepared for the Habitat Management Division, Department of Fisheries and Oceans, Vancouver, British Columbia.

Pleyte, K., S. D. Duncan, and R. B. Phillips. 1992. Evolutionary relationships of the genus *Salvelinus* inferred from the DNA sequences of the first internal transcribed spacer (ITS 1) of ribosomal DNA. Molecular Phylogenetics and Evolution 1: 223-230.

Roberts, B. C., and R. G. White. 1992. Effects of angler wading on survival of trout eggs and pre-emergent fry. N. Amer. J. Fish. Management 12: 450-459.

Taylor, E. B., C. J. Foote, and C. C. Wood. 1995. Molecular genetic evidence for parallel life history evolution in a Pacific salmon (sockeye salmon and kokanee, *Oncorhynchus nerka*). Evolution 49: in press.

Williams, J. E., J. E. Johnson, D. A. Hendrickson, S. Contreras-Balderas, J. D. Williams, M. Navarro-Mendoza, D. E. McAllister, and J. E. Deacon. 1989. Fishes of North America endangered, threatened, or of special concern: 1989. Fisheries 14: 2-20.

Wilson, C. C., and P. D. N. Hebert. 1993. Natural hybridization between Arctic char (*Salvelinus alpinus*) and lake trout (*S. namaycush*) in the Canadian Arctic. Can. J. Fish. Aquat. Sci. 50: 2652-2658.

10.0 APPENDIX — FIELD KEY TO THE THREE SPECIES OF SKAGIT CHAR

WARNING

These keys are meant as an aid to field identification in the upper Skagit system. They rely heavily on differences in pigment patterns that may not hold in other areas. Remember that about 10% of the fish in this area are F_1 hybrids. Consequently, there will be individuals that are either intermediate or a mosaic of characters. Because of this, the keys should be used with caution and, where possible, applied to a large enough sample to detect the bimodality characteristic of the presence of both species.

10.1 KEY TO NEWLY-EMERGED FRY (20-30 mm)

1 (2)Dense pigment at the base of anal fin; numerous small dark spots on dorsal surface in fish >30 mm ..... Brook trout

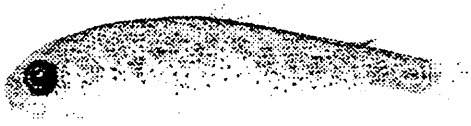
..... *Salvelinus fontinalis* (Introduced)

2 (1)No dense pigment at the base of anal fin; dorsal surface without small dark spots 3

3 (4) Parr marks distinct; posterior end of adipose fin densly speckled

..... Bull trout

..... *Salvelinus confluentus*

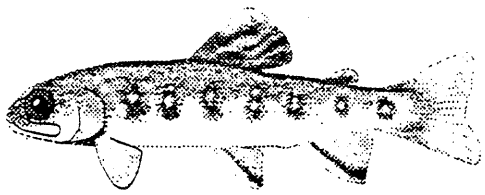


4 (3) Parr marks indistinct; posterior end of adipose fin lightly speckled

..... Dolly Varden

..... *Salvelinus malma*

10.2 KEY TO PARR (35-100 mm)



1 (2) Dorsal fin with bold, black spots; on fish 35-50 mm long the dorsal surface is covered in small,dark spots; on fish over 50 mm the anal fin has a white leading edge that contrasts sharply with the black pigment behind .....Brook trout

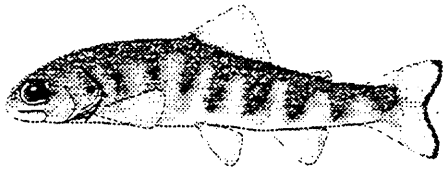
.....*Salvelinus fontinalis* (Introduced)

2 (1) Dorsal fin without well developed spots; on fish 35-50 mm long the dorsal surface is uniformly pigmented; on fish over 50 mm the anal fin is immaculate 3

3 (4) Snout pointed; caudal fin edged with a light dusting of pigment

.....Bull trout

.....*Salvelinus confluentus*

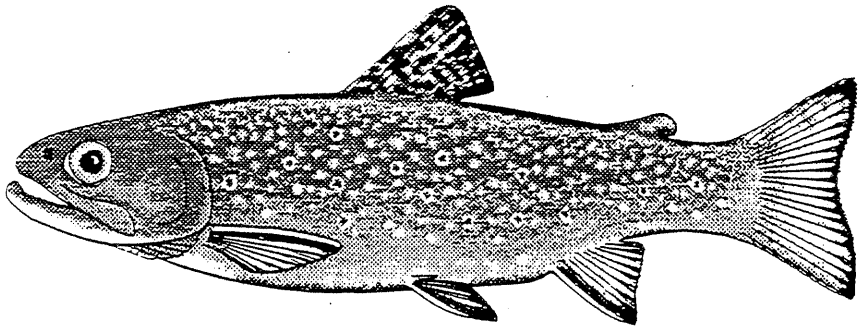


4 (3) Snout blunt; caudal fin clearly edged with black pigment

.....Dolly Varden

.....*Salvelinus malma*

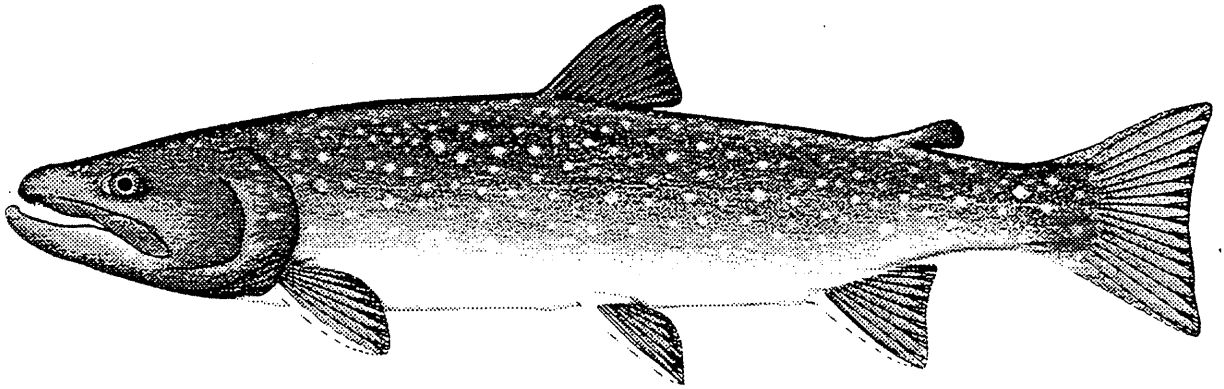
10.3 KEY TO JUVENILES AND ADULTS (>100 mm)



1 (2) Dorsal fin heavily mottled with black against a light background; coloured spots on flanks surrounded by blue haloes ..... Brook trout

..... *Salvelinus fontinalis* (Introduced)

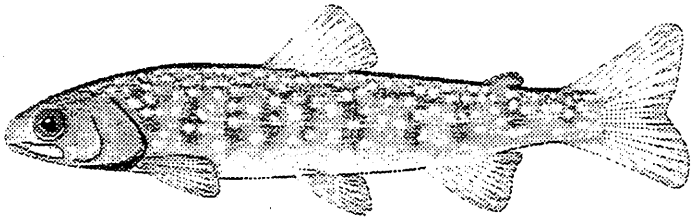
2 (1) Dorsal fin a uniform dusky colour; none of the coloured spots on flanks surrounded by blue haloes 3



3 (4) Snout pointed; ratio of upper jaw length to anal fin base about 1.7 (range 1.5-2.1); viewed from above, spots on back are relatively large and well separated

..... Bull trout

..... *Salvelinus confluentus*



4 (3) Snout blunt; ratio of upper jaw length to anal fin base about 1.2 (range 0.9-1.3); viewed from above, spots on back are small and crowded together

..... Dolly Varden

..... *Salvelinus malma*