VARIATION OF DOLLY VARDEN *SALVELINUS MALMA* IN NORTHWESTERN NORTH AMERICA

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Abstract

Dolly Varden (*Salvelinus malma* (Walbaum)) are native to northwestern North America and northeast Asia. Current taxonomical classifications within *S. malma* are ambiguous due to the morphological and ecological plasticity observed throughout their range. Uncertainty exists regarding the number and status of geographic variants of *S. malma* throughout their North American distribution. The objective of this study was to define the number and distribution of natural Dolly Varden taxa below the species level in northern North America, and to assign those taxa to an appropriate level. Morphological characteristics were explored using analysis of variance (ANOVA) and principal component analysis in order to assess the geographic patterns of variation. Allozyme frequency data were collected from the literature and were used to construct trees representative of phylogenetic relationships. The patterns of variation observed correspond to an existing hypothesis that differentiates between a northern and southern form. Phylogenetic analysis indicates the northern and southern forms represent different ancestral clades, which may be indicative of subspecific taxa. The location of the boundary between the northern and southern form suggests the variation may stem from an historic separation during the late Pleistocene glaciation.

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1.0 Introduction

1.1 Classification of Salmonids

Classification of fishes within the Salmonid family remains problematic for taxonomists despite thorough investigation (Grewe et al. 1990, Phillips and Pleyte 1991, Phillips and Oakley 1997). Members of the Salmonidae display a high frequency of hybridization and introgression, which complicates phylogenetic analysis (Taylor 2004, Phillips and Oakley 1997). In addition, there are few shared derived characters between branches of Salmonids, due to the rapid radiation following tetraploidization (Allendorf and Thorgaard 1984, Phillips and Oakley 1997, Grewe et al. 1990). Salmonids also display a wide range of genetic and phenotypic variation, both between and within species (reviewed in Willson 1997).

The classification of Salmonids is further complicated by glacial history, particularly in their north Pacific range. Repeated glacial events and subsequent recolonization of glaciated areas often results in low genetic divergence between species, creating taxa that do not fit the strict requirements of the biological species concept (Bernatchez and Wilson 1998). The high degree of variation within Salmonid taxa, coupled with low genetic divergence, can greatly complicate attempts to decipher intraspecific relationships. These complications have resisted attempts at establishing a general agreement as to relationships within the Salmonid family, perhaps most notably in the intensely debated genus *Salvelinus*, which includes the Dolly Varden (*Salvelinus malma* (Walbaum)) (Phillips and Oakley 1997). The objective of this study is to attempt

to define the presence, or absence, of subspecific taxa within North American Dolly Varden by describing the geographic pattern of genetic and morphological variation.

1.2 Classification of Dolly Varden

Dolly Varden, a salmonid charr species, has been the subject of many taxonomic studies, the result of which is the establishment of *S. malma* as a distinct species in North America (McPhail 1961, McPhail and Lindsey 1970, Behnke 1972, Behnke 1980, Morrow 1980). Discussions continue as to the status of Dolly Varden and it's subspecific taxa in Asia, however this paper will focus solely on North American Dolly Varden. Previously, northwestern *S. malma* were considered a subspecies of Arctic charr, *Salvelinus alpinus* (L.) (McPhail and Lindsey 1970) while *S. malma* at the south eastern edges of its range were confused with Bull Trout, *Salvelinus confluentus* Suckley (McPhail 2007). The Dolly Varden has been differentiated from its closely related species on the basis of morphology, ecology and life history. However, the strongest argument supporting Dolly Varden as a distinct species is that of sympatric reproductive isolation (McPhail 2007). Dolly Varden and Bull Trout coexist sympatrically, without evidence of hybridization, within the basins of the Fraser River, Skeena River, Nass River and Stikine River in British Columbia (Haas and McPhail 1991). However, there is evidence of interbreeding in other locales (McPhail 2007). Similarly, Dolly Varden and Arctic charr have been found to coexist without pervasive hybridization in southwestern Alaska (Reist et al.1991). Thus, sympatry provides support for Dolly Varden as a distinct species, within the biological species concept (Haas and McPhail 1991, Reist 1991).

1.3 Diversity within *S. malma*

The extensive phenotypic diversity of Dolly Varden and other members of the genus *Salvelinus* confuses the taxonomic understanding of the group. The morphological, ecological, genetic and karyological characteristics of Dolly Varden vary throughout their extensive North American range. Previous studies have suggested that the morphological variation within Dolly Varden essentially describes two forms: a Northern form with high counts of gill rakers (21-23) and vertebrae (66-68) and a Southern form with lower counts of gill rakers (15-19) and vertebrae (62-65) (Behnke 1980, 1984; Morrow 1980). Karyological variation also occurs between forms with the Southern form described as having a diploid number of 82 (2n=82) and the number of chromosomes arms, NF (nombre fondemental) = 98, while the Northern form has a diploid number of 78 (2n=78) and NF= 98 (Ueda 1991; Cavender and Kimura 1989; Phillips et al. 1999). Further chromosome analysis has observed differences in the location of the nucloear organizing region (NOR); present on a single submetacentric chromosome in the Southern form (Cavender 1984; Phillips et al. 1989) and present on a single acrocentric chromosome for a small (n=4) sample of Northern form fish (Phillips et al. 1999).

The suggested geographic range of the Northern form extends from west of the Mackenzie River in northwestern Arctic Canada, west along coastal north slope Alaska and then south to the Alaskan Peninsula and along the Aleutian Islands, as shown in Figure 1. The Southern form extends south of the Alaskan peninsula, along the coast of British Columbia southwards to Puget Sound, Washington (Behnke 1980, 1984; Morrow 1980). An alternative hypothesis suggested by Craig (1989) places the demarcation line between the two forms along the Kuskokwim River, north of the Alaskan Peninsula.

Figure 1. Distribution of Northern and Southern form Dolly Varden as proposed in Behnke (1980, 1984) and Morrow (1980).

It has been proposed that these two forms represent distinct taxonomic groups of a common ancestral species that was separated during the last glacial period (McPhail 1961, Behnke 1984). Continuing from this hypothesis, a review of the glacial history and geography of the northern North American range of Dolly Varden supports the possibility that more than two groups of Dolly Varden remained isolated during the Pleistocene glaciation. Reconstructed glacial scenarios suggest three possible areas of isolation (see

below). This isolation suggests there may be two or three distinct forms of Dolly Varden within Northwestern North America.

1.4 Life history of North American Dolly Varden

Dolly Varden in North America display three major life history types: 1) an anadromous form, which migrates between freshwater streams and the ocean; 2) a stream-resident form which remains in rivers and streams for the majority of its life, which can be further sub-divided into a) a small (residual) form that co-occurs with anadromous fish, and, b) stream-resident fish which are isolated either by falls or distance; and, 3) a lacustrine form, which remains within a single freshwater body, spawning in surrounding streams (McPhail 2007).

Life history type determines many aspects of Dolly Varden ecology. An example of this is the variable diet of Dolly Varden. Anadromous Dolly Varden feed on large crustaceans and small fish in the productive marine environment (Benhke 2002). In freshwater environments, Dolly Varden feed largely on nymphs and larvae of aquatic insects and snails, although they will feed on salmon eggs if available and have been known to prey on juvenile salmon (Benhke 2002). Lacustrine populations display a flexible diet, which fluctuates depending on co-existing species (McPhail 2007). Variation also occurs within life history types, such as spawning times among anadromous populations. Summer spawners overwinter in fresh water and travel upstream to spawning areas, only traveling to sea if overwintering in a non-natal stream. Thus the majority of summer spawners spend the whole of the spawning year in freshwater. Fall spawners migrate to sea to feed for the summer and then migrate that

autumn to natal spawning grounds in headwater streams to spawn and remain for the winter (DeCicco 1997).

The variation in life history further complicates our understanding of intraspecific taxa. Life history differences dictate when and where populations spawn, and therefore play an important part in the partial reproductive isolation of populations within the species. As well, populations which display different life histories are exposed to unique ecological factors, such as diet and size of the water body they inhabit, which affect the resulting morphology of the fish (Willis 1997). Thus, anadromous fish, which are sea run and eat a fish based diet, are much larger that residual fish which consume a lower energy diet and remain within freshwater streams. Therefore, life history variation must be accounted for in the analysis of variation within the species as a whole.

1.5 Glacial history of Northern North America

The North American range of Dolly Varden has experienced extensive glaciation. During the Pleistocene, it is estimated there were up to twenty glacial periods (Bernatchez and Wilson 1998). The most recent glacial period was the Wisconsin, which lasted from approximately 40 000 to 8000 years ago. This glaciation resulted in a lowering of sea level by down to 90 m, exposing a land bridge across the Bering Strait (Lindsey and McPhail 1986). The movement of glacial fronts altered and destroyed freshwater habitats, with fish surviving in newly created lakes and streams along glacial margins (Pielou 1991). Glacial activity may have isolated populations of Dolly Varden in several different refugia, resulting in phylogenetic divergence of these populations. It is suspected that the degree and length of glacial isolation may have been significant enough to create at least two discrete groups of Dolly Varden, possibly at the subspecific level.

During glaciation, fish of northern North America occurred in three major refugia; 1) Beringia, which included the north slope of Alaska, eastern Siberia and the Yukon River Basin, 2) Cascadia, existing south of glaciation along the Alaskan Peninsula, and, 3) the Mississippi River basin, consisting of interior areas well south of the main glacial areas (Figure 11) (Lindsey and McPhail 1986). The Southern form of Dolly Varden is thought to have derived from populations isolated south of the Alaskan glaciers, while the Northern form is thought to have been derived from populations isolated in Beringia (McPhail 1961). This Beringian population may have been further divided by glaciation and local relief into two major drainage basins, shown in Figure 2. Northern Beringia drained northwards into the Chukchi Sea via the Chukchee River and the Beaufort Sea via north slope rivers. Southern Beringia drained southwards via an extension of the current Yukon and Kuskokwim rivers into the Bering Sea. Thus, based upon this geological history, at least two (Beringia and Cascadia) and possibly three (Cascadia, North Beringia, and South Beringia) distinct areas containing Dolly Varden existed during glacial times. This geological history provides the basis for differentiation of Dolly Varden into two or more taxonomic forms as explored in this research.

1.6 Objectives

The primary objective of this study was to identify and define the number and geographic distribution of Dolly Varden taxa in northern North America. The secondary objective was to describe the nature of the variation observed and possible mechanisms responsible for the separation by placing the variation in the context of the Pleistocene

glacial history of this area.

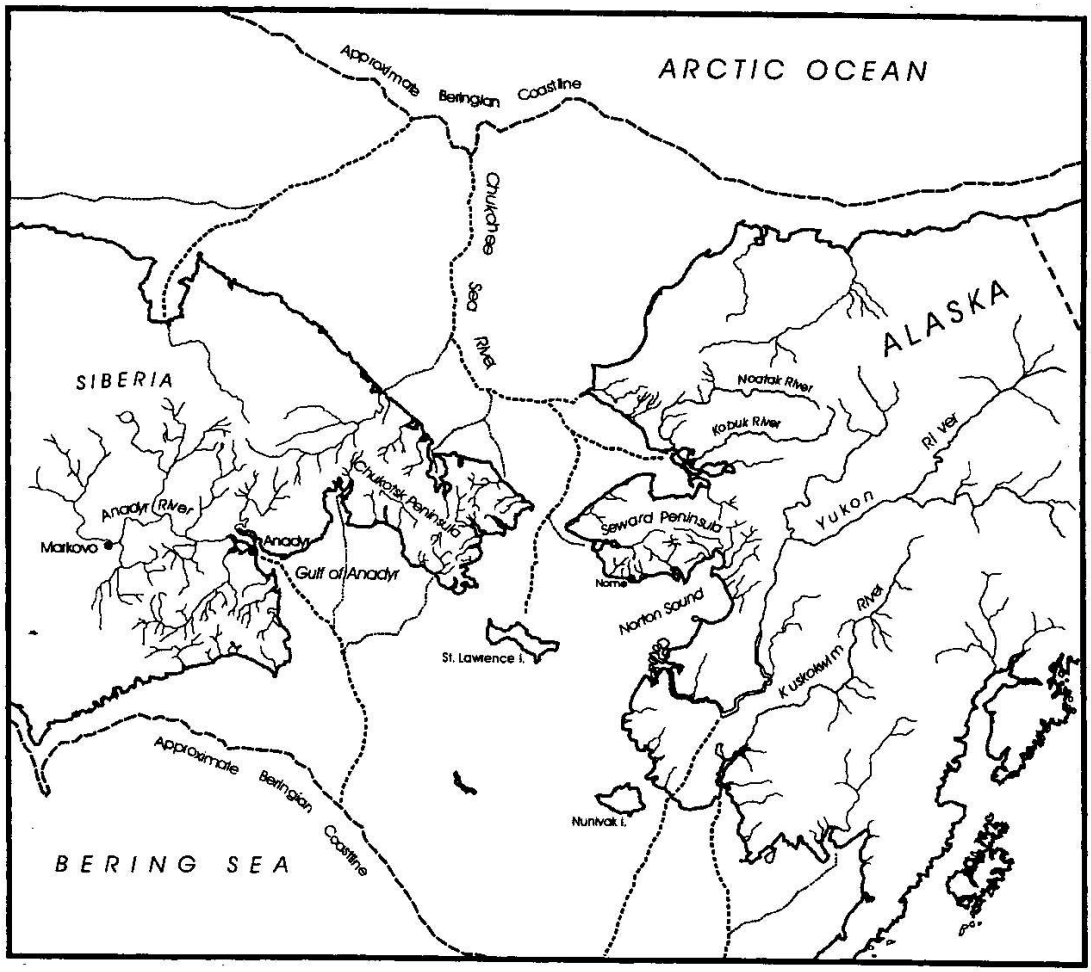

Figure 2. Drainage patterns of Beringia during the Late Wisconsin period. Dashed lines represent Beringian Coastline and dotted lines represent glacial rivers. From DeCicco and Reist (1999). Based on Hopkins (1972) and Lindsey and McPhail (1986).

2.0 Methods

2.1 Logic of the Study

Step 1) To examine the variation within Dolly Varden, data were collected from existing literature, including published peer-reviewed journals, books and fisheries reports with data recorded for as many characteristics as possible. This included morphological measurements such as fork length (FL), meristic counts, such as the numbers of gill rakers and vertebrae (see section 2.3 below), as well as karyological data. Genetic data were also collected, measured as the heterozygosity of 18 selected allozyme loci. Morphometric and meristic data were explored using univariate analysis to visualize patterns of variation. Allozyme frequency data were used to construct a parsimonious phylogenetic tree. These data were organized geographically and the location of each sample was plotted using ArcView 3.0 to reveal patterns in the variation.

Step 2) From the above analyses, more specific hypotheses of the number of subspecific taxa present were developed.

Step 3) The hypotheses developed were then tested with new morphological data for representative samples of Dolly Varden from all putative sub-specific taxa. Data for these new samples were taken from an archive held at the Freshwater Institute, the Department of Fisheries and Oceans Canada. Morphometric and meristic data were examined using a number of univariate methods including one-way ANOVA. The data were examined for each sample location individually, as well as for samples grouped into geographic categories (northern, southern, intermediate) that represented the suspected geographic and glacial divisions. The preliminary patterns were then tested using principal component analysis.

2.2 Sample selection

Literature samples were selected based on geographic location, with an attempt to collect data from as many samples as possible in the northern North American range of Dolly Varden (Figure 3, Table 2). Despite attempting to gather data from as wide a geographic distribution as possible in order to represent the diversity within the species, limited samples were available in the literature from the interior of Alaska and the Yukon River drainage basin. Another limitation was the condensed form of raw data found in published reports, which limited statistical analysis on the data collected from the literature. Therefore, the final analysis included only literature samples of allozyme data, as all other literature data were inadequate for analysis due to unavailability of raw data and extremely small samples sizes ($n < 10$).

Additional samples were selected from a morphological database for Dolly Varden at the Freshwater Institute (Table 1). These samples were also chosen by geographic location, which included a sample from the extreme northern (Canoe River, YK) and southern (Klutina River, AK) areas of the distribution (Figure 3). These two samples were taken, respectively, to represent the Northern and Southern forms of Dolly Varden as outlined in the present scientific literature based on their mean number of gill rakers and geographic location. In addition, two samples were selected from central locations within the distribution of *S. malma* (Nome River, AK and Becharof Lake, AK) as well as one sample that were presumed to represent an isolated life history type of the putative Northern form (Babbage River, YK) and one sample of unknown life history and affinity, in regards to northern or southern form (Blackstone River, YT). The four original samples within the Becharof Lake area were reduced to three samples because the Egegik

River sample location was known to have sympatric Arctic charr present. These fish, thus, may have been mistaken for Dolly Varden and to avoid confusion this sample was eliminated from further analysis. All samples, excepting Babbage River and Blackstone River were known to be anadromous, in order to reduce the potentially confounding effect of life history differences on any historical difference that may be present. The coastal samples in this part of the study were representative of populations within the major drainage basins of northwestern North America, that is, rivers to the Beaufort Sea basin, the Bering Sea basin (Norton Sound and Bristol Bay) and the Gulf of Alaska basin. These samples were chosen to represent the likely range of geographic variation and number of subspecific taxa possibly present in this area.

Sample locations from the literature were recorded in decimal degrees. Locations in UTM format were converted using the program UTM2LL. Locations in decimals (dd), minutes (mm) and seconds (ss) were converted to decimal degrees (dd.ff).

$$dd.ff = dd + mm/60 + ss/3600 \quad (2.0)$$

Literature samples without a recorded location (latitude/longitude or UTM) were identified by searching for the location name on the USGS map locator (Available at http://www.usgs.gov/).

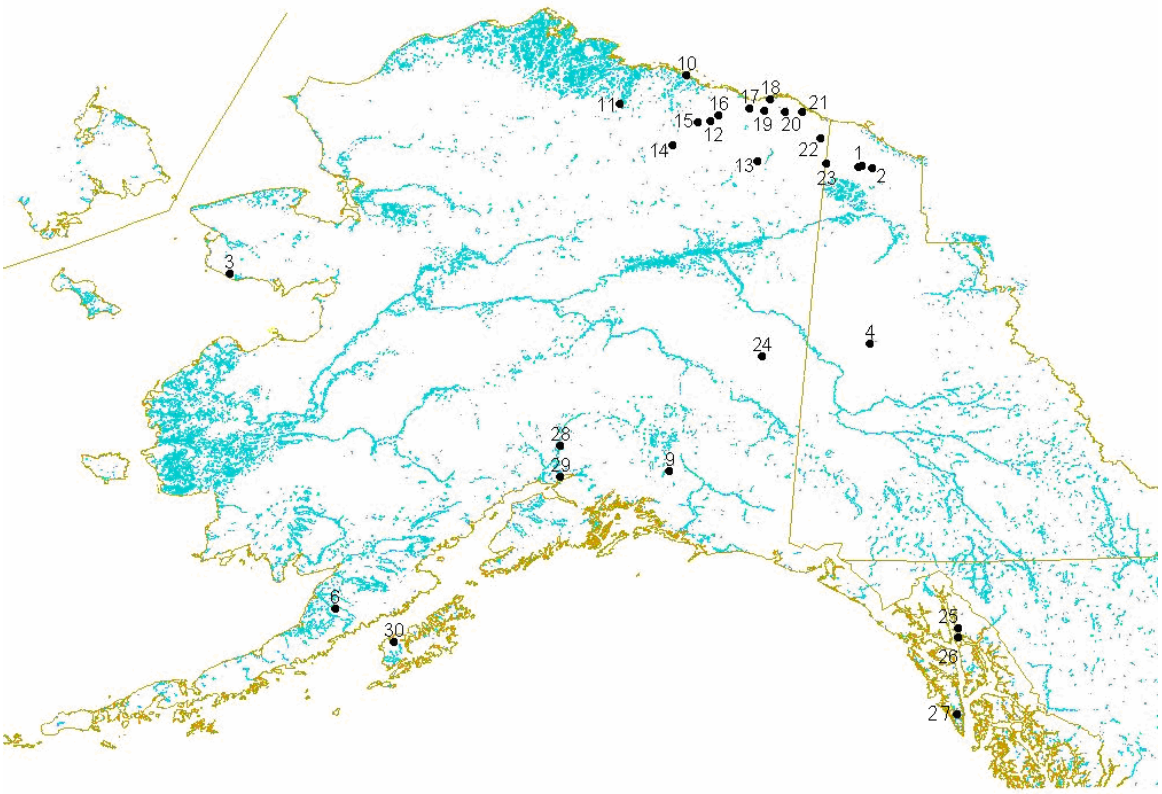

Figure 3. Location of morphological samples from Fisheries and Oceans (Samples 1-9, with location names provided in Table 1) and samples selected from the literature (Samples 10-30, with location names provided in Table 2).

2.3 Morphometrics and Meristic Data

Morphometric measurements and meristic counts were taken mostly from the left side of the fish, for the sake of consistency. Gill arch measurements and gill raker counts were taken from the right first gill arch. Eight meristic counts were included in the analysis: fin ray counts from the dorsal (DRC), anal (ARC), pectoral (PRC) and ventral (VRC) fins, as well as counts of branchiostegal rays (BRC); upper (UGR) and lower (LGR) gill raker counts were regarded as separate variables; and counts of pyloric caecae (PYL). Fifteen morphometric variables were analyzed, illustrated in Figure 4, including measurements of length along the fish's body: pre-orbital length (POL), orbital length (OOL), postorbital length (PSL), trunk length (TTL), dorsal length (DOL), lumbar length (LUL), anal length (ANL) and caudal peduncle length (CPL). Morphometric variables also included measurements of depth: head depth (HDD), body depth (BDD), caudal peduncle depth (CPD), interorbital width (IOW), maxillary length (MXL), maxillary width (MXW), and pectoral fin length (PCL).

2.4 Phylogenetic analysis

Allozyme data were compiled from the literature for a set of 30 loci (Gharret et al.1993, Crane et al. 1994, Everett et al. 1997, Reist et al. 1997). The data set was reduced to 18 loci (see Table 2), due to restrictions imposed by the program PHYLIP, which

Table 1. Sample location, size, date collected, life history type and suggested geographic form for samples selected from the Freshwater Institute database. Life history types are defined as I = isolated riverine fish, A = anadromous riverine fish, R= resident riverine fish and ? = unknown life history type. Suggested forms are N = Northern form, I = Intermediate form and S = Southern form.

Number	Location Name	Latitude	Longitude	Date	Life history	N	Form
1	Babbage River, YT	68.34	-139.19	1986	I	33	N
2	Canoe River, YT	68.46	-138.44	1986	A	70	N
3	Nome River, AK	64.67	-165.33	1991	A	54	I
4	Blackstone River, YT	64.77	-138.37	1992	R	36	U
5	Egegik River, Becharof Lake, AK			1998	?		I
6	Ruth Arm, Becharof Lake, AK			1998	A	16	I
7	Cleo Creek, Becharof Lake, AK			1998	A	14	I
8	Bear Creek, Becharof Lake, AK			1998	A	12	I
9	Klutina River/Lake, AK	61.45	-145.45	1991	A*	97	S

* As documented by Hocutt and Wiley 1986, although not recorded during collection of sample.

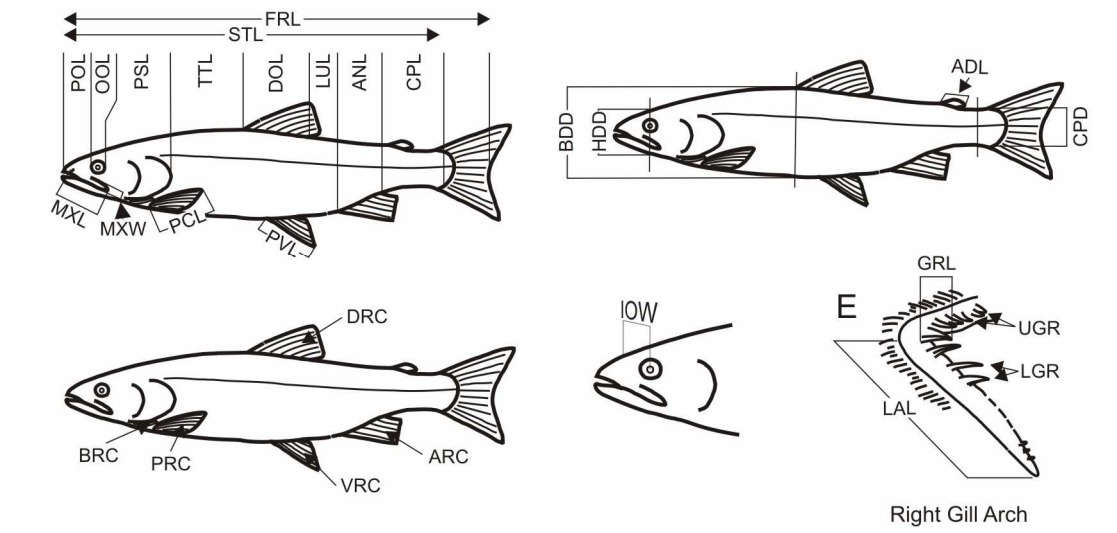

Figure 4. Morphometric measurements used for analysis. Description of acronyms found in the text. From Reist et al. (1991).

cannot accept variables with missing data points. The data were assessed for consistency according to Osinov (2001) which provided a comparison and summary of methods and alleles observed in various papers. Allozyme data were bootstrapped using Seqboot, a program from the PHYLIP package, to create one hundred pseudo-replicate data sets. Nei's distance matrices (Nei, 1972) were constructed for the bootstrapped pseudo-replicate data sets using Gendist of PHYLIP. The neighbour joining (NJ) method was applied to the pseudo-replicates using the Neighbor program of PHYLIP creating one tree for each pseudo-replicate set. The NJ method, developed by Saitou and Nei (1987), is based on the approach of minimum evolution (ME), which calculates the sum (S) of all branch lengths for each topology and selects the topology with the smallest value of S (Nei and Kumar 2000).

$$S = \sum_{I}^{T} \hat{b}_i \qquad (2.1)$$

The NJ method uses the ME approach at each stage of taxon clustering to produce a final tree with the smallest value of S (Nei and Kumar 2000). A bootstrap consensus tree was constructed using Consense, based on the concept of majority rule from the PHYLIP package. This sequence of analytical steps was completed for three sets of loci and compiled into a single tree.

Table 2. Sample location, data type, size, suggested geographic form and source for allozyme data collected from the literature. Suggested forms are N = Northern form, I = Intermediate form and S = Southern form.

Number	Location Name	Latitude	Longitude	N	Form	Source
10	Endicott channel, AK	70.37	-148.37	6	N	C
	West of Mackenzie riverine anadromous			631	N	R
	West of Mackenzie isolated riverine			84	N	R
	West of Mackenzie residual char			27	N	R
11	Anatuvuk River, AK *	68.98	-152.33	40	N	E
12	Ivishak River / Echooka River, AK*	69.08	-153.91	74	N	E
13	Ribdon River, AK *	68.65	-144.14	40	N	E
14	Lupine River, AK *	68.98	-154.42	48	N	E
15	Kavik River, AK *	69.33	-152.42	40	N	E
16	Canning River, YT*	69.58	-152.3	247	N	E
17	Sadlerochit Spring, AK*	69.67	-150.41	62	N	E
18	Hulahula River 1, AK*	70.02	-152.01	112	N	E
	Hulahula River 2, AK	69.76	-152.08	96	N	E
29	Aichilik River, AK *	69.34	-155.2	110	N	E
20	Egaksrak River, AK*	69.43	-154.64	41	N	E
21	Kongakut River, AK *	69.77	-153.26	130	N	E
22	Firth River, YT *	68.67	-140.92	161	N	E
1	Babbage River 1, YT*	68.63	-139.37	53	N	E
	Babbage River 2, YT*	68.67	-139.22	21	N	E
2	Canoe River, YT *	68.61	-150.73	35	N	E
3	Nome River, AK	64.67	-165.33	54	I ?	R
23	Gold Creek, AK	64.14	-142.4	66	I ?	G
24	Auke Creek, AK *	58.38	-134.63	26	S	C, G
25	Fish Creek, AK *	58.32	-134.58	50	S	C
26	Osprey Lake, AK*	56.4	-134.67	49	S	C
27	Klutina River, AK	61.75	-145.75	49	S	R
28	Montana Creek, AK	62.08	-150.08	27	S	G
29	Eagle River, AK	58.54	-134.82	34	S	G
30	Karluk River, AK	57.51	-154.27	60	S	G

* indicates samples used in phylogenetic consensus tree. C = Crane et al. 1994, E = Everett et al. 1998, G = Gharret et al. 1991, R = Reist et al. 1997.

2.4 Statistical analysis

Morphometric variables were adjusted individually to account for differences in body size. The linearity of each variable in relation to the standard length was tested by constructing scatter diagrams using the Statistical Package for the Social Sciences (SPSS Inc. 2001). Linearity was confirmed for all variables, thus, the standard length was used as a base to construct ratios for each measurement (Reist 1991). No meristic variables were adjusted for fish size.

Subsequent data analysis also used SPSS (SPSS Inc. 2001). Meristic and morphometric data were examined for outliers by creating scatter plots. Outliers which were suspected to be documentation errors were removed. Meristic and morphometric data were assessed for normality of sample distribution by creating histograms and Q-Q plots for each sample variable. Values of kurtosis and skewness were assessed, with values less than two standard deviations of skew considered to represent a normal distribution (Sokal and Rohlf 1995). The majority of morphometric variables was normally distributed, therefore, data were not transformed. Although most meristic variables were non-normal, the discrete nature and small samples sizes ($n<100$) for these data sets were likely responsible for results observed in the Wilks-Shapiro test. Given the robustness of the analytical techniques it is assumed this non-normality did not substantially affect the results.

Meristic and morphometric variables were analyzed with univariate and multivariate techniques in SPSS. The data sets for the eight sample locations were examined using one-way analysis of variance (ANOVA) to test the null hypothesis that

the population means for each population sampled were equal (Table 3). The sample data were then rearranged into four groups (putative Northern, Southern, Intermediate and unknown) and retested with one-way ANOVA to examine whether the population means for the different hypothetical forms were equal (Table 4). Both analyses included one population of unknown affinities (putative interior form from Blackstone River). A Bonferroni t test was used to assess which population means were significantly different. The critical level of significance accepted for all tests was $p = 0.05$.

3.0 Results

3.1 Phylogenetic analysis

Phylogenetic analysis of the allozyme frequency data from populations 10 – 30, as listed in Table 2, produced three consensus bootstrapped trees. One tree, shown in Figure 5, was constructed from data for five allozyme loci. This tree places all southern samples used for the analysis (Auke Creek, Fish Creek and Osprey Lake) on a single branch supported by a 99% bootstrap value as distinct from all other (i.e., northern) samples.

3.2 Morphological data

3.2.1 Univariate analysis

Univariate ANOVA testing of the 23 morphometric and meristic variables in populations 1 – 9, as listed in Table 1, found highly significant differences in the means of all nine locations ($P < 0.0001$) (Table 4). ANOVA testing for the morphometric and meristic variables by form (Northern, Southern, Intermediate and unknown) was statistically significant for all meristic variables and ratioed morphometric variables ($P<0.05$) with the exception of pyloric caecae ($P=0.093$) (Appendix 1).

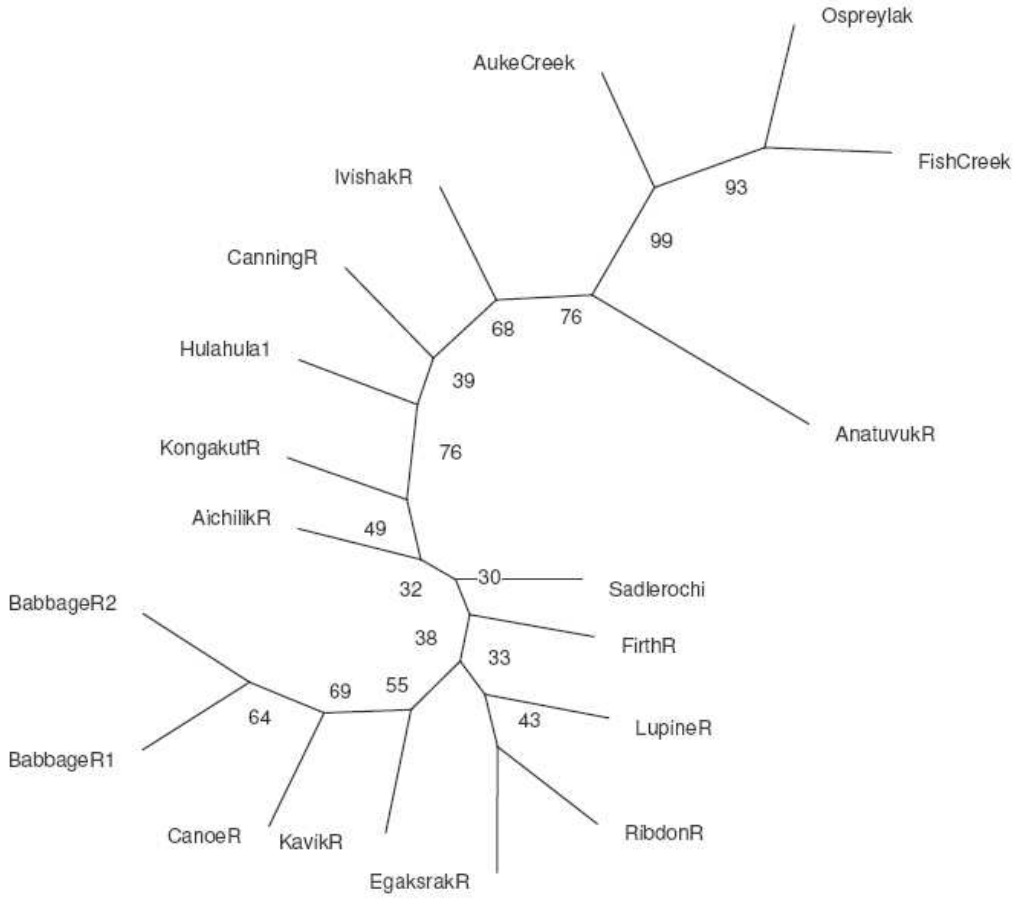

Figure 5. Bootstrapped consensus tree for loci IDDH, sAAT1,2, LDH-C, PGDH and SOD. Auke Creek, Fish Creek and Osprey Lake samples are located south of the Alaskan Peninsula, and therefore represent the Southern form. All other samples represent locations north of Seward Peninsula, thus representing the Northern form. See Table 2 for detailed sample locations. Numbers represent bootstrap values for each tree branch.

Table 3. Comparison of variable mean values for each location for samples selected from the Freshwater Institute database.

Site	1	2	3	4	6	7	8	9	
Variable				Area					P
	Babbage River	Canoe River	Nome River	Blackstone River	Ruth Arm	Cleo Creek	Bear Creek	Klutina River	
	N				I			S	
Meristic variables									
DRC	11.28	10.84	11.09	10.69	10.94	11.14	11.42	10.63	0.000
ARC	8.94	9.63	9.83	9.38	9.5	9.5	9.33	9.16	0.000
PRC	13.03	13.91	13.93	13.21	13.25	12.93	12.67	13.23	0.000
VRC	8.67	9.17	9.15	9.31	9.25	9	8.92	8.98	0.000
BRC	10.7	11.33	10.98	11.24	11.38	11.36	11.08	10.46	0.000
UGR	8.36	8.84	9.04	8.45	7.88	8.21	8.42	7.53	0.000
LGR	12.12	12.69	12.67	11.83	12.13	11.93	12.08	10.82	0.000
PYL	22.26	28.66	28.13	26.26	23.56	29.71	27.09	26.16	0.000
Ratioed morphometric variables (variable / STL (Standard length) x 100)									0.000
POL	47.78	59.56	51.47	51.36	42.5	56.15	42.09	44.92	0.000
OOL	47.2	30.16	29.22	42.67	27.1	27.86	26.51	41.43	0.000
PSL	130.66	124.59	123.93	135.81	120.11	124.02	115.42	126.39	0.000
TTL	242.38	230.1	230.91	223.88	249.05	239.56	246.7	233.79	0.000
DOL	130.15	121.94	122.41	126	137.96	138.45	139.99	124.23	0.000
LUL	138.54	167.31	170.57	149.21	166.11	158.87	162.36	146.45	0.000
ANL	96.29	86.76	88.73	94.09	99.83	101.84	107.56	80.13	0.000
CPL	143.04	169.69	173.92	157.48	178.55	170.27	166.24	155.05	0.000
HDD	118.67	104.77	93.26	113.83	107.21	123.57	113.66	100.81	0.000
BDD	215.25	204.41	172.3	207.92	215.06	215.91	203.7	232.34	0.000
CPD	89.94	79.98	77.43	83.53	89.83	86.69	89.42	87.9	0.000
IOW	76.87	75.85	73.87	69.23	91.2	94.07	88.71	60.01	0.000
MXL	86.36	95.2	88.71	95.83	92.35	87.82	91.2	88.34	0.000
MXW	21.71	16.11	12.74	17.95	15.97	16.72	15.75	16.7	0.000
PCL	167.02	152.81	144.42	175.42	145.16	154.52	148.92	171.4	0.000

DRC = dorsal fin rays, ARC = anal fin rays, PRC = pelvic fin rays, VRC = ventral fin rays, BRC = branchiostegal rays, UGR = upper gill rakers, LGR = lower gill rakers and PYL = pyloric caecae, POL = pre-orbital length, OOL = orbital length, PSL = post-orbital length, TTL = trunk length, DOL = dorsal length, LUL = lumbar length, ANL = anal length, CPL = caudal peduncle length, HDD = head depth, BDD = body depth, CPD = caudal peduncle depth, IOW = intraorbital width, MXL = maxillary length, MXW = maxillary width, and PCL = pectoral fin length .

Table 4. Comparison of variable mean values for each form for samples selected from the Freshwater Institute database.

Form	North	Intermediate	South	Unknown	P
Meristic variables					
DRC	10.98	11.11	10.63	10.69	0.000
ARC	9.41	9.67	9.16	9.38	0.000
PRC	13.63	13.51	13.23	13.21	0.001
VRC	9.01	9.11	8.98	9.31	0.004
BRC	11.13	11.11	10.46	11.24	0.000
UGR	8.69	8.65	7.53	8.45	0.000
LGR	12.5	12.4	10.82	11.83	0.000
PYL	26.69	27.47	26.16	26.26	0.093
Ratioed morphometric variables (variable / STL (Standard length) x 100)					0.000
POL	55.79	49.42	44.92	51.36	0.000
OOL	35.62	28.34	41.43	42.67	0.000
PSL	126.53	122.22	125.82	135.81	0.000
TTL	234.04	237.14	233.79	223.89	0.000
DOL	124.57	129.45	124.23	126	0.003
LUL	158.1	167.18	146.44	149.21	0.000
ANL	89.81	94.77	80.13	94.09	0.000
CPL	161.15	173.23	155.05	157.48	0.000
HDD	109.22	102.33	100.81	113.83	0.000
BDD	207.88	189.44	232.34	207.92	0.000
CPD	83.17	82.3	87.9	83.53	0.000
IOW	76.18	81.43	60.01	69.23	0.000
MXL	92.36	89.51	88.34	95.83	0.004
MXW	17.9	14.21	16.7	17.95	0.000
PCL	157.36	146.49	171.4	175.42	0.000
ADL	53.19	48.35	58.8	60.27	0.000
PVL	146.6	129.03	139.22	157.2	0.000

DRC = dorsal fin rays, ARC = anal fin rays, PRC = pelvic fin rays, VRC = ventral fin rays, BRC = branchiostegal rays, UGR = upper gill rakers, LGR = Lower gill rakers and PYL = pyloric caecae, POL = pre-orbital length, OOL = orbital length, PSL = post-orbital length, TTL = trunk length, DOL = dorsal length, LUL = lumbar length, ANL = anal length, CPL = caudal peduncle length, HDD = head depth, BDD = body depth, CPD = caudal peduncle depth, IOW = intraorbital width, MXL = maxillary length, MXW = maxillary width, and PCL = pectoral fin length. North includes Babbage River (1) and Canoe River (2), Intermediate includes Nome River (3), Ruth Arm (5), Cleo Creek (6) and Bear Creek (7), South includes Klutina River (8) and Unknown includes Blackstone River (5).

3.3.2 Multiple comparison procedures

For 23 morphological variables (8 meristic and 15 morphometric), the results of Bonferroni tests for pairwise comparisons between nine sample locations were as follows (Figure 6): The greatest number of significant differences (21 variables) were observed between Babbage River and Canoe River (6 meristic and 15 morphometric). These two samples are located in the same drainage basin, within the northern range, yet maintain different life histories. Canoe River fish are anadromous while Babbage River fish are an isolated riverine population, separated from the sea by impassable waterfalls.

Canoe River Dolly Varden were found to be significantly different from Klutina River fish for 19 variables (6 meristic and 13 morphometric), which reinforced the discontinuity observed between northern and southern populations in the phylogenetic analysis. Therefore, Canoe River was considered representative of the Northern form of Dolly Varden and Klutina River was considered representative of the Southern form.

Comparisons of the intermediately located sample, Nome River, with the northern and southern representative populations showed closer similarity to Canoe River (6 meristic and no morphometric variables) than to Klutina (20, 7 meristic and 13 morphometric variables). The other intermediate group, Becharof Lake (includes samples Ruth Arm, Cleo Creek and Bear Creek) also displayed a closer similarity to Canoe River (9, 7, and 8 total differences, respectively) than to Klutina River (16, 11, 15 total differences). Following from this result, the intermediate samples Nome River and Becharof Lake displayed a close morphological resemblance, with Becharof Lake samples from Ruth Arm, Cleo Creek and Bear Creek different from Nome River for

7,11,12 and 10 differences, respectively, with an average of 10. A small number of meristic differences were noted among these variables, specifically 1, 3, 2, and 1, respectively for these pairwise comparisons.

Within Becharof Lake, comparisons of Ruth Arm, Cleo Creek and Bear Creek displayed few differences, with a maximum difference of 3 variables (between Ruth Arm and Cleo Creek), and comparisons of Bear Creek and Ruth Arm exhibiting no significant morphological difference.

Blackstone River fish were significantly different from other sample locations for at least 11 morphological variables in all pairwise comparisons. The lowest number of overall differences was Blackstone River compared with Klutina River and Becharoff Lake samples (11 variables in total).

	Location	1	2	3	4	5	6	7
1	Babbage River							≤ 5
2	Canoe River	6 15						≤ 10
3	Nome River	5 13	0 6					≤ 15
4	Blackstone River	3 9	3 13	2 12				> 15
5	Ruth Arm, BL	1 9	3 6	3 8	0 11			
6	Cleo Creek, BL	1 6	1 6	2 10	1 10	1 2		
7	Bear Creek, BL	1 8	1 7	1 9	1 10	0 0	0 2	
8	Klutina River	5 10	6 13	7 13	3 8	2 14	3 8	3 12

Figure 6. Pairwise comparison of the number of significantly different meristic and morphometric variables. The numbers within cells indicate the number of significant meristic variables (out of eight) (left number), and morphometric variables (out of 17) (right number). The legend shows the total number of significant morphological variables ranked into colored categories. BL = Becharof Lake.

3.3.3. Multivariate analysis

Meristic and ratioed morphometric data sets passed the Bartlett's test of sphericity ($P<0.0001$) and the KMO (Kaiser-Meyer-Olkin measure of sampling adequacy) test with a value of 0.731 and 0.826, respectively. Principal component analysis (PCA) of meristic data extracted three principal components with eigenvalues greater than one. Analysis of the ratioed morphometric data set extracted five principle components with eigenvalues greater than one. The PCAs of both the meristic and ratioed morphometric data set revealed a clear distinction between the Northern form, represented by Canoe River and the Southern form, represented by Klutina River (Figure 7, 8, 9 and 10). Nome River was grouped with Canoe River in all plots, which reinforced the relationship between these samples observed in the multiple comparison procedure. PCAs of meristic data placed Becharof Lake intermediately between Klutina River and Canoe River (Figure 7 and 8), yet PCAs of ratioed morphometric data displayed alternative results. In Figure 9, Becharof Lake was clustered with Canoe and Nome River, yet Becharof Lake forms a distinct, nearly isolated group in Figure 10. These results reaffirmed the morphological similarity between Becharof Lake and northern samples Canoe River and Nome River; however, the inconsistency of this relationship suggests a more complex association among populations.

Blackstone River displays distinct clustering with Klutina River in Figure 10, yet showed no clear relationships with other samples in the other PCAs.

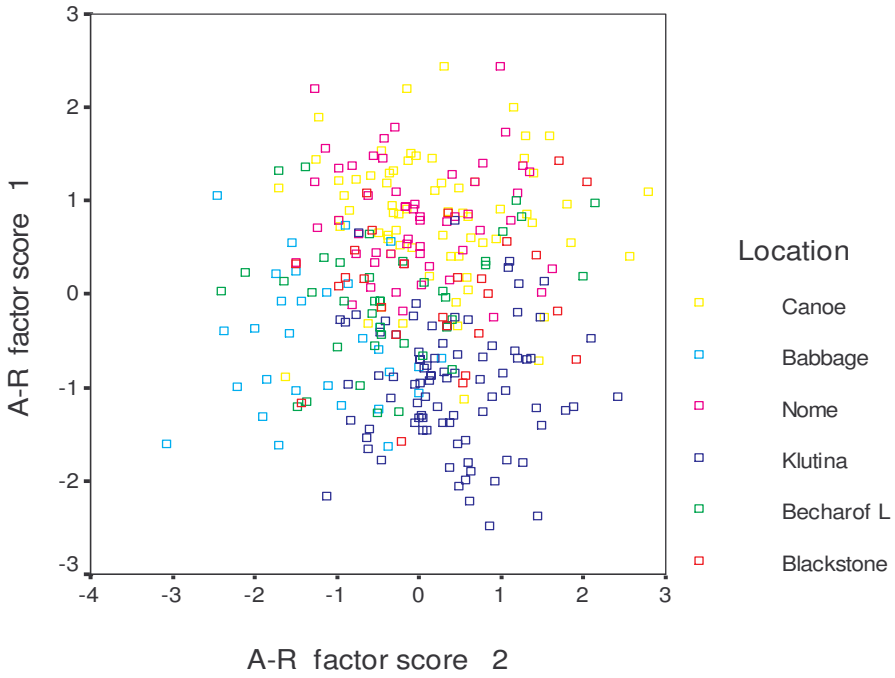

Figure 7. Scatter plot of principal component one (28.1% of variance) by principal component two (15.5% of variance) for meristic variables by sample location.

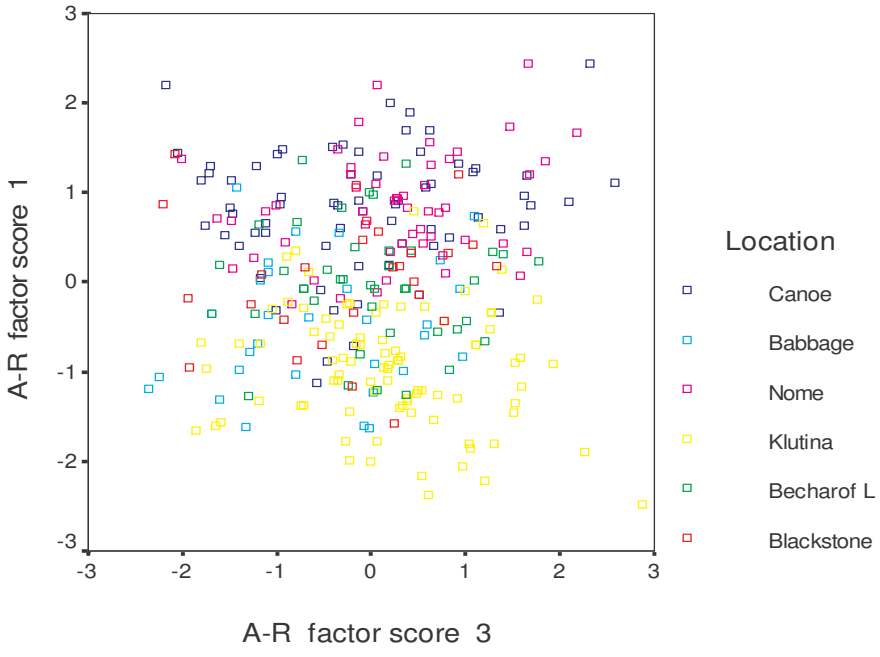

Figure 8. Scatter plot of principal component one (28.1% of variance) by principal component three (13.1% of variance) for meristic variables by sample location.

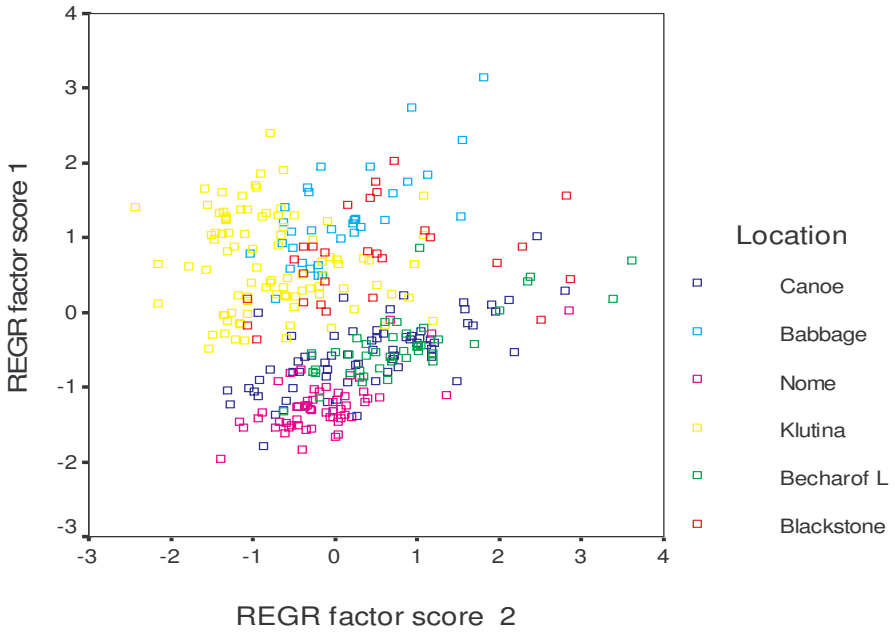

Figure 9. Scatter plot of principal component one (30.1% of variance) by principal component two (19.4% of variance) for ratioed morphometric variables by sample location.

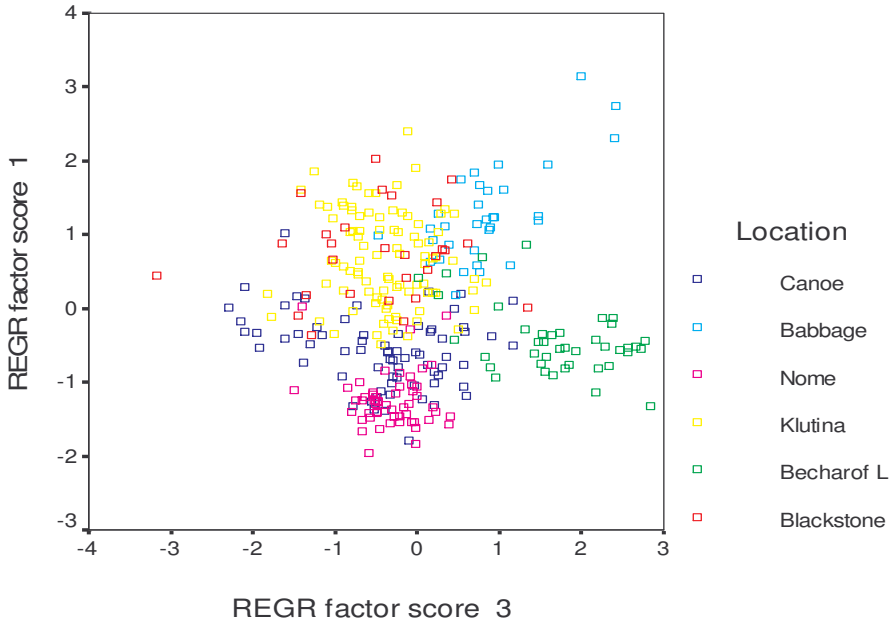

Figure 10. Scatter plot of principal component one (30.1% of variance) by principal component three (12.9% of variance) for ratioed morphometric variables by sample location.

4.0 Discussion

4.1 Phylogenetic Analysis of Genetic Data

The grouping of the three southern samples onto a single tree branch based on the allozyme frequency data is biologically meaningful, as these samples are in close geographic proximity. This is most notable when compared to the northern samples, all of which are north of Norton Sound. The high bootstrap value of this branch suggests a clear divergence between the extreme northern and southern samples. Further support for the genetic separation of the Northern and Southern forms of Dolly Varden was provided by Crane et al. (1994), who found fixed alternate alleles for the PEP-LT* locus between north slope *S. malma* and southern populations from Fish Creek, Auke Creek and Osprey Lake, Alaska.

The grouping of Fish Creek (Sample 25) and Osprey Lake (Sample 26) does not appear to be an accurate portrayal of genetic divergence. Osprey Lake is located on an island on the southwestern coast of Alaska and is unlikely to have an influx of alleles from other populations, whereas Fish Creek and Auke Creek are less than 10 km from each other, and would likely experience gene flow between populations (Crane et al. 1994). It was expected that Fish Creek and Auke Creek be grouped together in the analysis. The observed lack of expected association may be a result of the small number of alleles used for analysis.

The northern populations of *S. malma* did not display any groupings. As all northern samples were proposed to be of Northern form, the absence of distinct groups fits our expectation of the low genetic variation between populations. It is well

documented that northern populations in general show less genetic diversity than their southern counterparts within a species (reviewed in Martin and McKay 2004). This pattern has been observed in populations of Dolly Varden, with northern populations reported to display significantly less allozyme variation than southern populations (Salmenkova and Omelchenko 2000, Osinov 2001). An explanation for this is the biological advantage of a warmer climate to the south, whereas northern populations of Dolly Varden endure extreme overwintering conditions causing a reduction in survival (Omelchenko et.al. 2002). This differential survival reduces the genetic diversity of the northern fish. An alternative, or perhaps additional historical explanation could be that extensive glaciation in the northern reaches of their range may have reduced the effective population size of northern *S. malma* populations, resulting in the low genetic variation (Osinov 2001).

The phylogenetic tree was constructed using Nei's genetic distance (1978), which may not be the best model for analysis of phylogeny of *S. malma*. Nei's calculations assume the population fits the infinite alleles theory (Kimura 1968), which implies equal effective population sizes. Glacial events have played a selective role in the evolution of *S. malma*, therefore the species may not fit the assumption of equal effective population sizes (Crane et al. 1994). In this case, genetic drift may provide a better explanation for the allelic variation observed (Soule 1976 in Crane et al. 1994). A model that would account for this is Cavalli-Sforza and Edwards chord distance (Swofford and Olsen 1990). Preliminary trees using the Cavalli-Sforza and Edwards chord distance displayed similar results to the tree described in this study.

4.2 Morphological diversity

All samples of Dolly Varden were significantly different from one another morphologically, with the exception of two samples (Bear Creek and Ruth Arm) both taken from Becharof Lake. However, comparison of the remaining samples within Becharof Lake displayed at least two significant morphological variables. All Becharof Lake samples were collected from inlet rivers surrounding the lake. Becharof Lake Dolly Varden are known to be anadromous, which use the lake as a pathway on their migration to sea (Reist pers. Com.). It was expected that samples from a single lake system would show no differences, yet significant variation was observed between these samples (Figure 6). This difference observed between samples may be accounted for by the strong fidelity Dolly Varden show to natal spawning streams, thereby some degree of isolation is maintained between the populations despite using a common path to sea. However, considering that Bear Creek and Cleo Creek are less than 5 km apart, and the small samples sizes that were used, the difference between the samples may reflect natural variation within the greater Becharof Lake population.

The greatest difference observed was between the geographically proximate samples of Canoe River and Babbage River. As these populations are separated by impassable falls, yet are within the same drainage basin, the large degree of variation observed between these populations likely reflects the morphological specialization of different ecological life history types, rather than the evolutionary history of the populations. It is important to acknowledge the strong morphological differences between life histories of Dolly Varden, as this may confuse efforts to investigate historic evolutionary events. For this reason, the major comparisons of this study were made

between populations of anadromous fish, to reduce the confounding effect of different life histories.

Morphological differences between Canoe River and Klutina river, described by multiple comparisons procedures and principal component analysis, provided further support for the consideration of Northern and Southern forms as distinct taxa within Dolly Varden. The greater similarity observed between the intermediate samples, Nome River and Becharof Lake, with Canoe River, rather than with Klutina River suggests these three samples all represent a form distinct from Klutina River. The greater disparity between Becharof Lake and Klutina River despite their more geographically proximate location compared to Canoe River, points to a discontinuity in the pattern of morphological variation. This discontinuity may be the result of glacial periods of reproductive isolation.

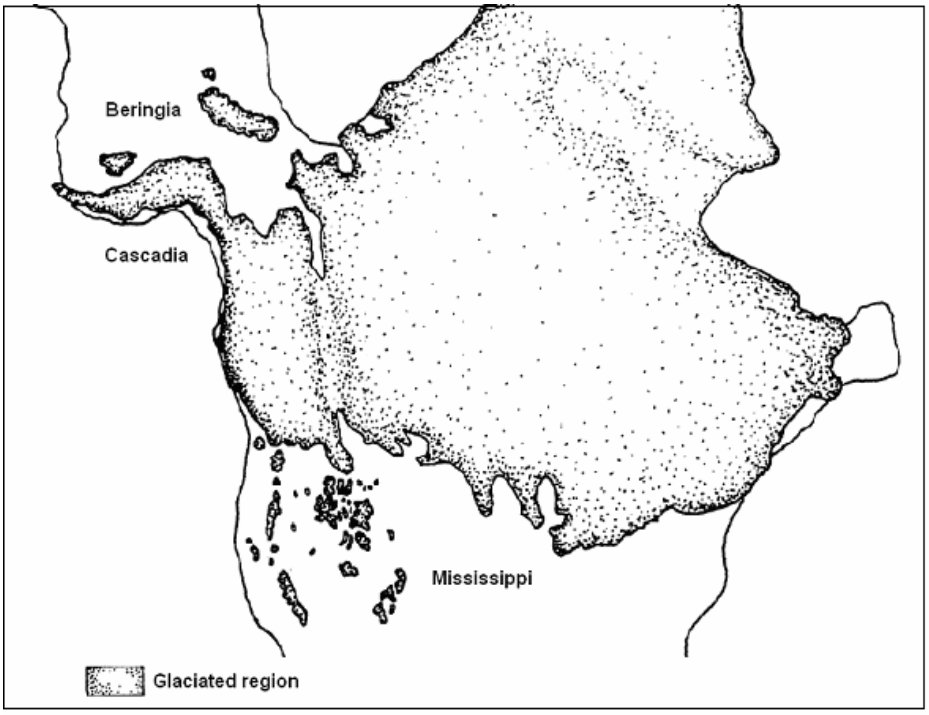

Figure 11. Location of ice-free refugia during Wisconsin. Based on Rogers et al. (1991).

4.3 Phylogeographic Analysis

The location of major glacial masses and ice-free freshwater habitat during glacial maximum correspond to the break observed in the pattern of morphological and genetic variation (Figure 11). During the Illinoian glacial period, the North American Cordilleran ice sheet extended from the Rocky Mountains to the Aleutian Islands, isolating Beringia from southern refugia (McPhail and Lindsey 1986). More recently, during the Wisconsin, glaciation was much reduced from the earlier Illinoian, yet appears to have remained a significant barrier to dispersal of fish between Beringia and refugia south of the Alaskan glaciation. A band of glaciation stretched from the Rocky Mountains westwards along the Alaskan Peninsula, with ice-free refugia remaining north of the glaciation around Bristol Bay and the northern side of the Alaskan Penisula (Lindsey and McPhail 1986) as well as several smaller ice-free regions south of the glacial band (Carrara et al. 2007).

Populations of Dolly Varden were likely present both north and south of the glaciation; north in the Beringian refugium, and to the south, either along the southern margin of the Cordilleran ice sheet or possibly within smaller refugia along the coast of southeastern Alaska and northern British Columbia. The isolation of these populations presumably led to the morphological and genetic differentiation observed between the Northern and Southern forms. Thus, it can be suggested with confidence that these forms represent distinct taxa within the species *S. malma*.

This study has not resolved the possibility of a third taxon within Dolly Varden, presumed to be created by glacial and drainage division's within the Berinigan refugium. Characteristic morphological and biological differences have been observed that suggest partial isolation between populations north and south of the Seward Penisula. DeCicco

and Reist (1999) observed a significant difference in size of adult anadromous spawners between Nome River and Pilgrim River fish. Nome River fish migrate southwards to Norton Sound while Pilgrim River fish, shown to be larger, migrate westward into Imuruk Basin. Life history differences are also noted both north and south of the Seward Peninsula, with stream-resident Dolly Varden common south of Norton Sound yet not seen in northern populations, except where geographic barriers prevent seaward migrations (DeCicco and Reist 1999). These characteristics indicate ecological specializations that may represent periodic allopatry during the Pleistocene, thus suggesting the possibility of further sub-divisions within the Northern form of Dolly Varden.

4.5 Future Recommendations

There was a noticeable lack of studies on populations of Dolly Varden from the western coastal regions of Alaska and within the Yukon and Kuskokwim drainage basins. The lack of data from these regions has resulted in a geographic gap in documented variation of Dolly Varden. The absence of data is likely due to the relative inaccessibility of these regions and the expense required to travel in these areas. The collection of data in these regions needs to become a priority in order to resolve the question of whether a third taxon may exist within the Northern form of Dolly Varden. Future studies addressing the phylogeography of Dolly Varden would receive much benefit from the addition of data from these western and interior populations. As well, future studies should attempt to gather both genetic and morphological data from the same populations in order to reduce the complications of phenotypic plasticity with evolutionary history. An alternative hypothesis for the differentiation of Northern and Southern forms also

requires further examination. On the basis of ribosomal ITS1 sequences, Phillips et al. (1999) suggest that Dolly Varden may be paraphyletic, with the Northern form of Dolly Varden diverging from Arctic charr (*Salvelinus alpinus*) prior to the Southern form of Dolly Varden. This hypothesis suggests more interesting and complex relationships may exist within *Salvelinus* than previously considered.

5.0 Conclusions

1. Genetic and morphological divergence exists between Northern and Southern form Dolly Varden.

2. The pattern of morphological variation suggests the division between the Northern and Southern forms lies between Becharof Lake and Klutina River in southern Alaska.

3. The location of this divide corresponds to the established understanding of glacial refugia during the Wisconsinan glacial period.

4. Based on these results, it is likely the Northern and Southern form represent natural intraspecific taxa created by isolation during the Wisconsinan.

5. Discontinuous patterns of biological variation of Seward Peninsula populations coupled with the glacial history of the north slope of Alaska suggest the possibility of a third taxa within Dolly Varden.

6. There is a need for more samples from the interior and intermediate coastal regions of Alaska, such as the Kuskokwim and Yukon River basins, in order to explore the possibility of a third taxon within Dolly Varden.

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Appendix 1. Enzyme loci and sources. Tissues used for extraction (M = muscle, L = liver, E = eye). Buffer systems as described in Crane et al. (1994). Enzyme names follow the International Union of Biochemistry, Nomenclature Committee (IUBNC 1984).

Enzyme	IUBNC No.	Locus	Tissue	Buffer	Source
Aspartate aminotransferase	2.6.1.1	sAAT-1,2*	M	2	C, E, G
Esterase	3.1.1.1	EST-3*	L	3	C
Fumarate hydratase	4.2.1.2	FH*	F	1	C
L-Iditol dehydrogenase	1.1.1.14	IDDH*	L	3	C, E, G
Lactate dehydrogenase	1.1.1.27	LDH-C*	E	3	C, E, G
Mannose-6-phosphate isomerase	5.3.1.8	MPI*	L	2	C
Tripeptide aminopeptidase	3.4._._	PEPB-1*	M	4	C
Proline dipeptidase	3.4.13.9	PEPD-1,2*	M, E	2	C
Peptidase	3.4._._	PEP-LT*	M, E	4	C
Phosphogluconate dehydrogenase	1.1.1.44	PGDH*	L	2	C
Superoxide dismutase	1.15.1.1	SOD*	L	4	C, E, G

C = Crane et al. 1994, E = Everett et al. 1998, G = Gharret et al. 1991.

Appendix 2. Allozyme frequency for polymorphic loci in populations of Dolly Varden. Samples numbers correspond to locations in Figure 3.

	Locus			sAAT-1,2		EST-3		FH		IDDH		LDH-C	
Sample Number	Location	Form	N	70	60	100	96	100	78	100	43	100	96
24	Auke Creek, AK	S	26	1.000	0.000	0.930	0.060	0.850	0.150	0.000	1.000	0.750	0.250
25	Fish Creek, AK	S	50	1.000	0.000	0.990	0.010	0.860	0.140	0.000	1.000	0.730	0.270
26	Osprey Lake, AK	S	49	1.000	0.000	0.970	0.030	0.930	0.070	0.000	1.000	0.440	0.560
11	Anatuvuk River, AK	N	40	0.918	0.082	n/a	n/a	0.000	1.000	0.875	0.125	1.000	0.000
12	Ivishak River / Echooka River, AK	N	74	0.966	0.034	n/a	n/a	0.000	1.000	0.913	0.087	0.973	0.027
13	Ribdon River, AK	N	40	0.950	0.050	n/a	n/a	0.000	1.000	1.000	0.000	0.923	0.077
14	Lupine River, AK	N	48	0.956	0.044	n/a	n/a	0.000	1.000	0.989	0.011	1.000	0.000
15	Kavik River, AK	N	40	1.000	0.000	n/a	n/a	0.000	1.000	1.000	0.000	0.988	0.012
16	Canning River, AK	N	247	0.908	0.092	n/a	n/a	0.000	1.000	0.912	0.088	0.995	0.005
17	Sadlerochit Spring	N	62	0.867	0.133	n/a	n/a	0.000	1.000	1.000	0.000	1.000	0.000
18	Hulahula River 1, AK	N	112	0.950	0.050	n/a	n/a	0.000	1.000	0.914	0.086	0.989	0.011
29	Aichilik River, AK	N	110	0.918	0.082	n/a	n/a	0.000	1.000	0.965	0.035	0.946	0.054
20	Egaksrak River, AK	N	41	0.951	0.049	n/a	n/a	0.000	1.000	0.986	0.014	0.929	0.071
21	Kongakut River, AK	N	130	0.912	0.088	n/a	n/a	0.000	1.000	0.959	0.041	0.965	0.035
22	Firth River, YT	N	161	0.95	0.050	n/a	n/a	0.000	1.000	0.985	0.015	0.973	0.027
1	Babbage River 1, YT	N	53	1.000	0.000	n/a	n/a	0.000	1.000	1.000	0.000	1.000	0.000
2	Canoe River, YT	N	35	1.000	0.000	n/a	n/a	0.000	1.000	1.000	0.000	1.000	0.000

Appendix 2. (continued)

Sample Number	Location	Form	N	Locus: MPI 100	MPI 91	MPI 67	PEP-B1 100	PEP-B1 70	PEPD-1 77	PEPD-1 84	PEPD-2 77	PEPD-2 122	PEPD-2 139	PEPD-2 119
24	Auke Creek, AK	S	26	0.042	0.938	0.021	0.867	0.133	0.940	0.060	0.020	0.880	0.060	0.040
25	Fish Creek, AK	S	50	0.010	0.906	0.083	0.806	0.194	0.920	0.080	0.000	0.820	0.180	0.000
26	Osprey Lake, AK	S	49	0.000	0.875	0.125	1.000	0.000	0.690	0.310	0.000	0.990	0.000	0.010
11	Anatuvuk River, AK	N	40	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	Ivishak River / Echooka River, AK	N	74	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
13	Ribdon River, AK	N	40	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
14	Lupine River, AK	N	48	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
15	Kavik River, AK	N	40	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
16	Canning River, AK	N	247	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
17	Sadlerochit Spring	N	62	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
18	Hulahula River 1, AK	N	112	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
29	Aichilik River, AK	N	110	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
20	Egaksrak River, AK	N	41	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
21	Kongakut River, AK	N	130	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
22	Firth River, YT	N	161	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1	Babbage River 1, YT	N	53	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Canoe River, YT	N	35	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Appendix 2. (continued)

Sample Number	Location	Form	N	PEP-LT 126	PEP-LT 56	PGDH 110	PGDH 95	SOD 178	SOD 152	SOD 200
	Locus									
24	Auke Creek, AK	S	26	1.000	0.000	1.000	0.000	0.815	0.185	0.000
25	Fish Creek, AK	S	50	1.000	0.000	1.000	0.000	0.730	0.270	0.000
26	Osprey Lake, AK	S	49	1.000	0.000	1.000	0.000	0.550	0.450	0.000
11	Anatuvuk River, AK	N	40	n/a	n/a	1.000	0.000	1.000	0.000	0.000
12	Ivishak River / Echooka River, AK	N	74	n/a	n/a	0.993	0.007	0.972	0.000	0.028
13	Ribdon River, AK	N	40	n/a	n/a	0.988	0.012	0.888	0.000	0.112
14	Lupine River, AK	N	48	n/a	n/a	0.989	0.011	0.911	0.000	0.089
15	Kavik River, AK	N	40	n/a	n/a	1.000	0.000	1.000	0.000	0.000
16	Canning River, AK	N	247	n/a	n/a	1.000	0.000	0.974	0.000	0.026
17	Sadlerochit Spring	N	62	n/a	n/a	1.000	0.000	1.000	0.000	0.000
18	Hulahula River 1, AK	N	112	n/a	n/a	1.000	0.000	0.914	0.000	0.086
29	Aichilik River, AK	N	110	n/a	n/a	1.000	0.000	0.947	0.000	0.053
20	Egaksrak River, AK	N	41	n/a	n/a	1.000	0.000	0.943	0.000	0.057
21	Kongakut River, AK	N	130	n/a	n/a	1.000	0.000	0.976	0.000	0.024
22	Firth River, YT	N	161	n/a	n/a	1.000	0.000	0.996	0.000	0.004
1	Babbage River 1, YT	N	53	n/a	n/a	1.000	0.000	0.944	0.056	0.000
2	Canoe River, YT	N	35	n/a	n/a	1.000	0.000	0.957	0.028	0.015