MOLECULAR AND OTOLITH TOOLS INVESTIGATE POPULATION OF ORIGIN AND MIGRATION OF ARCTIC CISCO FOUND IN THE COLVILLE RIVER, ALASKA

PROGRESS REPORT

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Abstract

The U. S. Minerals Management Service (MMS) defined specific questions concerning Arctic cisco in the Colville River, Alaska, and requested that the U.S. Geological Survey implement a study developing and applying scientific tools and techniques to address those questions (see below Problem Statement and Justification**)**. We used genetics and otolith microchemisty to assess population structure and movements of Arctic cisco collected from the Colville River subsistence fishery. Nine previously published coregonid microsatellite loci, two newly optimized microsatellite loci, and one segment of the mtDNA locus were included in our genetic analyses. All microsatellite loci conformed to Hardy-Weinberg equilibrium and there was no evidence of linkage disequilibrium between loci. No genetic differentiation was found in pairwise F_{st} analyses between collections made in the Colville River subsistence fishery and either of the two populations sampled on the Mackenzie River (Arctic Red and Peel rivers). There were no significant genetic differences found among age-classes (ages 5 through 8) of Arctic cisco in the Colville River in 2006. Additional support for high levels of gene flow among Arctic cisco collected in the Colville and Mackenzie rivers was found using STRUCTURE simulations which supported one genetic cluster across all locations (K = 1). Twenty unique mtDNA haplotypes were sequenced from a 594 nucleotide segment of the ATPase 6 gene. Seventeen haplotypes aligned closely with other Arctic cisco. There were four fish collected in the Colville River with highly divergent mtDNA compared to other Arctic cisco samples. Three of these samples (1 in 2005; 2 in 2006) aligned exactly with Yukon River Bering cisco haplotype LAU001 suggesting species miss-identification in the field or introgression between these species based on mtDNA signatures. The species affiliation for the other highly divergent haplotype collected from the Colville River in 2006 has not been determined.

We measured otolith growth of 722 cisco captured in subsistence fisheries in the Colville River between 1986 and 2006 representing 24 year classes (1978 – 2002). There was a significant positive relation between otolith radius and fork length ($r^2 = 0.85$, $P < 0.0001$). Significant inter-annual variation in otolith growth was evident in the first 7 annual growth increments (one-way ANOVA for each annulus, $P < 0.05$). Because the greatest growth rates occur in the first year, we analyzed first year otolith growth rates in relation

to environmental variables using linear regression with each point weighted by sample size. First year growth was positively correlated with the winter Arctic Oscillation index (Nov-Mar, $r^2 = 0.14$, $P < 0.05$), mean summer air temperature at Inuvik in the Mackenzie River Delta (May-Aug, $r^2 = 0.17$, $P < 0.05$), mean speed of east wind at Barrow, Alaska (July-Aug, $r^2 = 0.18$, $P < 0.05$), and mean Mackenzie River discharge at Ft. Simpson lagged 2 years (April-June, $r^2 = 0.32$, $P < 0.01$). These results suggest that changes in the physical environment may influence cisco growth rates directly and indirectly as biologically mediated shifts in lower trophic level productivity.

Results from this study provided information on multiple scales about the origins and movements of Arctic cisco in the Colville River using both genetic and otolith tools. The application of both techniques demonstrated important findings addressing objectives put forth by MMS on Colville River Arctic cisco. This report represents the second technical report submitted to MMS addressing these objectives.

Key words: Arctic cisco, genetic population structure, otolith growth, microsatellites, migration, age.

Problem Statement and Justification

Arctic cisco or Qaaktaq (*Coregonus autumnalis*) are an important subsistence resource for the village of Nuiqsut, Alaska. Subsistence users have expressed concern over declines in harvests and size of Arctic cisco from the Colville River (Figure 1). Issues concerning population of origin, life history and recruitment variation over time, and changes in migration and diet of Arctic cisco ranked high in a list of critical research elements developed by the local community in a workshop hosted by MMS in Nuiqsut, November 18-20, 2003 (MBC 2004). Six questions specific to Arctic cisco in the Colville River were identified by MMS:

1) Can we assign Arctic cisco from the Colville River back to a population of origin with high confidence?
2) Can we define genetic relationships among Colville and Mackenzie rivers' Arctic cisco populations?
3) Are there year-to-year genetic variations among Arctic cisco recruiting to the Colville River?
4) Has growth in Arctic cisco decreased over recent time?
5) Have migration routes to and from coastal habitats changed in Arctic cisco?
6) Can growth inferred from otoliths be used as an indicator of changes in diet over time?

This pilot project developed tools and tested the efficiency and rigor of two scientific approaches, molecular genetics and otolith microchemistry, to address these questions. Seven objectives were listed in the original pilot study plan for genetics; these are listed below with an overview of results based on data derived from collections of Arctic cisco from the Colville River (2005 and 2006), and the Arctic Red and Peel rivers, Canada (2007).

1) Search the available literature for molecular markers developed for other *Coregonus* species that may be useful for this study. **RESULT**: We tested a total

of 27 microsatellite loci primer sets from the published literature and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) for *Coregonus* species. Seven of these were selected for use in this pilot study based on ease of amplification in Arctic cisco, preliminary statistical analyses of polymorphism, and consistent allelic product amplification.

2) Test genetic diversity of molecular loci developed from other species in DNA amplifications from Colville River Arctic cisco. **RESULT**: For this report we developed locus specific descriptive statistics using seven previously available loci and four loci developed from Arctic cisco for this study. Allelic sizes for previously published microsatellite loci that were amplified from Arctic cisco were within expectations for the loci reported for other species.
3) Develop new microsatellite markers directly from Arctic cisco DNA. **RESULT:** Five microsatellite loci were developed and optimized for Arctic cisco in last year's pilot study (Nielsen et al. 2007). One of those loci (*Aut062*) was dropped from subsequent analyses since allelic structure in the 2005 Colville River sample did not conform to Hardy-Weinberg equilibrium. *Aut066* was also dropped due to ambiguous amplification products, and *Aut135* was dropped because of low allelic diversity. Two new microsatellite loci developed from Arctic cisco (*Aut134* and *Aut189*) were optimized in 2007 and added to our list of loci. Four microsatellite loci developed specifically for Arctic cisco were included in the panel of loci used to assess genetic structure for this report.
4) Examine the rigor and applicability of microsatellite loci developed from Colville Arctic cisco in comparison with molecular markers from the current literature. **RESULT**: We used WHICHRUN (Banks et al. 2003) and allele frequency differential methods of WHICHLOCI (Shribver et al. 1997) to determine the relative contribution of each of our loci to the observed diversity – those drawn from the literature and those developed in our laboratory for this study. Both sets of loci contributed significant information on allelic diversity found in our extended sample collection. Four loci developed in our laboratory and seven loci taken from the published literature of GENEBANK met strict criteria for the number of alleles, allelic richness, and heterozygosity for population genetic

structure analyses. All loci conformed to Hardy Weinburg Expectations (HWE) and we found no evidence of linkage disequilibrium among the loci.

5) Determine the usefulness and rigor of selected nuclear microsatellite and mitochondrial DNA loci to determine population structure in Colville River Arctic cisco. **RESULT**: Microsatellite and mtDNA data provided significant rigor to examine genetic population structure over time in Colville River Arctic cisco. However, the question of population of origin remains ambiguous. Arctic cisco captured in the Colville River were not significantly different from Mackenzie River Arctic cisco collected from the Arctic Red and Peel rivers. Two Mackenzie River populations also lacked distinct genetic structure suggesting a high level of gene flow between these two putative spawning stream populations. Genetic analyses of additional sampling locations from the Mackenzie River in 2008 will help confirm or refute panmixia for Arctic cisco stocks found in the Mackenzie River.
6) Test genetic differentiation among samples based on molecular genotypes of samples of Arctic cisco from the 2005 harvest (samples made available by LGL under contract to ConocoPhillips). **RESULT**: Pairwise F_{st} analyses did not support significant genetic differentiation among sample locations, sample years within the Colville River or among age classes in the 2006 Colville River sample. We used the Bayesian program STRUCTURE to assess the number of genetic clusters present within the total Arctic cisco sample collection analyzed to date (Colville River 2005-2006; Peel 2007; Arctic Red 2007). When all samples were evaluated results support one genetic cluster for Arctic cisco (K = 1).
7) Establish the best suite of molecular markers to address the putative stream of origin for Arctic cisco in the Colville River. **RESULT**: Both microsatellites and mtDNA sequence analyses proved efficient and rigorous in depicting a high degree of genetic population structure in the Arctic cisco analyzed in our laboratory. Allelic and haplotype frequencies were not significantly different in pairwise comparisons between sample collections, sample years or age classes of Arctic cisco. However, four fish with highly divergent haplotypes were found in Arctic cisco collected from the Colville River. Three of these fish carried

haplotypes that aligned with a Yukon River Bering cisco haplotype, species identification for the fourth fish's haplotype remains unknown. These results may represent misidentification of fish in the field or the presence of other coregonid mtDNA haplotypes in Arctic cisco from a previous hybridization event(s).

Three objectives listed in the original pilot study plan for otolith microchemistry are listed below with an overview of preliminary results developed from the pilot study.

8) Develop a protocol to compare length at age of Arctic cisco captured in the Colville River so that age and growth histories can be compared. **RESULT**: Protocols for the identification of annuli and measurement of otolith growth were developed and implemented to reconstruct growth chronologies of Arctic cisco captured in the Colville River. Otoliths from an archive of samples collected from 1986 to the present were aged and measured. There is a significant relation between otolith growth and fish length indicating that otolith growth can be used as a surrogate for fish length at age. Age-specific growth varied among years. To date, we have focused on growth during the first year and find a pattern that mimics the Arctic Oscillation Index. Analysis is ongoing to determine the relation of local-scale environmental variables and otolith growth. Changes in growth characteristics (von Bertalanffy growth coefficients) suggest that growth patterns have changed over the record analyzed.

9) Using otolith strontium to calcium ratios (Sr:Ca), determine if the chronology of migrations between freshwater, brackish water, and saltwater environments can be documented in Arctic cisco. **RESULT:** As previously reported, otolith Sr:Ca patterns confirmed the presumed pattern of migration between freshwater and marine waters. A sub-sample of otoliths will be analyzed over the next year to increase the sample size and temporal coverage.

10) Describe the variability of multi-elemental signatures of otoliths to determine the feasibility of these methods to identify tributary of origin. **RESULT:** Preliminary results of multi-elemental analyses were included in the previously reported pilot study. Now that all otoliths are prepared, multi-elemental analyses will be conducted in 2008 – 2009.

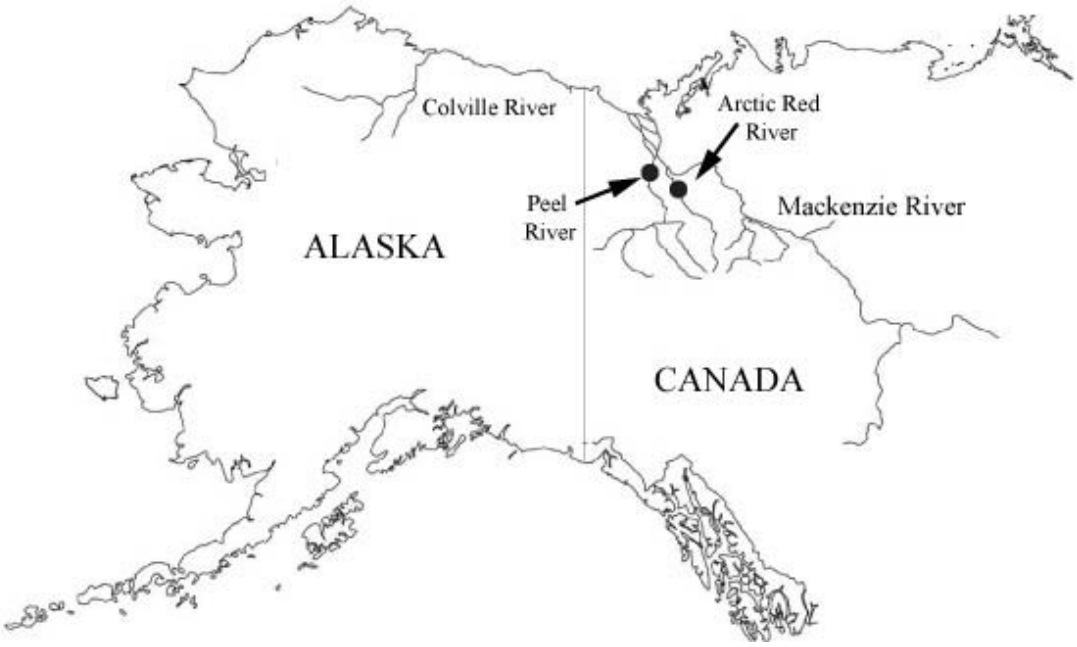

Figure 1. Map showing collection locations for Arctic cisco from the Colville and Mackenzie rivers 2005-2007.

Genetics

Materials and Methods

Sample Collections

Arctic cisco sampling collections included the Colville River, Alaska 2005 and 2006 (N = 86 and 176, respectively), the Arctic Red River, Canada 2007 (N = 60), and the Peel River, Canada 2007 (N = 54; Figure 1). Both Colville River collections were a sub-sample of the Nuiqsut subsistence fishery collected by LGL Limited and were sent to the U.S. Geological Survey (USGS) Alaska Science Center for genetic analyses. Colville River samples collected in November 2005 consisted of fish heads (N = 82) and four whole Arctic cisco. The Colville River 2006 collection consisted of 176 Arctic cisco collected from October 23 - November 10, 2006, and were received by USGS individually bagged, labeled, and frozen. Ages for the 2006 Colville River Arctic cisco samples were estimated by LGL Limited using otoliths. The Arctic Red River 2007 and Peel River 2007 collections were made by USGS from tributaries of the Mackenzie River, Northwest Territories, Canada (August 8 – 16, 2007). Arctic cisco were caught using 7.62 cm stretched mesh monofilament gill nets. Whole heads were collected, individually bagged, labeled, and placed on ice in the field. Samples were later frozen and transferred to the USGS laboratory. Bering cisco (*C. laurettae*) samples were collected in 2003 from the Yukon River by U.S. Fish and Wildlife Service as fin clips preserved in ethanol (N = 5).

DNA Extraction and Microsatellite Amplification

Genomic DNA was extracted from fin, gill, or muscle tissue from the Colville River 2005 and 2006 samples using the DNeasy Tissue kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Genomic DNA was extracted from gill tissue from Arctic Red and Peel samples using the Purgene DNA extraction kit (Gentra Systems Inc., Minneapolis, MN) following the manufacturer's protocol. Genomic DNA was extracted from Yukon River Bering cisco fin tissues using the Purgene DNA extraction kit protocol.

Two previously published microsatellite loci developed by USGS specifically for Arctic cisco (Ramey et al. 2008), and two newly optimized Arctic cisco microsatellite loci were included in our panel of loci (Table 1). Seven additional microsatellite loci developed by other researchers from different *Coregonus* species were also amplified for population genetic analyses (Table 1). Five additional microsatellite loci were amplified, but were excluded after preliminary statistical analyses because of low allelic polymorphism (*Aut135*, *Bom21c*, *Cisco-90*, *Cocl-Lav6* and *Cocl-Lav8*).

Microsatellite loci developed for this study were amplified using the polymerase chain reaction (PCR) as described in Ramey et al. (2008) with the exception of *Aut189* which was amplified at an annealing temperature of 52°C. Microsatellite loci developed from other *Coregonus* species were amplified using PCR in 10 μL volumes containing 4 – 50 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, 3.7 – 4.0 μM each unlabeled primers, 0.1 – 0.3 μM labeled tail primer, 2 mM each dNTP and 0.3 – 0.5 U of Taq DNA polymerase. PCR reactions were carried out in MJ Research (BIORAD) or MWG thermocyclers (MWG Biotech Inc.) with an initial denaturation time of 2 min at 94°C followed by 30 cycles of 94°C for 30 s, 51 - 57°C for 30 s, 72°C for 1 min and a final 10 min elongation step at 72°C.

PCR products were separated on 6% polyacrylamide gels using a LI-COR Model IR2 automated fluorescent DNA sequencer (Lincoln, NE). Forward primers were either directly fluorescently labeled to visualize alleles (*Bom3c*, *Bom17c*, *Bom22b*, *LGL BWF2*, *Cisco-151*, *Cisco-181*, and *Cocl-Lav4*) or were synthesized with universal tails on the 5' ends and visualized by adding fluorescently labeled complementary tailed sequences to the PCR reaction (*Aut134*, *Aut139*, *Aut151*, and *Aut189*). Allele sizes for specific samples at each locus were determined relative to the M13 phage single nucleotide ladder and used as internal size standards in subsequent gels. GeneImagIR v4.05 (Lincoln, NE) software was used to assign allele scores. A minimum of 10% of all samples were independently amplified and scored to verify allele sizes across all loci for quality control.

Table 1. Characteristics of 11 microsatellite loci used to assess genetic structure of Arctic cisco in this study.

Locus	Source	Cloned repeat motif	Primer sequence (5' → 3')	Developed in	Accession no.
Aut134	this study	$(CA)_{35}$	F: TCATGAAATCACACACCC R: CTCACGCATCACACACC	*Coregonus autumnalis autumnalis*	EU603399
Aut139	Ramey et al. 2008	$(CA)_{41}$	F: GGGTAAAGCAGAATGAC R: GGGAGTGTGTTTGTCT	*C. autumnalis autumnalis*	EF192595
Aut151	Ramey et al. 2008	$(CA)_{27\ \text{imperfect}}$	F: GACCAGTTGTTTATGAAGC R: GTAGCATAGGGGGTTTTA	*C. autumnalis autumnalis*	EF192596
Aut189	this study	$(CA)_{17}$	F: TTATACTGCATGGTGC R: GCCTGGCTCAATTTGT	*C. autumnalis autumnalis*	EU603400
Bom3c	unpublished	$(CA)_6$ TA $(CA)_{40}$	F: GTGGACTGCTGCTTTGTTG R: GCTGTATCTTTAACCTCCC	*C. autumnalis migratorius*	AY507115
Bom17c	unpublished	$(GT)_{21}$	F: GGCAACAGACAGAAATAAG R: TATGTCACAAGGGGCAGTG	*C. autumnalis migratorius*	AY507114
Bom22b	unpublished	$(GA)_{15}$	F: GGATGCCTTCTTGATGCTT R: CATTTATCAGCCTCTCAGCC	*C. autumnalis migratorius*	AY507121
Cisco-157	Turgeon et al. 1999	$(GT)_{17}$	F: CTTAGATGATGGCTTGGCTCC R: GGTGCAATCACTCTTACAACACC	*Coregonus* spp.	N/A
Cisco-181	Turgeon et al. 1999	$(GATA)_{36}$	F: GGTCTGAATACTTTCCAAATGCAC R: CCATCCCTTTGCTCTGCC	*Coregonus* spp.	N/A
Cocl-Lav4	Rogers et al. 2004	$(CA)_{13}$	F: TGGTGTAATGGCTTTTCCTG R: GGGAGCAACATTGGACTCTC	*C. clupeaformis*	AY453197
LGL BWF2	Patton et al. 1997	$(GA)_{16}$ $(N)_{95}$ $(TG)_{13}$	F: CGGATACATCGGCAACCTCTG R: AGACAGTCCCCAATGAGAAAA	*C. nasus*	N/A

Mitochondrial DNA Sequencing

A 594 base pair (bp) fragment of the mitochondrial ATP synthase VI subunit gene (ATPase 6) reported to be variable between Arctic cisco collected from the Colville and Peel rivers (Turgeon and Bernatchez 2003) was sequenced for 376 samples collected for this study and five Bering cisco samples. Previously published primers (H9208 and L8558) were used in this sequence analyses (Giuffra et al. 1994). Primers were modified on the 5' ends with universal M13 tails, and sequences were visualized by adding fluorescently labeled complementary tailed sequences to the sequencing reaction. Mitochondrial DNA (mtDNA) was amplified using a MJ Research thermocycler using PCR conditions adapted from Giuffra et al. (1994) as follows: initial denaturation at 94ºC for 1 min followed by 35 cycles of 94ºC for 1 min, 62ºC for 1 min, and 72ºC for 1 min ending with a final elongation step at 72ºC for 10 min. The sequencing reaction was conducted using a RoboCycler ® (Stratagene, La Jolla, CA) with the following conditions: initial denaturation at 92ºC for 2 min followed by 30 cycles of 92ºC for 30 s, 60ºC for 30 s and 70ºC for 1 min. ATPase 6 sequence data were proofed and aligned using AlignIR software (LI-COR, Lincoln, NE). Nucleotide (nt) positions for sequences amplified from *C. autumnalis* were confirmed by alignment with the published sequence for the complete mtDNA genome of *C. lavaretus* (Miya and Nishida 2000; GenBank accession no. AB034824). The frequency of mtDNA haplotypes, haplotype diversity, and nucleotide diversity were calculated using ARLEQUIN v3.1 (Excoffier et al. 2005) for each collection and for all collections combined. ARLEQUIN was also used to generate of minimum spanning network of all haplotypes, and to calculate pairwise F_{ST} values based on haplotype frequencies between collections.

Population Genetic Statistics

The allelic size ranges for each locus, total number of alleles (N_A), allelic richness (A_R), and observed and expected heterozygosity by locus (H_o and H_e, respectively) were generated using FSTAT v 2.9.3 (Goudet et al. 2001). Pairwise linkage disequilibrium between all pairs of loci was tested using FSTAT, and a Bonferroni correction based on the number of comparisons was applied to evaluate significance ($p \leq 0.0009$). Probability tests for Hardy-Weinberg equilibrium (HWE) were done per locus using

GENEPOP v3.4 (Raymond and Rousset 1997), and significance was assessed after a Bonferroni correction for the number of loci ($p \leq 0.0045$). Weir and Cockerham's (1984) locus-specific measure of departure from HWE (F_{IS}) was generated to assess heterozygosity excess (negative F_{IS} values) or deficit (positive F_{IS} values) using FSTAT. A global F_{IS} value over all loci and collections was generated using FSTAT. Pairwise F_{ST} comparisons between collections and age classes were calculated using ARLEQUIN, and a Bonferroni correction based on the number of comparisons was used to evaluate significance ($p \leq 0.0083$). Age classes determined by LGL Limited for the Colville River 2006 collection included ages five (N = 5), six (N = 32), seven (N = 97), eight (N = 25), and nine (N = 1). The age nine fish was not included in our pairwise F_{ST} analysis.

The program STRUCTURE was explored as an alternative approach to assess population structure using a Bayesian clustering method (Pritchard et al. 2000; Pritchard and Wen 2004). Correlated allele frequencies (Falush et al. 2003) were simulated using the admixture model to infer the number of genetic clusters (K) present among the 376 samples in the database. STRUCTURE simultaneously delineates clusters of individuals based on their multilocus genotype and assigns individuals to identified clusters using the Monte Carlo Markov chain (MCMC) approach (Manel et al. 2005). STRUCTURE assumes HWE and linkage equilibrium among loci and infers the number of clusters (populations) by comparing the posterior probability for different numbers of putative clusters. The number of clusters (K) for Arctic cisco was assessed for values of K = 1 – 5, using 100,000 burn-in steps and 250,000 MCMC repetitions. Simulations for each K were conducted five times each. The probability that the data (X) fits the number of inferred clusters [Ln Pr (X | K)] for each of the five runs was averaged, and the posterior probability for each possible value of K was calculated. The number of clusters (K) was inferred when the posterior probability was equal to one.

Results

Microsatellites

Two Arctic cisco microsatellite loci previously reported in the pilot study report were dropped from these analyses due to lack of conformity to HWE in the Colville River 2005 collection (*Aut062*), and ambiguous amplification products (*Aut066*; Nielsen

et al. 2007). The number of alleles (N_A) ranged from 13 (*LGL BWF2*) to 41 (*Aut139*; Table 2), and average N_A = 25.5. Allele richness (A_R) was considerably lower than N_A for each locus, and average A_R = 15.1. Average observed and expected heterozygosity across all loci were virtually the same (average H_O = 0.775 and average H_E = 0.774). All microsatellite loci met HWE when collections were analyzed independently or combined as a single group. Significant heterozygosity excess or deficit was not detected for any locus, and global F_{IS} = 0.000 (p = 0.491) for all loci and collections combined. No linkage disequilibrium was detected between loci. STRUCTURE's analysis of population structure inferred one genetic cluster (K = 1) among all of our sample collections. No statistically significant pairwise F_{ST} differences were detected between sample collections ($p \geq 0.21$; Table 3) or age classes ($p \geq 0.09$; Table 4).

Mitochondrial DNA

Twenty haplotypes and 29 variable nucleotide (nt) positions were amplified from a 594 nt fragment of ATPase 6 sequenced from 381 samples (Table 5). Complete sequence information available for the common whitefish (*C. lavaretus*) was used to standardize the nucleotide numbering scheme presented in Table 5 (Miya and Nishida 2000; GenBank accession no. AB034824). Haplotype diversity among the 17 Arctic cisco haplotypes (AMN) was 0.414 ± 0.030 and nucleotide diversity averaged across all positions equaled 0.0011 ± 0.0009. No statistically significant differences were detected between haplotype frequencies for sample collections using pairwise F_{ST} comparisons.

There were three samples collected from the Colville River subsistence fishery (N = 1 in 2005 and N = 2 in 2006) that aligned exactly with Bering cisco haplotype LAU001. Bering cisco haplotype LAU001 occurred in four Yukon River samples. One Yukon River sample generated haplotype LAU002. The LAU002 Bering Cisco haplotype was not found among the 376 samples collected for this report. The unknown haplotype collected in 2006 from the Colville River was divergent from ATPase 6 sequences from the common whitefish (*C. lavaretus*), Arctic cisco (*C. autumnalis*) and Bering cisco (*C. laurettae*; Figure 2).

Table 2. Locus-specific descriptive statistics resulting from the analysis of 376 Arctic cisco collected from the Colville and Mackenzie rivers, 2005-2007.

Locus	N	N_A	A_R	H_o	H_e	F_{IS}	allelic size range
Aut134	374	28	18.04	0.758	0.826	+0.082	144-198
Aut139	376	41	20.87	0.887	0.878	-0.011	131-221
Aut151	376	28	17.79	0.894	0.864	-0.035	88-168
Aut189	369	33	16.16	0.777	0.778	+0.002	99-173
Bom3c	376	34	21.67	0.898	0.903	+0.006	273-345
Bom17c	376	20	11.96	0.814	0.803	-0.013	181-231
Bom22b	376	15	6.96	0.591	0.573	-0.033	104-146
Cisco-157	376	21	14.14	0.797	0.790	-0.009	125-167
Cisco-181	376	30	23.41	0.936	0.948	+0.012	90-270
Cocl-Lav4	376	17	8.93	0.564	0.565	+0.001	139-181
LGL BWF2	376	13	5.93	0.612	0.587	-0.043	152-178

N = number of samples; N_A = number of alleles; A_R = allelic richness; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{IS} = HWE variance estimate or inbreeding coefficient

Table 3. Pairwise F_{ST} values between samples collected in 2005 and 2006 from the Colville River, Alaska, and from the Arctic Red and Peel rivers in Canada, 2007. All pairwise comparisons were not significantly different ($p \geq 0.21$ for all comparisons).

	Colville 2005	Colville 2006	Arctic Red 2007
Colville 2006	-0.0012		
Arctic Red 2007	0.0001	0.0001	
Peel 2007	-0.0001	0.0008	-0.0007

Table 4. Pairwise F_{ST} values between age classes of Arctic cisco collected in 2006 from the Colville River. All pairwise comparisons were not significantly different ($p \geq 0.09$ for all comparisons).

	age 5	age 6	age 7
age 6	0.0093		
age 7	0.0032	0.0013	
age 8	-0.0021	0.0033	0.0033

A minimum spanning network was constructed to graphically depict relationships between haplotypes sequenced from 381 samples (Figure 3). The most common haplotype was represented in 74% (N = 280) of the Arctic cisco samples and had a relative frequency of 0.73 to 0.78 in all sample collections (Table 6). Ten AMN haplotypes (relative frequency ≤ 0.012) sequenced from Colville River samples were not found in the Arctic Red or Peel rivers. Two haplotypes (AMN009 and AMN010) identified in the Arctic Red River were not found in the Colville or Peel rivers.

Discussion

Anadromous Arctic cisco have a unique life history that differs significantly from other anadromous salmonids (Figure 4). A major feature of this life history is a form of anadromy (amphidromy) where the fish migrate to rich, productive coastal waters to feed during the summer and return to estuarine-like habitats to overwinter (Bond and Erickson 1992). Arctic cisco are thought to overwinter in the Colville River for 5-7 years before reaching reproductive maturity and returning to the Mackenzie River to spawn (Gallaway et al. 1983). Their migration under the ice to overwintering coastal pools that do not freeze solid during the winter on the Colville River provides the opportunity for traditional subsistence harvest. One primary goal of these genetic analyses was to test for population(s) of origin for Arctic cisco collected in the subsistence harvest on the Colville River. Multiple genetic analyses performed on two sample collections from the Colville River (2005, 2006) and on two collections from the lower Mackenzie River (Peel and Arctic Red rivers) supported inference for one randomly mating, panmictic population of Arctic cisco from the Mackenzie River contributing to recruitment in the Colville River over-wintering populations.

Global F_{IS} across all sample locations and years was equal to zero supporting stable genetic structure in Arctic cisco sampled for this study, adding additional inference for one interbreeding population of Arctic cisco. Previous genetics analyses supported the Mackenzie River as the primary population of origin for Arctic cisco in the Colville River subsistence fishery, i.e., the “Mackenzie Hypothesis” (Gallaway et al. 1983; Gallaway et

Figure 2. Mitochondrial DNA sequence alignment of 594 nucleotides (nt) of the ATPase6 gene from three *Coregonus* species and the unknown *Coregonus* haplotype (*Coregonus* spp.) collected from the Colville River in 2006. Complete sequence data available for the Common whitefish (*C. lavaretus*) was used to standardize nucleotide numbering (Miya and Nishida 2000; GenBank accession no. AB034824). Sequences for Arctic cisco (*C. autumnalis*; AMN001) and Bering cisco (*C. laurettae*; LAU001) represented the most frequently occurring haplotypes by species from this study.

```
C. lavaretus      25 TTTATAAGCCCCACATACCTGGGCATCCCACTCATTGCTGTAGCACTAACCCTCCCATGA 84
C. autumnalis     25 TTTATGAGCCCCACATACCTGGGTATTCCACTCATTGCTGTAGCACTAACCCTCCCATGA 84
C. laurettae      25 TTTATGAGCCCCACATACCTGGGTATTCCACTCATTGCTGTAGCACTAACCCTCCCATGA 84
Coregonus spp.    25 TTTATGAGCCCCACATACCTGGGTATTCCACTCATTGCTGTAGCACTAACCCTCCCATGA 84
                          *                  *  *

C. lavaretus      85 ATTCTCTTCCCAACCCCCTCTGCCCGGTGACTAAACAACCGCCTTATCACACTACAAGGG 144
C. autumnalis     85 ATTCTCTTCCCAACCCCCTCTGCCCGATGACTAAACAACCGCCTTATCACACTACAAGGA 144
C. laurettae      85 ATTCTCTTCCCAACCCCCTCTGCCCGATGACTAAACAACCGCCTTATCACACTACAAGGA 144
Coregonus spp.    85 ATTCTCTTCCCAACCCCCTCTGCCCGATGACTAAACAACCGCCTTATCACACTACAAGGA 144
                                                *                                 *

C. lavaretus     145 TGGTTTATCAACCGATTCACCCAACAGCTTCTTCTCCCCTTGAACCTAGGAGGCCATAAG 204
C. autumnalis    145 TGATTCATCAACCGATTCACCCAACAGCTTCTCCTCCCCTTAAACCTAGGAGGCCATAAG 204
C. laurettae     145 TGATTCATCAACCGATTCACCCAACAGCTTCTCCTCCCCTTAAATCTAGGAGGCCATAAG 204
Coregonus spp.   145 TGATTCATCAACCGGTTCACCCAACAGCTTCTCCTCCCCTTAAACCTAGGAGGCCATAAG 204
                       *  *        *                  *        *  *

C. lavaretus     205 TGAGCAGTAATGCTGACCTCTTTAATACTATTCCTAATTACCCTGAATATATTAGGCCTT 264
C. autumnalis    205 TGAGCAGTAATACTAACCTCTTTAATACTATTCCTAATTACCCTAAATATATTAGGCCTT 264
C. laurettae     205 TGAGCAGTAATACTGACCTCTTTAATACTGTTCCTAATTACCCTAAATATATTAGGCCTT 264
Coregonus spp.   205 TGAGCAGTAATACTGACCTCTTTAATACTGTTCCTAATTACCCTAAATATATTAGGCCTT 264
                                  *  *              *              *

C. lavaretus     265 CTTCCATACACCTTCACCCCGACCACGCAGCTCTCCCTAAATATGGGGCTTGCAGTTCCA 324
C. autumnalis    265 CTTCCATATACCTTCACCCCGACCACGCAGCTCTCCCTAAATATAGGGCTTGCAGTTCCA 324
C. laurettae     265 CTTCCATATACCTTCACCCCGACCACGCAGCTCTCCCTAAATATAGGGCTTGCAGTTCCA 324
Coregonus spp.   265 CTTCCATATACCTTCACCCCGACCACGCAGCTCTCCCTAAATATAGGGCTTGCAGTTCCG 324
                              *                                  *              *

C. lavaretus     325 CTATGACTTGCAACAGTAATTATCGGTATACGAAACCAACCAACTGCCGCCCTGGGACAC 384
C. autumnalis    325 CTATGACTTGCAACGGTAATTATCGGTATGCGAAACCAACCAACTGCCGCCCTGGGGCAC 384
C. laurettae     325 CTATGGCTTGCAACGGTAATTATCGGTATACGAAACCAACCAACTGCCGCCCTGGGGCAC 384
Coregonus spp.   325 CTATGGCTTGCAACGGTAATTATCGGTATACGAAATCAACCAACTGCCGCCCTGGGGCAC 384
                          *        *              *     *                    *

C. lavaretus     385 CTCTTACCTGAAGGAACCCCTGTCCCACTTATCCCGGTTCTTATCATCATCGAAACAATT 444
C. autumnalis    385 CTCTTACCTGAAGGAACCCCAGTCCCACTTATCCCGGTTCTTATCATCATCGAAACAATT 444
C. laurettae     385 CTCTTACCTGAAGGAACCCCGGTCCCACTTATCCCGGTTCTTATCATCATCGAAACAATT 444
Coregonus spp.   385 CTCTTACCTGAAGGAACCCCGGTCCCACTTATTCCAGTTCTTATCATCATCGAAACAATT 444
                                             *           *  *
```

Figure 2. Cont.

```
C. lavaretus     445 AGCCTCTTCATCCGCCCCCTTGCTCTAGGCGTACGGCTTACAGCCAACCTTACGGCAGGC 504
C. autumnalis    445 AGCCTCTTCATCCGCCCCCTTGCTCTAGGCGTACGGCTTACAGCCAACCTTACAGCAGGC 504
C. laurettae     445 AGCCTCTTCATCCGCCCCCTTGCTCTAGGCGTACGGCTTACAGCCAACCTTACAGCAGGC 504
Coregonus spp.   445 AGCCTCTTCATCCGCCCCCTTGCTCTAGGCGTACGGCTTACAGCCAACCTTACAGCAGGC 504
                                                                            *

C. lavaretus     505 CACCTTCTAATTCAACTAATTGCAACAGCAGCCTTTGTTCTTCTACCCCTGATACCAACA 564
C. autumnalis    505 CACCTTCTAATTCAACTAATTGCAACAGCAGCCTTTGTTCTTCTACCCATGATACCAACA 564
C. laurettae     505 CACCTTCTAATTCAACTAATTGCAACAGCAGCCTTTGTTCTTCTACCCATGATACCAACG 564
Coregonus spp.   505 CACCTTCTAATTCAACTAATTGCAACAGCAGCCTTTGTTCTTCTACCCATGATACCAACG 564
                                                                        *         *

C. lavaretus     565 GTGGCAATCCTTACTGCTATTGTCCTATTCCTGCTTACCCTTCTTGAGATCGCC 618
C. autumnalis    565 GTGGCAATCCTTACTGCTCTTGTCCTATTCCTGCTTACCCTTCTTGAGATCGCC 618
C. laurettae     565 GTAGCAATCCTTACTGCTCTTGTCCTATTCCTGCTTACCCTTCTTGAGATCGCC 618
Coregonus spp.   565 GTAGCAATCCTTACTGCTCTTGTCCTATTCCTGCTTACCCTTCTTGAGATCGCC 618
                       *               *
```

Table 5. Mitochondrial haplotypes and variable nucleotide (nt) positions identified within a 594 nt segment of the ATPase6 gene. Variable nucleotides listed in bold differ from AMN001, the most common Arctic cisco haplotype. Nucleotide numbering scheme was based on the complete published sequence for the *C. lavaretus* ATPase6 gene (GenBank accession no. AB034824; Miya and Nishida 2000). Haplotype classification: AMN = Arctic cisco; LAU = Bering cisco; unknown = not determined.

	Variable nucleotide position																												
Haplotype classification	36	38	87	111	159	162	189	219	232	234	249	285	324	325	330	348	354	359	360	372	378	405	417	420	555	564	565	567	615
AMN001	C	C	T	A	A	C	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN002	C	C	T	A	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN003	C	C	T	A	A	C	**T**	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN004	C	C	T	A	A	C	C	A	C	A	A	G	A	C	A	**T**	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN005	C	C	T	A	A	C	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	**A**	A	G	G	C
AMN006	C	C	T	A	A	C	C	A	C	A	A	G	A	**A**	A	C	G	A	C	**T**	G	A	C	G	G	A	G	G	C
AMN007	C	C	T	A	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	**T**	C	**A**	A	C	G	G	A	G	G	C
AMN008	C	**T**	T	A	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	**T**	C	**A**	A	C	G	G	A	G	G	C
AMN010	**T**	C	T	A	A	C	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN011	C	C	T	A	A	**T**	C	A	**T**	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN012	C	C	T	**G**	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN013	C	C	**C**	A	A	C	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN014	C	C	T	A	A	C	C	A	C	A	A	G	A	C	A	C	G	**G**	C	C	G	A	C	G	G	A	G	G	C
AMN016	C	C	T	A	A	**T**	C	A	C	A	A	**A**	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN017	C	C	T	A	A	C	C	A	C	A	**G**	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	C
AMN018	C	C	T	A	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	G	G	**T**
AMN019	C	C	T	A	A	**T**	C	A	C	A	A	G	A	C	A	C	G	A	C	C	G	A	C	G	G	A	**A**	G	C
LAU001	C	C	T	A	A	C	**T**	**G**	C	**G**	A	G	A	C	**G**	C	**A**	A	C	C	G	**G**	C	G	G	**G**	G	**A**	C
LAU002	C	C	T	A	A	**T**	**T**	**G**	C	**G**	A	G	A	C	**G**	C	**A**	A	C	C	G	**G**	C	G	G	**G**	G	**A**	C
UNKNOWN	C	C	T	A	**G**	C	C	**G**	C	**G**	A	G	**G**	C	**G**	C	**A**	A	**T**	C	G	**G**	**T**	**A**	G	**G**	G	**A**	C

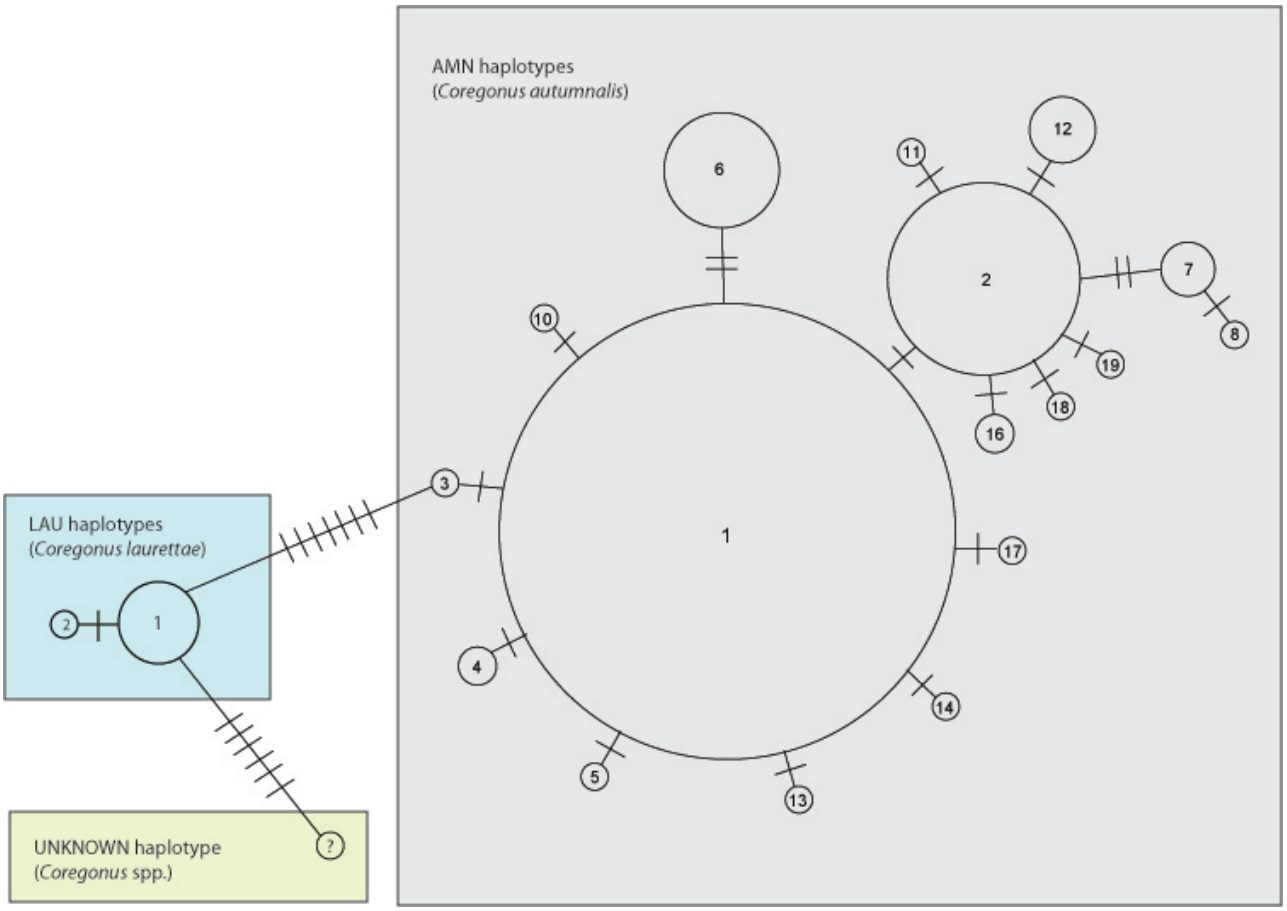

Figure 3. Minimum spanning network of mtDNA haplotypes identified within a 594 nucleotide fragment of the ATPase6 gene. Circle sizes are proportional to haplotype frequency and line lengths are proportional to the number of nucleotide differences between haplotypes. Each cross hatch indicates one nucleotide difference. AMN numbers in the grey box and LAU numbers in the blue box correspond to haplotype classifications listed in Table 5. A question mark in the green box represents the unknown haplotype sequenced from the Colville River 2006 collection.

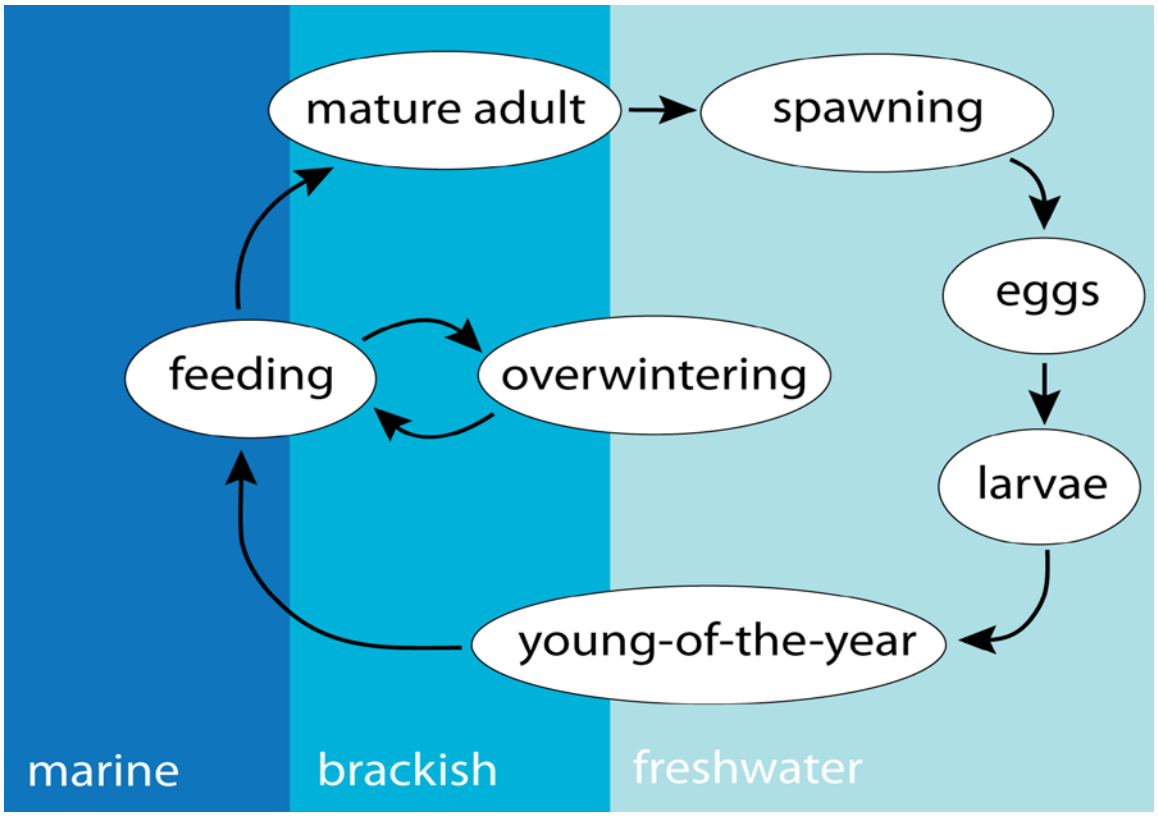

Figure 4. Generalized life history for anadromous Arctic cisco.

Table 6. *Coregonus autumnalis* mitochondrial ATPase VI haplotype frequencies for each sample collection.

Haplotype	Colville 2005 (N = 85)	Colville 2006 (N = 173)	Arctic Red 2007 (N = 60)	Peel 2007 (N = 54)
AMN001	0.765	0.728	0.783	0.778
AMN002	0.153	0.139	0.100	0.130
AMN003	0.012	0	0	0
AMN004	0.012	0.006	0	0
AMN005	0.012	0	0	0
AMN006	0.024	0.069	0.017	0.056
AMN007	0.012	0.012	0.017	0
AMN008	0.012	0	0	0
AMN009	0	0	0.017	0
AMN010	0	0	0.017	0
AMN011	0	0.006	0.050	0.037
AMN012	0	0.006	0	0
AMN013	0	0.006	0	0
AMN014	0	0.012	0	0
AMN015	0	0.006	0	0
AMN016	0	0.006	0	0
AMN017	0	0.006	0	0

al. 1989; Bickham et al. 1989; Morales et al. 1993; and Fechhelm et al. 2007). Our current genetic analyses concur with this hypothesis.

It is unknown if over-wintering Arctic cisco return to the same habitat year-after-year until they reach spawning maturation. Our current database included only one year with assigned year classes of Arctic cisco (Colville River 2006). The level of genetic differentiation reported among year classes was not significant. Many studies report the rapid drop of assignment success once F_{st} falls below 0.05 (Bernatchez and Duchesne 2000; Berry et al 2004; Mank and Avise 2004). Recently, the level of genetic differentiation required to successfully use Bayesian assignment tests to demonstrate independence of isolated populations has been challenged (Hauser et al. 2006; Bradbury et al. 2008). North American eel populations were successfully assigned to population of origin using baseline information despite low differentiation (F_{st} = 0.013; Mank and Avise 2004). Hauser et al. (2006) differentiated hatchery and wild steelhead with successful assignment tests despite low differentiation (F_{st} = 0.02). F_{st} pairwise analyses

of 2006 Colville River Arctic cisco year-classes did suggest possible fine scale genetic structuring between year classes 5 and 6, despite low differentiation (F_{st} = 0.01). Additional year-class data and increased sample sizes will be available for the 2007 collection from the Colville River through analyses underway by Dr. Zimmerman. Multiple year-class data available for our next report will allow tests based on Bayesian assignment tests among year-classes to address the question of year-to-year overwinter site fidelity among age-class cohorts.

If over-wintering site fidelity across age classes of Arctic cisco can be demonstrated in the Colville River, it will be important to determine what physical or biochemical parameters contribute to natural conditions leading to migration and site fidelity. If Arctic cisco reside in brackish waters throughout the winter, do they experience resmoltification and under go physiological changes necessary for fresh/salt water adaptation in their fall and spring transitions to and from the Beaufort Sea (see similar life history traits described in anadromous Arctic char (*Salvelinus alpinus*) by Aas-Hansen et al 2005)? Do changing temperature or wind regimes alter these conditions and affect recruitment and survival back to the Mackenzie River? Bayesian landscape genetic analyses (Foll and Gaggiotti 2006) can be used to reveal dominant physical or biochemical influences on gene flow at temporal scales. Biochemical and genetic processes present important questions with significant implications for local adaptation in these stocks and sustainable recruitment in the face of climate change on the North Slope.

Anadromous Arctic cisco have been shown to migrate as much as 1,000 km upstream to spawn (Roguski and Komarek 1971). Mature male and female Arctic cisco have been observed in tributaries of the Mackenzie River (Gallaway et al. 1983; McLeod and O'Neil 1983). However, actual spawning events often occur in remote locations during harsh climatic conditions and have only been inferentially reported. The concept of natal stream fidelity in Arctic cisco was first reported by Bickham et al. (1989). Their analyses differentiating collections from the Arctic Red and Peel rivers were based on allelic frequency distributions derived from allozyme analyses at two loci (Mpi and α-Gdp-1). However, these analyses were based on small sample sizes (N = 15-17) and changes in abundance for low-frequency alleles using Chi square analyses of

significance. These factors limit the rigor of their conclusions. Indeed, Bickham et al. (1992) concluded that the spawning sites on the Arctic Red and Peel rivers were only "somewhat differentiated from one another" and they go further to state that there was no "*strong* genetic differentiation, in the form of fixed alleles" (italics their own) in any of their Arctic cisco samples.

A review of genetic studies of the Arctic cisco produced by Bickham et al.(1992), contained a draft manuscript listed as submitted to *Science* where Alaskan coastal samples (Colville and Sagavanirktok) were most similar genetically to fish from the Peel River (no subsequent journal publication was found). These authors also speculated on the advantage of Peel River juvenile Arctic cisco transport timing and process from the plume of the Mackenzie River to Alaskan waters (Bickham et al. 1992; see page 70). Our current genetic analyses did not support genetic independence for the Peel River spawning population from the Mackenzie River. Both microsatellite allelic frequency data from 11 highly polymorphic loci and mtDNA sequence data from the ATPase 6 gene support genetic similarity between Arctic cisco collected from two putative spawning locations on the Arctic Red and Peel rivers. Further, neither putative spawning location differed genetically from subsistence harvest collections from the Colville River over two years (2005-2006). Sufficient sample size and analytical rigor drawn from these genetic data support the inference of one source population for Arctic cisco in the Mackenzie River with high levels of gene flow between putative spawning sites sampled for this report.

The Arctic Red and Peel rivers enter the mainstem of the Mackenzie River only ~150 km upstream from the mouth at Mackenzie Bay. This short geographical distance may contribute to gene flow and explain the lack of genetic variability detected between these spawning sites. Bickham et al. (1992) did report significant genetic differences based on Chi square tests for two allozyme loci between coastal Arctic cisco samples (Colville and Sagavanirktok) and 'upstream stocks" in the Mackenzie River including the Mountain and Carcajou rivers. Additional sampling during the summer of 2008 at these "upstream" locations will allow further comparisons of genetic diversity for Arctic cisco in the Mackenzie River and their relative contributions to the Colville subsistence fishery.

Genetic differentiation based on geographic separation (isolation by distance, IBD based on standard Mantel tests; Smouse et al. 1986) has been reported for many salmonid populations with high levels of spawning site fidelity (Allendorf and Waples 1996; Olsen and Seeb 1998; Carlsson and Nilsson 2000; Castric and Bernatchez 2003; Primmer et al. 2006). Isolation by distance is characterized by increasing genetic divergence and decreasing gene flow with increasing geographic distance (Crispo and Hendry 2005). Mantel tests and partial Mantel tests can be used to test pairwise differences in a diversity of ecological factors which may influence genetic diversity over time (Storfer et al. 2007; Dionne et al. 2008). Collections from the Mountain, Carcajou and Great Bear rivers located ~500 – 700 km from the mouth of the Mackenzie River will allow us to investigate genetic structure among Arctic cisco across a broader geographical scale, and to determine at what scale geographic distance affects, if at all, anadromous Mackenzie River Arctic cisco populations. This type of analyses will help determine the relative scale of local adaptation in Arctic cisco spawning populations in the Mackenzie River over time and space. Exploring the processes that underlie fine-scale population divergence in Arctic cisco from the Mackenzie River is a crucial step toward understanding the origin and maintenance of biodiversity in this population, especially in a changing climate.

Arctic cisco populations from different Mackenzie River tributaries may represent unique ecophenotypes derived from the same interbreeding population (Rogers et al. 2002; Saint-Laurent et al. 2003; Turgeon and Bernatchez 2003). The study of adaptive radiation and functional genomics is a growing field that may also shed light on trade-offs and phenotypic divergence among interbreeding groups of Arctic cisco with different geographic distributions at different life history stages (Singer et al. 2002; St-Cyr et al. 2008). The study of patterns of genetic transcription and inter-individual variation across natural environments with rapidly changing conditions may be the best approach to setting near-term conservation goals for fish and fisheries in the Arctic.

The distribution and genetic population structure of high-latitude fishes has been strongly influenced by climate oscillations and periods of glaciation (Phillips and Ehlinger 1995; Turgeon and Bernatchez 2001; Politov et al. 2004; Dodson et al. 2007). Mitochondrial DNA has been used successfully to describe population structure, the

influence of historical events (i.e., glaciation) on evolutionary patterns, and the processes involved in coregonid fish distributions in the Arctic region (Patton et al. 1997; Bernatchez and Wilson 1998; Turgeon and Bernatchez 2003). Sequence analyses of mtDNA demonstrated 19 unique haplotypes for the ATPase 6 gene in this report. However, two of these haplotypes sequenced from putative Arctic cisco collected in the Colville River were highly divergent; one aligned to Bering cisco sequence data and the other remains unknown. The haplotype that aligned with Bering cisco mtDNA differed by eight nucleotide (nt) substitutions from the most common Arctic cisco haplotype, and the unknown haplotype was 14 nt different. This suggests the possibility of other coregonid mtDNAs in comparison with the rest of the haplotype diversity found in our Arctic cisco collection. We suggested two possible factors that may have contributed to this mtDNA diversity: 1) past hybridization events; or 2) misidentified samples taken in the field.

The mitochondrial DNA sequence from one of the divergent haplotypes identified in three Colville River samples was congruent with a Bering cisco haplotype (LAU001) from the Yukon River sequenced in our laboratory. Congruence between haplotypes could indicate hybridization between these two species at any point in the past and retention of the Bering cisco haplotype after backcrossing among viable progeny to produce the Arctic cisco phenotype. Introgressed mitochondrial genomes have been identified in several species of white fish (Bernatchez et al. 1989; Wilson and Hebert 1993; Bernatchez et al. 1995; Turgeon and Bernatchez 2001; Rogers et al. 2007), including Arctic cisco (Bickham et al. 1997; Yakhnenko and Mamontov 2006). Natural hybridization between Arctic and Bering ciscoes was reported by Bickham et al. (1989, 1992 and 1997). Bickham et al. (1997) identified two fish with a Bering cisco mtDNA restriction pattern, but gill raker counts were within the range of Arctic cisco and outside of the range of Bering cisco. Allozyme alleles from these two fish were present in both species and the authors concluded that these fish were not Bering ciscoes misidentified as Arctic ciscoes, but rather fish of hybrid origin. Alternatively, Bering cisco have been found to comprise as much as 10% of the ciscoes taken in the Colville River subsistence catch (Moulton et al. 1988). It is possible that the three fish possessing the LAU001 haplotype in our study were actually Bering cisco that were misidentified in the field.

Highly polymorphic loci, such as microsatellites, provide the potential to investigate species-specific alleles or allelic size ranges that can be diagnostic for characterizing ambiguous samples (hybrids). Diagnostic nuclear markers have been used to resolve steelhead–cutthroat trout hybrids (Wenburg et al. 1998; Ostberg and Rodriguez 2002; Williams et al. 2005). However, to our knowledge no nuclear DNA markers are available to discern Arctic cisco from Bering cisco. Genotyping additional Bering cisco from the Yukon River (our current collection N = 28) will be used to investigate species-specific diagnostic alleles or allelic size ranges between these two species. Discovering microsatellite markers that can resolve *Coregonus* species ambiguities will enable a thorough genetic characterization of suspect hybrids potentially misidentified in the field (Nielsen et al. 2003).

Species affiliation for the most divergent haplotype identified in the 2006 Colville River collection has not yet been determined. ATPase 6 gene nucleotides for this haplotype aligned directly with other *Coregonus* species (Figure 2) supporting the conclusion that the unknown haplotype represents a *Coregonus* species. There were six pairwise differences between the unknown haplotype and Bering cisco haplotype LAU001. This level of variation could be explained by a higher level of divergence within Bering cisco compared to that found in Arctic cisco in this study. Analysis of a larger number of Bering cisco will demonstrate the extent of divergence found in the ATPase 6 gene for this species. However, genetic affiliation with another *Coregonus* species cannot be ruled out. Reports of hybridization involving Arctic cisco are not limited to Bering cisco (Svardson 1998; Yakhnenko and Mamontov 2006). Sequencing the ATPase 6 gene from other coregonids that co-occur within the distribution of Arctic cisco, such as the least cisco (*C. sardinella*), broad whitefish (*C. nasus*), and humpback whitefish (*C. pidschian*) may help resolve the species classification for this unknown *Coregonus* spp. haplotype.

New changes in climatic patterns in the Arctic may have strong influence on species distributions and population genetic structure in the near term (McCloud and O'Neil 1983). Genetic data on Arctic cisco in the Colville River provide an excellent baseline for opportunities to further our understanding of population structure, Arctic cisco genomics, and interactions among adaptive mechanisms responding to climate

change. It is important to understanding the mechanisms of how variation in genes affect an organisms' resilience to change and how selection and adaptation at the cellular level combine to impact variation at the individual phenotype and population levels.

Otolith Analysis

Methods

Otolith samples and preparation

All otoliths examined were collected from subsistence fisheries or monitoring efforts in the Colville River. An archive of otoliths from 1986 to 2005 was provided by Larry Moulton (MJM Research, Lopez Island, WA) and otoliths captured in the 2006 subsistence fishery were provided by LGL Limited (Anchorage, AK). At least thirty otoliths per capture year were analyzed for each year between 1986 and 2006 with the exception of 1989, 2002, 2003 and 2004 (years when otoliths were not collected). The total number of otoliths examined was 768 and 283 of these were paired with fish length data (Table 7). All otoliths had been previously removed from fish, cleaned, dried and stored in vials.

Otoliths were embedded in epoxy (Buehler Ltd., Lake Bluff, Illinois, USA), sectioned on a transverse plane through the nucleus with a low speed wafering saw (Isomet, Buehler Ltd.), and affixed to glass slides with cyanoacrylate glue. After drying, slides were polished for clarity on 1200 grit sandpaper with water. Following slide preparation, digital images of each otolith were captured at a magnification of 40x using a Leica DFC280 digital camera attached to a Leica MZ6 dissection microscope.

Age and growth

Age determination and annulus measurements were conducted using ImageJ image analysis software (National Institute of Health). Annulus measurements were made along the longest possible radius between the center of the nucleus and the ventral edge of the transverse section. To test reader consistency, 10% of otoliths were aged and

measured twice. Successive age estimates were compared using a non parametric Wilcoxon signed rank sum test that does not assume a normal distribution (Proc univariate in SAS, $P < 0.05$). Repeat annulus measurements were compared using a paired t-test (Proc ttest in SAS, $P < 0.05$).

Table 7. Table of samples collected from the Colville River, AK, USA by year. Numbers in parentheses indicate samples that were not included in the analysis.

Collection Year	*Samples Collected (removed)*	*Samples with known length (removed)*
1986	30 (1)	
1987	54 (4)	24 (2)
1988	32 (0)	2 (0)
1989	-	-
1990	59 (1)	29 (1)
1991	130 (2)	99 (2)
1992	43 (9)	13 (8)
1993	38 (3)	8 (3)
1994	30 (2)	-
1995	39 (4)	9 (2)
1996	30 (3)	-
1997	30 (3)	-
1998	30 (0)	-
1999	30 (0)	-
2000	30 (3)	-
2001	31 (2)	-
2002	-	-
2003	-	-
2004	-	-
2005	46 (0)	16 (0)
2006	83 (6)	83 (3)
Total	**765 (43)**	**283 (21)**

To confirm that otolith growth was correlated with fish body growth, fish of known fork length were compared to otolith radius using the Fraser-Lee method (Frances 1990). Once the relationship between otolith radius and fish length had been described, an Arctic cisco growth model was developed using the von Bertalanffy growth curve to model cisco growth. To obtain the growth coefficient (K) and the maximum length (L_∞) for the von Bertalanffy growth curve, the radius of the otolith at time t (r_t) was regressed against the radius of the otolith at time t+1 (r_{t+1}). The slope of this regression was used to solve for K. To obtain L_∞ the value of r_t where $r_t = r_{t+1}$ was used in the previously obtained relationship (otolith radius and fish length) as otolith radius and L_∞ was calculated as the resulting fish length.

In addition, cohort specific von Bertalanffy growth curves based only on otolith radius data (no length at capture information) were developed for cohorts with more than 40 individual otoliths to examine possible changes in the growth curve parameters over time. Because length at capture was not included, the parameters K and L_∞ could not be estimated and r_∞, the maximum otolith radius, was substituted for L_∞. Instead, these parameters were determined simply by least squares non-linear regression of the otolith radius vs. age plot, with the otolith radius plotted against each age within each individual. Least squares regression was used to calculate the mean value and the associated error for each parameter. A modified one-way ANOVA for summary statistics with a Tukey's post hoc test was used to analyze differences in each parameter (Larson 1992).

The relationship between otolith radius and fish length was not used to back calculate fish lengths due the extra assumptions associated with back calculation (Francis 1990). Instead growth analyses were preformed directly on the annulus measurements. Annulus measurements were compared by growth year using a one-way analysis of variance (ANOVA) for each annulus (PROC GLM in SAS, $P < 0.05$). The mean widths of successive annuli in the same growth year were compared using simple linear regression to determine if a correlation between annuli was present.

Environmental covariates

Environmental covariates analyzed included the winter Arctic oscillation index as a broad measure of environmental change and air temperature, wind speed, and river

discharge as local environmental indicators. Because otoliths used in this study were originally collected for monitoring the age structure of the catch, local environmental variables were not recorded during the time of otolith collection. Consequently local environmental indicators used in this study were selected from data available from outside sources based two criteria: (1) Records for the entire period of study, 1978-2006, and (2) Proximity to arctic cisco habitat. Water temperature was not available for this time series in the Beaufort Sea, Mackenzie River, or Colville River so air temperature was used as a proxy for water temperature. In Canada, air temperature was available from Inuvik in the Mackenzie River delta and river discharge was available from Ft. Simpson for the Mackenzie River (Environment Canada and Water Survey of Canada). The Ft. Simpson site was chosen for inclusion because only one main channel is present at this point. In Alaska, air temperature and wind speed and direction were available for Barrow (National Climate Data Center (NCDC)). Because juvenile cisco migrate during their first year from Canada to Alaska, first year growth was compared to the average temperature during the summer growing season (May-August) in both Inuvik, NWT and Barrow, Alaska. The proportion and speed of east wind during the open water season (July-August), when wind can influence water current and therefore migration was also compared to growth. Finally, the mean flow rate (m^3/s, April-June) of the Mackenzie river was compared to growth with and without time lags as river flow rate may impact growth as a physical factor with no time lag (e.g., within seasons changes in salinity or water temperature) or a biological factor with a time lag through changes in carbon input to the nutrient limited Beaufort sea (Carmack et al. 2006, Dunton et al. 2006, Forest et al. 2008). At this time, covariates were compared only to growth of the first annulus using simple linear regression weighted by sample size per growth year. Correlations between growth of other annuli and environmental covariates will be included in future reports.

Results

Age and growth

Of the 768 samples collected for analysis, 43% (6%) were not included in the study because of vaterite replacement (rendering them unreadable), genetic analyses suggested the fish were of a different species, or the fish were aged older than 10 years. Fish older

than 10 years were excluded, as Arctic cisco typically return to the Mackenzie River by age 10. Analysis of otolith microchemistry will be used to characterize migration of these fish for comparison with known Arctic cisco migration patterns (to be completed in 2008).

For samples that were aged and measured twice, neither age nor annulus width differed between subsequent assignments ($n = 74$, $P > 0.05$). Age assignments were made for individuals 1 to 9 years old and 93% of individuals were assigned the same age in each trial. Of the remaining 7% of samples, ages differed by one year. There was a significant positive relation between otolith radius and fork length for individuals of known length ($y = 3.6x + 358.4$, $r2 = 0.89$, $P < 0.0001$, Figure 5). Otolith radius at time t and time t+1 were significantly related ($y = 0.84x + 154.1$; $r^2 = 0.96$; Figure 6) and used to solve for the von Bertalanffy coefficient K. For all samples combined, the von Bertalanffy coefficient (K) was 0.16 and L_∞ was 498 mm and these estimates were used to describe to growth of cisco (Figure 7, $r^2 = 0.82$, $P < 0.0001$). Cohort specific growth curves were developed for seven cohorts, 1980, 1985, 1986, 1987, 1990, 1994 and 1999. Overall, cohorts differed significantly in r_∞ and K. Post hoc tests were used only to examine difference between successive years. Post hoc tests confirmed that the 1980, 1985, and 1986 cohorts were not significantly different for r_∞ or K. Similarly the 1987, 1990, 1994, and 1999 cohorts were not significantly different for r_∞ or K. A significant difference was present between the 1986 and 1987 cohorts for both r_∞ and K, indicating a switch to higher growth rates, but lower maximum otolith growth in the later cohorts (Figure 8). It is important to note that these parameter values should not be compared to the K and L_∞ obtained for the overall study since this information is related to fish lengths and not otolith growth.

Significant inter-annual variation in otolith growth was evident in the first 7 annual growth increments (one-way ANOVA for each annulus, $P < 0.05$). Figures 8 and 9 display growth residuals up to annulus 6, older annuli were not graphed due to lower sample sizes at older ages including growth years with no samples present. The average growth of annulus 1 was not correlated with the average growth of annulus two in the same growth year ($p>0.05$). However growth of annuli 2-6 was correlated with the

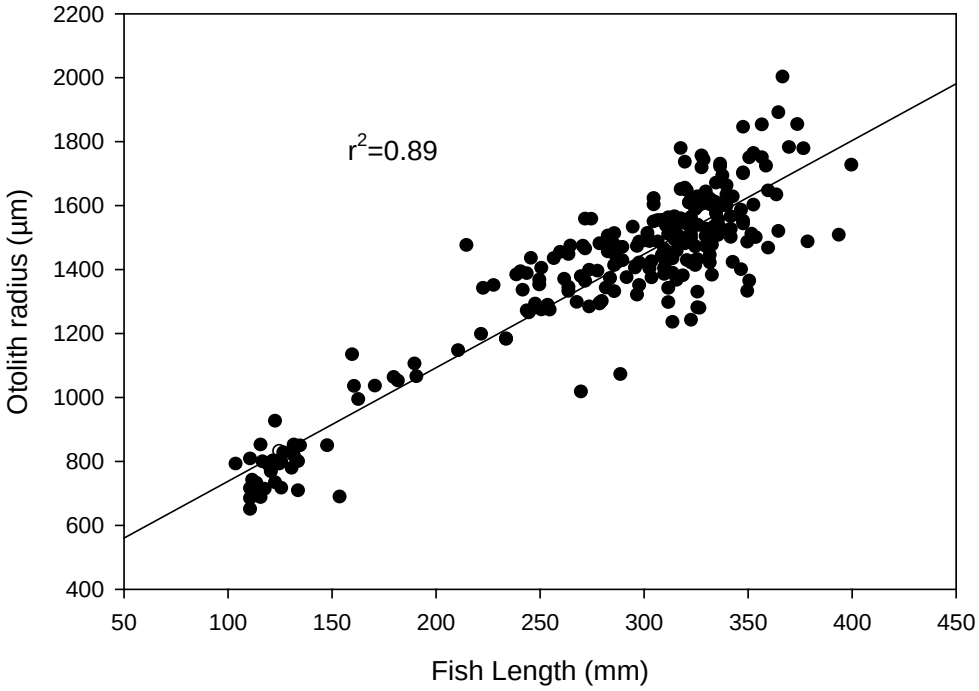

Figure 5. Linear regression of fish length and otolith radius for fish of known length for fish captured in the Colville River, Alaska, USA (1986-2006).

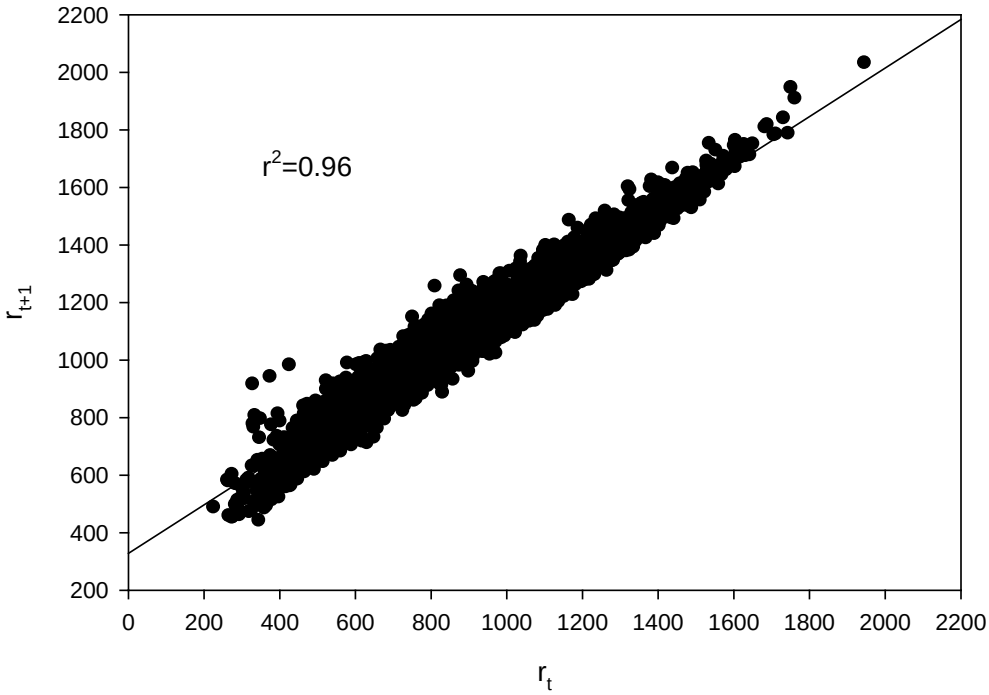

Figure 6. Otolith radius at time t versus otolith radius at time t+1 for all annuli measurements from Arctic cisco captures in the Colville River, Alaska, USA (1986-2006).

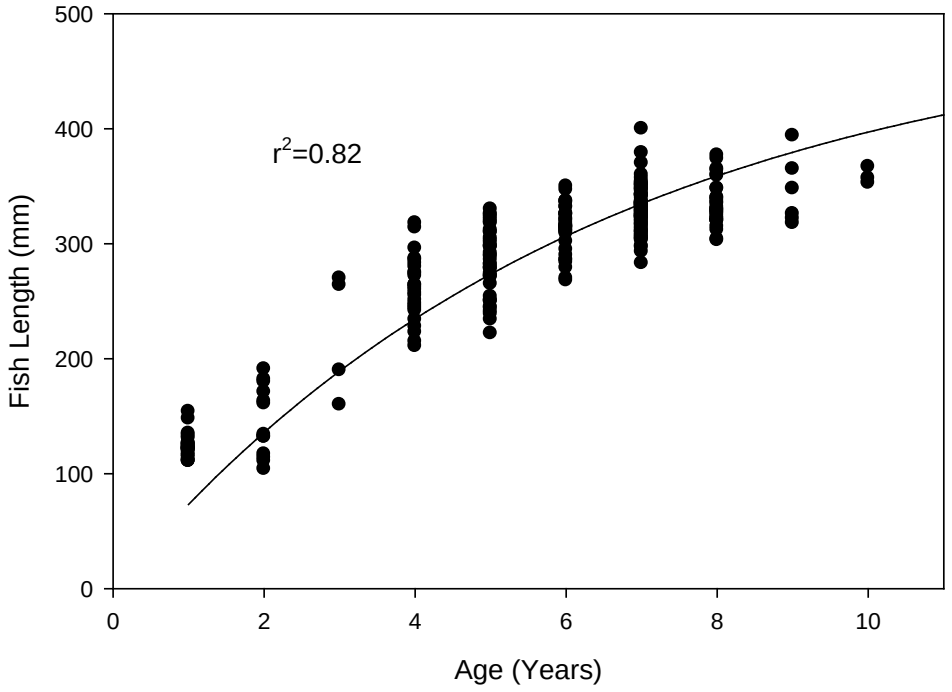

Figure 7. The von Bertalanffy growth curve for Arctic cisco captured in the Colville River, Alaska, USA (1986-2006). K=0.16 and L_∞=498mm.

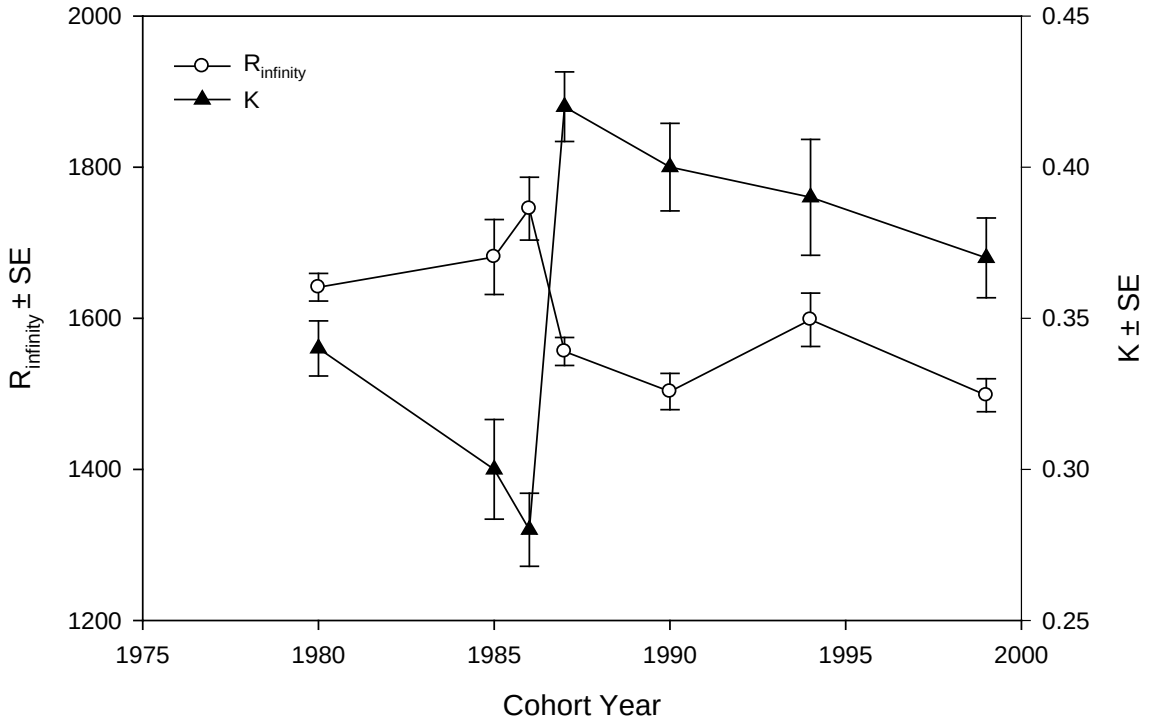

Figure 8. The von Bertalanffy growth parameters, K (solid triangle) and R_∞ (open circle), for individual Arctic cisco cohorts captured in the Colville River, Alaska (1986-2006).

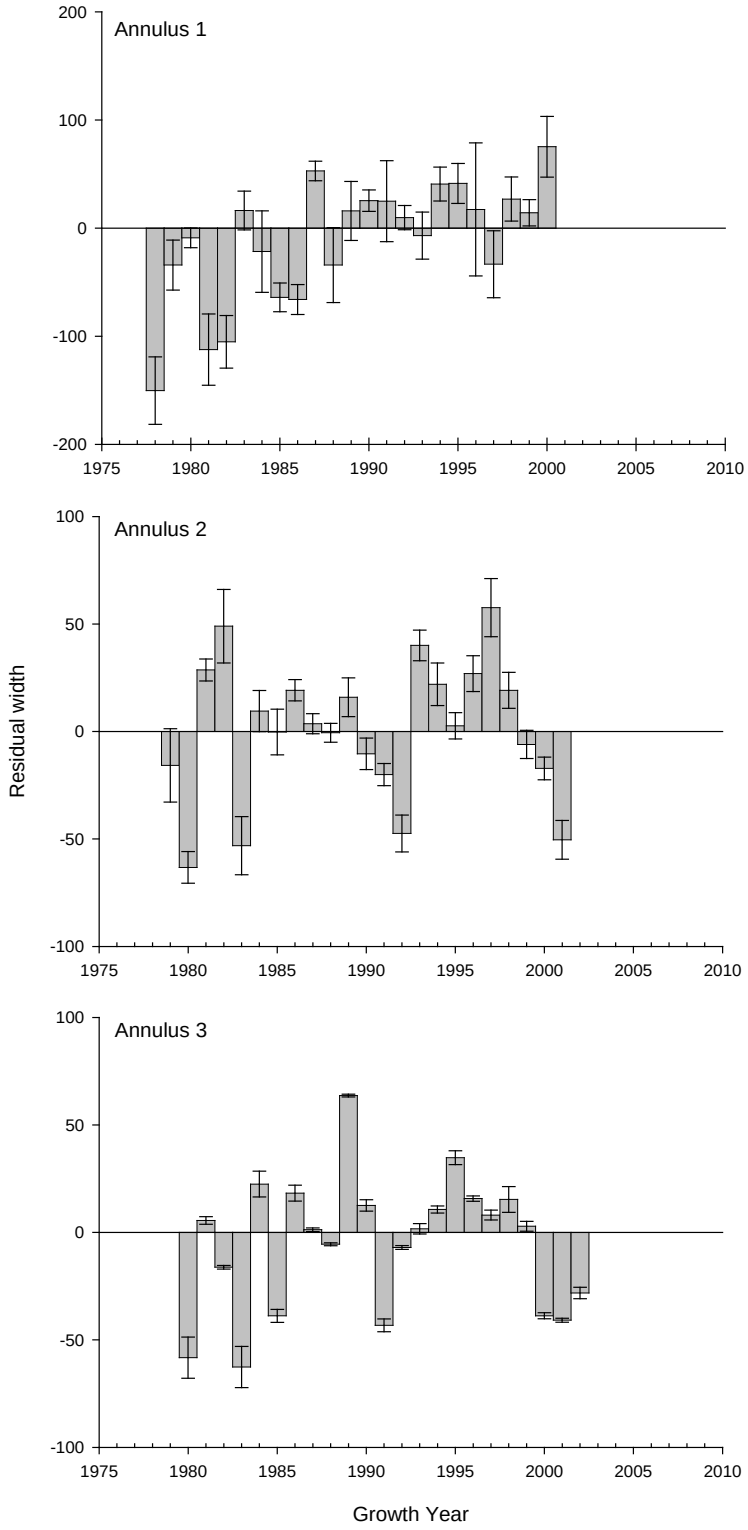

Figure 9. Residuals of annulus growth for the first three annuli by growth year ± SE from Arctic cisco captures in the Colville River, Alaska, USA (1986-2006). Note that the axis for the graph of annulus 1 differs.

successive annulus (all $p<0.05$, annulus 2 vs. annulus 3 $r^2 = 0.38$, annulus 3 vs. annulus 4 $r^2 = 0.41$, annulus 4 vs. annulus 5 $r^2 = 0.75$, annulus 5 vs. annulus 6 $r^2 = 0.58$).

Environmental covariates

The growth of the first annulus was positively correlated with the winter Arctic oscillation index ($r^2 = 0.18$, $P = 0.04$). At a more local scale, air temperature in Inuvik was positively correlated with growth ($r^2 = 0.20$, $P = 0.03$, Figure 10A), but temperature in Barrow had no effect ($P = 0.43$). The proportion of east winds during the open water period was not correlated with growth ($P = 0.74$), but the speed of east winds did have a positive effect ($r^2 = 0.22$, $P = 0.03$, Figure 10B). River flow did not correlate with growth unless lagged two years (no lag $P = 0.72$; 1 year lag $P = 0.17$; 2 year lag $r^2 = 0.32$, $P = 0.005$, Figure 10C).

Discussion

A strong positive relationship is present between otolith radius and fish length in Arctic cisco, suggesting that changes in otolith growth do reflect overall fish length (Figure 5). Arctic cisco growth, as determined by otolith growth, did differ among years (Figures 9 and 10). Cohort specific growth curves also demonstrate that the pattern of growth differs among cohorts (Figure 8). While a clear regime shift was not present in the growth rate data, residual values indicate that growth rates of the first annulus were generally lower than average during the 1980s and higher than average in the 1990s with a shift occurring sometime in the late 1980s (Figure 9). Growth rates were positively associated with the Arctic oscillation index, temperature, speed of east winds and river flow lagged by two years (Figure 11).

The observed difference in cohort specific growth parameters indicate that fish from cohorts after 1986 may be growing faster, but not reaching the same size as fish hatched during the first part of the 1980s and late 1970s (Figure 8). The mean difference between r_∞ for cohorts prior to 1987 and those later expressed in terms of fish length is approximately 50 mm, as determined by the equation relating otolith radius to fish of known length. Concurrent with a shift toward lower r_∞ was an increase in K or a measure of growth rate. This shift in growth parameters is particularly relevant as residents of

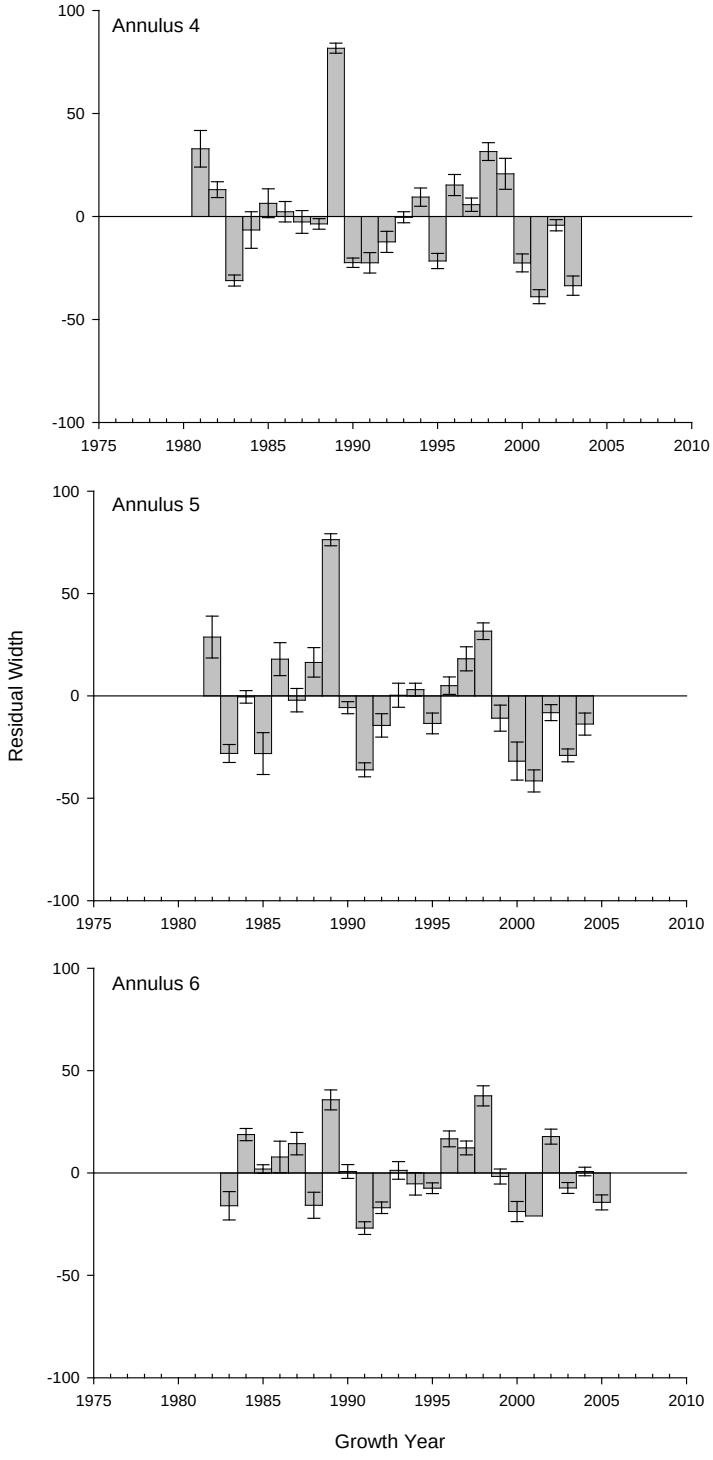

Figure 10. Residuals of annulus growth for the fourth, fifth and sixth annuli by growth year ± SE from Arctic cisco captures in the Colville River, Alaska, USA (1986-2006).

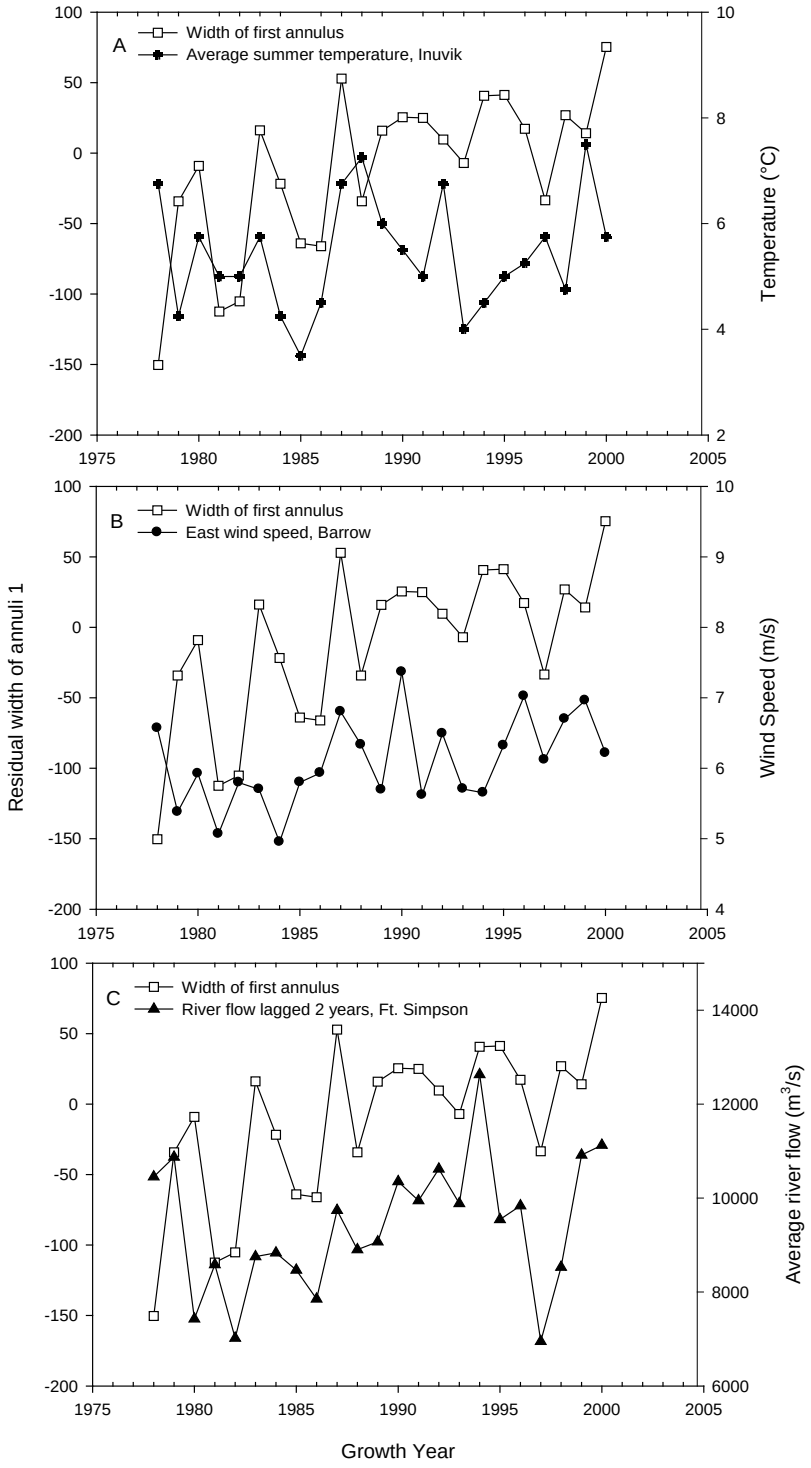

Figure 11. Residual width of the first annulus (open squares) plotted with environmental parameters. Top panel: Average summer temperature (May-August) in Inuvik, Canada (solid plus). Middle panel: Average speed of east winds during the open water period (July-August, solid circles). Bottom panel: Average Mackenzie River flow rate as measured at Ft. Simpson, Canada (April-June, solid triangles).

Nuiqsut, where much of the subsistence harvest of cisco occurs, believe that the size of cisco has decreased over time. Thus the changes in r_∞ documented here align well with the local knowledge.

Growth rates do show strong year-to-year variation for arctic cisco in the first seven annuli with the greater variation in the first annulus as compared to later annuli (Figures 10 and 11). The significant correlations between successive older annuli (annuli 2-6), but not between annulus 1 and 2 suggest that different factors impact age-0 fish as compared to fish > 1 year old. This result is not surprising given that age-0 fish spent part of the year in a different location from older fish. Among older fish, the correlations between successive growth years may indicate that environmental changes impact these individuals in similar ways. In other words, a change in the environment that benefits growth during the second year of life also benefits growth during the third year of life.

While the impact of a changing environment can differ across age groups, the youngest age groups typically respond to changes in a quicker and more pronounced manner. Part of this trend is due to the fact that older and larger individuals have a lower mass-specific metabolic rate and can possess proportionally larger on board energy stores so that during periods of poor environmental conditions growth may continue longer than that of a younger and smaller individual (Clarke and Johnston 1999). Due to these physiological factors, annuli laid down during later years of life may be less variable as growth rates slow or individuals in these older ages may be better at buffering against environmental change, thus, making correlations at older ages more difficult to detect.

The growth of the first annulus was correlated with a number of environmental parameters. The Arctic Oscillation Index was significantly correlated with otolith growth, but due to the number of parameters incorporated in this index, determining a potential mechanism for this correlation is difficult. At the local level, temperature was positively correlated with first year growth. Temperature is known to increase metabolic rate and growth in a multitude of fish species including salmonids, provided there is an adequate food supply (Neilson and Geen 1985, Rogers and Ruggerone 1993, LeBreton and Beamish 2000).

The speed of east wind, but not the proportion of east wind, correlated with first year cisco growth. Previous research has found that the proportion of east wind impacts

recruitment to the Colville River (Gallaway et al. 1983). However, the lack of correlation with growth suggesting that the proportion of east wind does not impact growth or condition.

The strongest correlation with the first year growth was with river flow lagged two years. The lag in this correlation suggested that river flow influenced cisco growth but the process was biologically mediated through lower trophic levels. Dissolved organic carbon (DOC) provides the basis of the food web and levels are positively correlated with river discharge (Largier 1993, Holmes et al. 2008). Arctic cisco juveniles feed predominately on copepods and juvenile mysids that graze phytoplankton which are capable of utilizing DOC (Knutzen et al. 1990). Each of these trophic links requires a particular amount of time and together may explain the two year time lag present between river discharge and cisco growth. Zooplankton surveys in the Prudhoe Bay region found that available copepod species for Arctic cisco prey included the *Acartia spp.*, *Pseudocalanus minutus*, *Calanus glacialis* and *C. hyperborelis* (Horner et al. 1974). For *C. glacialis* the life cycle has been recorded as one or two years depending on location and at least two to four years for *C. hyperboreus* (Hirche and Bohrer 1987, Prokopowicz and Fortier 2002). The dominate mysid in the region is *Mysis relicata* (Knutzen et al. 1990). *M. relicata* also has a life cycle of two years (Kjellberg et al. 1991, Chess and Stanford 1998). Even in temperate climates, time lags have been documented between river flow rates and animal growth. In the Mediterranean Sea, polychaete growth correlated with discharge of the Rhône River with time lags of two months to three years depending on the polychaete species (Salen-Picard et al. 2002). While variation in prey species composition, life cycle length and the stage that cisco consume prey are all important factors that would assist in determining how long of a time lag we would anticipated between changes river flow and cisco growth, these finding suggest that the presence of the significant trend between river flow and cisco growth lagged two years provides evidence of a bottom-up biological control on growth.

At this point in our study we have provided evidence to support the relationship between otolith radius and fish length. We have also documented year to year changes in the pattern of growth and the growth rates for the first seven annuli. Finally, we have found evidence of physical and biological controls on the first year growth of juvenile

Arctic Cisco. The results of this study confirm that the use of otoliths is a valuable tool to address the questions surrounding differences in Arctic cisco over time.

We are continuing to analyze samples, including more than 100 otoliths collected during the 2007 spawning season from Mackenzie River tributaries, the Peel and Arctic Red Rivers, and more than 130 otoliths collected from the subsistence fishery in Nuiqsut, Alaska during fall 2007. Growth information from these samples will be used to update the analyses reported here. In addition we will be expanding our analyses to more thoroughly investigate the pattern of growth in older annuli, possible environmental controls during later ages, and multivariate analyses. Finally growth data and environmental covariates will be statistically compared with subsistence catch data to investigate the possibility of building predictive catch models for Arctic cisco.

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