Alaska Fisheries Technical Report

Number 48 September 1998

Genetic Assessment of Inconnu (*Stenodus leucichthys*) from the Selawik and Kobuk Rivers, Alaska, using PCR and RFLP Analyses

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Genetic Assessment of Inconnu (*Stenodus leucichthys*) from the Selawik and Kobuk Rivers, Alaska, Using PCR and RFLP Analyses

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Abstract: Population structure of inconnu in the Kobuk and Selawik rivers in western Alaska was assessed using genetic methods. Collections from each of the rivers were performed in 1993 and 1994 in conjunction with an inconnu tagging study. Nonlethal sampling methods were used to collect samples of boney fin and scales (with epithelium attached) for DNA extraction. The polymerase chain reaction (PCR) was used to amplify three segments of the mitochondrial DNA (mtDNA) and one segment of the nuclear DNA (nucDNA). Restriction site analysis was used to assess genetic variation among individual fish for the cytochrome-B segment of the mtDNA and the growth hormone-1 segment of the nucDNA. Frequencies of mtDNA genotypes were significantly different between the Kobuk and Selawik collections. A total of four mtDNA genotypes were observed; all four occurred in the Selawik collections and two were in the Kobuk collections. The two mtDNA genotypes that were unique to the Selawik collections occurred at ≤8% each. Three nucDNA genotypes composed of two alleles occurred in both the Kobuk and Selawik collections at significantly different frequencies. Both the mtDNA and nucDNA revealed similar stock relationships and indicated that the Kobuk and Selawik rivers each support different stocks of inconnu that do not routinely interbreed. Further support for different stocks came from a multiyear tagging study showing that fish tagged in one river were not recaptured in the other river. Inconnu of the region support sport, commercial, and subsistence fisheries and have significant cultural importance. Our results can be used to address concerns about the overharvest of the smaller Selawik stock in mixed stock winter fisheries and to help formulate management plans to achieve harvest and conservation goals.

Introduction

Inconnu (*Stenodus leucichthys*) of northwest Alaska support culturally important fisheries and contribute to ecosystem vitality. In 1980, Congress recognized the importance of inconnu, also known as sheefish, in the Alaska National Interest Lands Conservation Act by specifically naming the species as one to be conserved as part of the formation of the Selawik National Wildlife Refuge. Refuge lands are managed to conserve fish and wildlife populations and maintain their natural diversity.

From a geological perspective, the inconnu is a recent addition to Alaskan fauna (Morrow 1980). Eight stock assemblages are currently recognized in major western Alaskan rivers and tributaries, with evidence suggesting that the range of inconnu is expanding (Alt 1987). Some life history forms are anadromous while others are considered freshwater residents. Inconnu of the Kobuk and Selawik rivers (Figure 1) rear in the esturine habitats of Selawik Lake, Hotham Inlet, and Kotzebue Sound and ascend the Kobuk and Selawik rivers to spawn (Alt 1987). The Kobuk and Selawik rivers contain the largest inconnu in Alaska with fish reaching 24 kg and living to 20 years of age (Alt 1987).

The Kobuk River population of spawners is about eight times larger than that of the Selawik River. Abundance estimates from 1996 indicated the Kobuk River supported 43,036 spawners (Taube 1996), while the Selawik River was at 5,157 (Underwood et al. 1998).

Inconnu support subsistence, sport, and commercial fisheries in the Kotzebue Sound region. Fishermen from villages in the region (e.g., Ambler,

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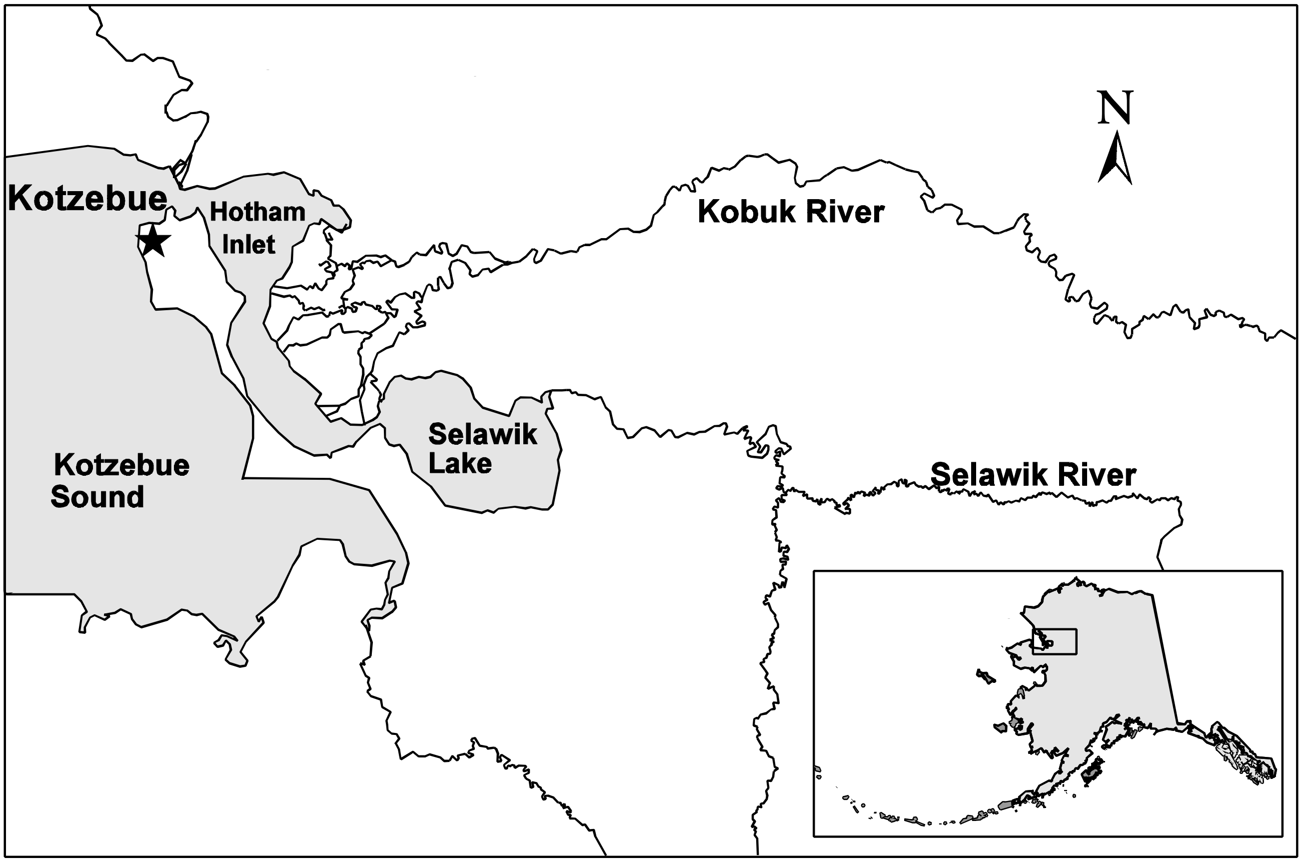


Figure 1. Map showing the hydrography of the study area including the Kobuk and Selawik rivers.

Buckland, Deering, Kiana, Kivalina, Kobuk, Kotzebue, Noatak, Noorvik, and Selawik) harvest inconnu during all seasons of the year. Annual subsistence harvests have been as high as 31,292 fish in the 1960s and have ranged from 2,180–6,651 fish during 1981–1991 (Lean et al. 1993). Annual sport catches have ranged from 403–3,678 fish with annual harvests ranging from 150–1,904 fish for 1990–1994 (Howe et al. 1995). A small commercial fishery has annually harvested from 26–2,600 fish during 1981–1991 (Lean et al. 1993).

The inconnu of the Kobuk and Selawik rivers are currently managed as a single stock; however, in a literature review, Alt (1987) speculated that multiple stocks may share the Selawik Lake overwintering area. The possible mixed-stock nature of the winter fishery raises concerns about possible overharvest of weaker stocks such as the apparently smaller Selawik stock.

Clearly, knowledge of population structure and stock relationships is important in formulating management plans to achieve harvest and conservation goals. Managers need to know whether inconnu of the Kobuk and Selawik rivers represent one big stock or multiple, smaller stocks. Further, if multiple, smaller stocks are present, managers also need to know the number of stocks and their boundaries. For example, if the inconnu of the Kobuk and Selawik rivers represented one big stock, overharvest in one river might be compensated by recolonization from the other river, with relatively rapid recovery and no loss of genetic diversity. On the other hand, if each river supported a different stock, that same overharvest might result in a long-term loss of production, slower recovery, and loss of genetic diversity.

Our objective was to use genetic methods to determine if there was evidence of population structure of inconnu in the Selawik and Kobuk rivers, i.e., whether there was one big stock or multiple stocks. This study was designed to establish an initial foundation of biological information from which more in-depth studies can be launched and to chart a course for further work.

Methods

Scales (with epithelial tissue attached) or pectoral fin clips were collected from inconnu of the Selawik and Kobuk rivers and stored in individually numbered vials with 70% ethanol until processed (Table 1). Inconnu fin samples from the Arctic Red River in the Mackenzie River drainage, Northwest Territories, were used as an outgroup (N=10; 10/12/93).

Table 1. Collection summary.

River	Date	N
1993		
Kobuk	9/4–10	115
Selawik	9/11–16	76
1994		
Kobuk	9/21–22	100
Selawik	8/3–16	68

Nucleic acids were extracted from about 25 mg of tissue incubated in 500 µL of STE buffer (0.1 M NaCl, 10 mM Tris [pH 8.0]–HCl, 1 mM EDTA), 50 µL 10% SDS, and 25 µL of proteinase–K (10 mg•mL^{-1}) at 65ºC for ≥60 minutes. Ammonium acetate (250 µL, 7.5 M at 4ºC) was then added and the samples incubated on ice for 60 minutes, centrifuged at 9000 X g for 10 minutes, and 500 µL of supernatant transferred to new tubes. Ethanol (1 mL, 95%) was added to the supernatant to precipitate the nucleic acids. The DNA pellets were washed with 70% ethanol, air–dried, and dissolved in 100 ul TE buffer (10 mM Tris [pH 7.6], 1 mM EDTA). DNA samples were electrophoresed in 0.8% agarose gels cast in TBE buffer (Sambrook et al. 1989), stained with ethidium bromide, and photographed with Polaroid® film on an ultra–violet light table.

One nuclear DNA (nucDNA) and three mitochondrial DNA (mtDNA) segments, were amplified using the polymerase chain reaction (PCR) with the following primers:

growth hormone 1* (*GH1**; nucDNA)
5'–ATCGTGAGCCCAGTCGACAAGCAC–3'
5'–GGGTACTCCCAGGATTCAATCAGA–3'

cytochrome B* (*cyt*B*; mtDNA)
5'–GAAAAACCA(CT)CGTTGT(TA)ATTCAACT–3'
5'–GAGCTACTAGGGCAGGCTCA–3'

*Dloop** (mtDNA)
5'–TACACTGGTCTTGTAAACC–3'
5'–TTGGGTTTCTCGTATGACCG–3'

NADH dehydrogenase 1* (*ND1**; mtDNA)
5'–ACCCCGCCTGTTTACCAAAAACAT–3'
5'–GGTATGAGCCCGATAGCTTA–3'

Previous assessments of these segments of DNA in rainbow trout, chinook, chum, sockeye, and coho salmon had revealed variation (Cronin et al. 1993; Fobes et al. 1993; Patton 1993). mtDNA primers were complimentary to conserved tRNA and rRNA sequences which flank the amplified fragment (Cronin et al. 1993). *GH1** primers were complimentary to sequences in the coding region flanking the *GH1** non–coding intron region (Fobes et al. 1993).

Each PCR reaction was composed of 0.1–0.5 µg of genomic DNA, 5 µL of 10X buffer (0.1 M tris–HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl, 1 µg• µL^{-1} bovine serum albumin), 5 µL of dNTP mix (2 mM each of dATP, dTTP, dCTP and dGTP in 10 mM Tris–HCl, pH 8.0), 1 µL of a 10 µM solution of each of two primers, and 2.0 units of *Taq* polymerase, with deionized water added for a final volume of 50 µL. The amplification cycle for mtDNA fragments consisted of 95ºC for 45 seconds, 50ºC for 30 seconds, and 70ºC for 2 minutes and 30 seconds, cycled 32 times. The amplification for the *GH1** segment consisted of 92ºC for 60 seconds, 62ºC for 60 seconds, and 72ºC for 1 minute and 30 seconds with this portion of the cycle extended one additional second for each of the 34 cycles. PCR products were electrophoresed on 1.4% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light.

A pilot screening was initially conducted to evaluate the performance of the primers and optimize PCR conditions for each DNA segment. *GH1**, *cyt*B*, and *ND1** could be consistently amplified. *Dloop** amplified consistently with the DNA extracted from fin, but inconsistently with DNA extracted from scale epithelium and was excluded from further analyses.

To initially identify different genotypes or polymorphisms, each DNA segment was screened with 30 restriction enzymes (RE) for 20 samples from the Alaska collections and the 10 samples from the Mackenzie outgroup (Appendix 1). Each RE recognized a unique sequence of four to six bases and cuts the DNA at that site. For example, if the *GH1** segment from one fish had two restriction sites and another fish had only one restriction site, those fish had different genotypes. Polymorphisms were only detected in the *GH1** and *cyt*B* segments, so these were then used with the remaining samples. RE digests were electrophoretically separated on 2.5% agarose gels, stained with ethidium bromide, and photographed.

Sizes of restriction fragments were estimated by comparison to a 100 kilobase (KB) ladder and a Phi standard (lambda c1857 Sam 7 DNA digested with *Hin*d III mixed with Phi X–174 DNA digested with *Hae* III; Appendices 2 and 3). Restriction fragment patterns were visually identified from gels and photographs. *cyt*B* composite genotypes and *GH 1** genotypes were defined from the restriction fragment patterns for the segment–RE combinations (Lansman et al. 1981).

Goodness-of-fit tests using the χ^2 statistic (Richardson et al. 1986) were used to evaluate Hardy–Weinberg (HW) equilibrium for *GH1** genotype frequencies for each collection using BIOSYS (Swofford and Selander 1989). This was done to test the assumption that each collection represented a single stock rather than a mixture of different stocks. Significant tests (P<0.05) would indicate excessive deviation from the genotypic proportions expected under HW law.

Hierarchical tests of heterogeneity using the log likelihood ratio statistic (G; Sokal and Rohlf 1981) were used to assess differentiation of allele frequencies among collections. Frequencies were considered significantly different if P<0.05. Collections between years and within rivers were tested to determine if pooling data across years and within rivers was feasible.

Genetic relatedness of stocks within and among rivers was illustrated with neighbor-joining (Saitou and Nei 1987) cluster analysis of matrices of pairwise Cavalli-Sforza and Edwards (1967) chord genetic distances.

Results

Collection and Processing

The nonlethal techniques for collecting scales and fin were simple and easily applied in the field. Scale epithelium and fin both yielded adequate amounts of DNA; however, DNA from fin amplified more consistently with all primer sets. Other extraction techniques, such as phenol–chloroform, may yield better quality DNA from scale epithelium than the ammonium acetate method; however, further tests are needed to confirm that. We originally used the ammonium acetate protocol for DNA extractions because of its simplicity and lack of hazardous waste. The 70% ethanol worked well for tissue preservation and storage.

Polymorphisms

The mtDNA *cyt*B* segment was about 1150 bases long. Two of 30 REs revealed genetic variation among individuals; three genotypes with *Dde* I and two with *Mse* I (Appendix 2). These individual genotypes occurred in four different combinations to form composite genotypes AA, AB, BA, and CA (Table 2) where the first letter of the composite genotype is the genotype for *Dde* I and the second letter is for *Mse* I.

The mtDNA *ND1** segment was about 2000 bases long. None of the 30 REs revealed variation among individuals, i.e., only one genotype was observed for each of those REs.

The nucDNA *GH1** segment was about 700 bases long. Of 30 REs tested, one revealed variation in the form of two alleles, A and B. These two alleles combined to form three different genotypes; the homozygotes AA and BB, and the heterozygote AB (Table 2; Appendix 2).

mtDNA Analyses

Selawik and Kobuk shared the common genotype AA and the rarer BA (Table 2). Genotypes AB and CA occurred in the Selawik but did not occur in the Kobuk. The outgroup Mackenzie had only the CA genotype.

Hierarchical tests of heterogeneity of the *cyt*B* genotype distributions showed significant differences among the four collections from the Selawik and Kobuk rivers (Table 3). Most of the total G (30.6) was contributed by the differences between the Selawik and Kobuk collections (21.3 or 70%) with the balance (9.3 or 30%) attributed to differences between years (Table 3). The Selawik 1993 and 1994 collections were also different due to the presence of the CA genotype in 1993 but not 1994 (Tables 2 and 3).

nucDNA Analyses

The Selawik and Kobuk collections contained both alleles while the Mackenzie sample contained

Table 2. Proportions of growth hormone (*GH1* *) genotypes and alleles, and cytochrome B (*cyt*B *) genotypes for inconnu from the Selawik and Kobuk rivers, and an outgroup from the Mackenzie River, Canada.

		GH1 * (nucDNA)					*cyt*B * (mtDNA)			
		Genotype			Allele		Genotype			
Year	N	AA	AB	BB	A	B	AA	AB	BA	CA
Selawik R.										
1993	76	0.68	0.28	0.04	0.82	0.18	0.83	0.03	0.07	0.08
1994	65	0.72	0.26	0.02	0.85	0.15	0.85	0.06	0.09	—
Pooled	141	0.70	0.27	0.03	0.84	0.16	0.84	0.04	0.08	0.04
Kobuk R.										
1993	86	0.44	0.45	0.10	0.67	0.33	0.95	—	0.05	—
1994	97	0.60	0.37	0.03	0.78	0.22	0.93	—	0.07	—
Pooled	183	0.52	0.41	0.07	0.73	0.27	0.94	—	0.06	—
Mackenzie R.										
1993	10	1.00	—	—	1.00	—	—	—	—	1.00

Table 3. Results of hierarchical tests of heterogeneity using the log likelihood ratio statistic (*G*; Sokal and Rohlf 1981). The sum of *G* values from tests of within and pooled variation equal the total *G* value for the test of variation among all four collections, e.g., 17.4=0.5+6.1+10.8.

			*cyt*B *	
Test		*GH1* *	All Genotypes	CA Genotype Dropped
Among Selawik R and Kobuk R. (all four collections)	*G*	17.4	30.6	12.8
	df	3	9	6
	P	0.001***	0.001***	0.046*
Within Selawik R. (1993 vs. 1994)	*G*	0.5	8.8	1.1
	df	1	3	2
	P	0.475	0.032*	0.569
Within Kobuk R. (1993 vs. 1994)	*G*	6.1	0.5	0.5
	df	1	1	1
	P	0.014**	0.463	0.463
Between Selawik R. pooled and Kobuk R. pooled	*G*	10.8	21.3	11.2
	df	1	3	2
	P	0.001***	0.001***	0.004**

only allele A. Genotype proportions were not different from those expected under Hardy–Weinberg law (Table 4) indicating that each collection appeared to represent a single stock and not a mixture of multiple stocks.

Table 4. Results of goodness-of-fit tests for deviations of nucDNA genotype proportions from Hardy–Weinberg law.

	χ^2	df	*P*
Selawik 1993	0.223	1	0.636
Selawik 1994	0.149	1	0.699
Selawik pooled	0.023	1	0.878
Kobuk 1993	0.047	1	0.829
Kobuk 1994	0.857	1	0.355
Kobuk pooled	0.271	1	0.603

Hierarchical tests of heterogeneity for the alleles indicated highly significant differences among the four collections from the Selawik and Kobuk rivers ($P \leq 0.001$; Table 3). Only a small amount of the total *G* value (17.4) was attributable to differences between years in the same river (6.6 or 38%). Most (62%) of the total *G* was contributed by differences between rivers (Table 3). There was a significant difference (P=0.014) between 1993 and 1994 Kobuk collections.

Genetic Distance

Genetic relationships based on genetic distance were similar for *cyt*B* and *GH1* *, showing the Kobuk collections most similar with each other and different from the Selawik collections (Figure 2). The Mackenzie collection showed the greatest divergence from the Alaska collections.

Discussion

For management purposes it is important to understand the dynamics of inconnu life history in order to determine which model best describes population structure and stock relationships. The nature of the population structure could determine which management strategy is best. For example, if separate stocks exist then unique density–dependent and –independent factors could influence each stock to yield stock–specific population dynamics. This model would require stock–specific management regimes. Overharvest of one stock could mean the loss of a unique gene pool and a long period of time to rebuild numbers or for recolonization from other stocks.

Alternatively, if a single stock exists instead of multiple stocks, that indicates fish movement and gene flow across a broad geographic area to yield a large genetically homogenous stock. Hypothetically, a single management regime could be applied across a broad area. Overharvest in one portion of the area could be compensated by rapid recolonization from other portions of the area by individuals that are genetically similar to those harvested.

In most cases it is a combination of simple population models that best describes population structure. For example, population structure of chinook salmon in the Yukon River drainage can be described by a subpopulation model nested within an isolation–by–distance model (Wilmot et al. 1992). Imprinting and homing to natal spawning grounds result in the maintenance of subpopulations or stocks, but with straying or gene flow highest among nearby stocks and lowest with distant stocks.

Patterns of genetic differentiation can reflect current gene flow, historic events, or a combination of influences and permit determination of an appropriate population model. For example, complete reproductive isolation between two stocks can permit genetic drift, mutation, and natural selection to act independently within each of the stocks and result in genetic differences occurring over time. However, reproductive isolation does not necessarily ensure that genetic differences will be seen for several reasons. First, genetic drift is a random effect that can result in divergence or convergence of allele frequencies. Second, natural selection may demand the presence of certain alleles in certain combinations. Third, we are analyzing a small portion of the genome and thus genetic differences may not be observed when they really exist.

Do the Selawik and Kobuk collections represent different stocks? The results of this study demonstrated genetic differences between collections from the Selawik and Kobuk drainages. Based on our data, these drainages support different stocks. Supporting evidence of different stocks includes the presence of two genotypes in the Selawik collections that did not occur in the Kobuk. *cyt*B* genotypes AB and CA were unique to the Selawik collections, though they occurred at low frequencies. The presence of some unshared genotypes in collections can be strong evidence of low gene flow. However, since those genotypes were relatively uncommon in the Selawik collections they could be present but rare in Kobuk inconnu pointing to the possibility that the collections were not large enough to detect the presence of those mtDNA genotypes. For example, the CA genotype that occurred in the Selawik 1993 collection did not occur in the 1994 collection. Thus, the presence of unshared genotypes suggests low gene flow and population structure, but in this case it is not conclusive.

Additional evidence of different stocks includes the different *GH1* * allele frequencies between

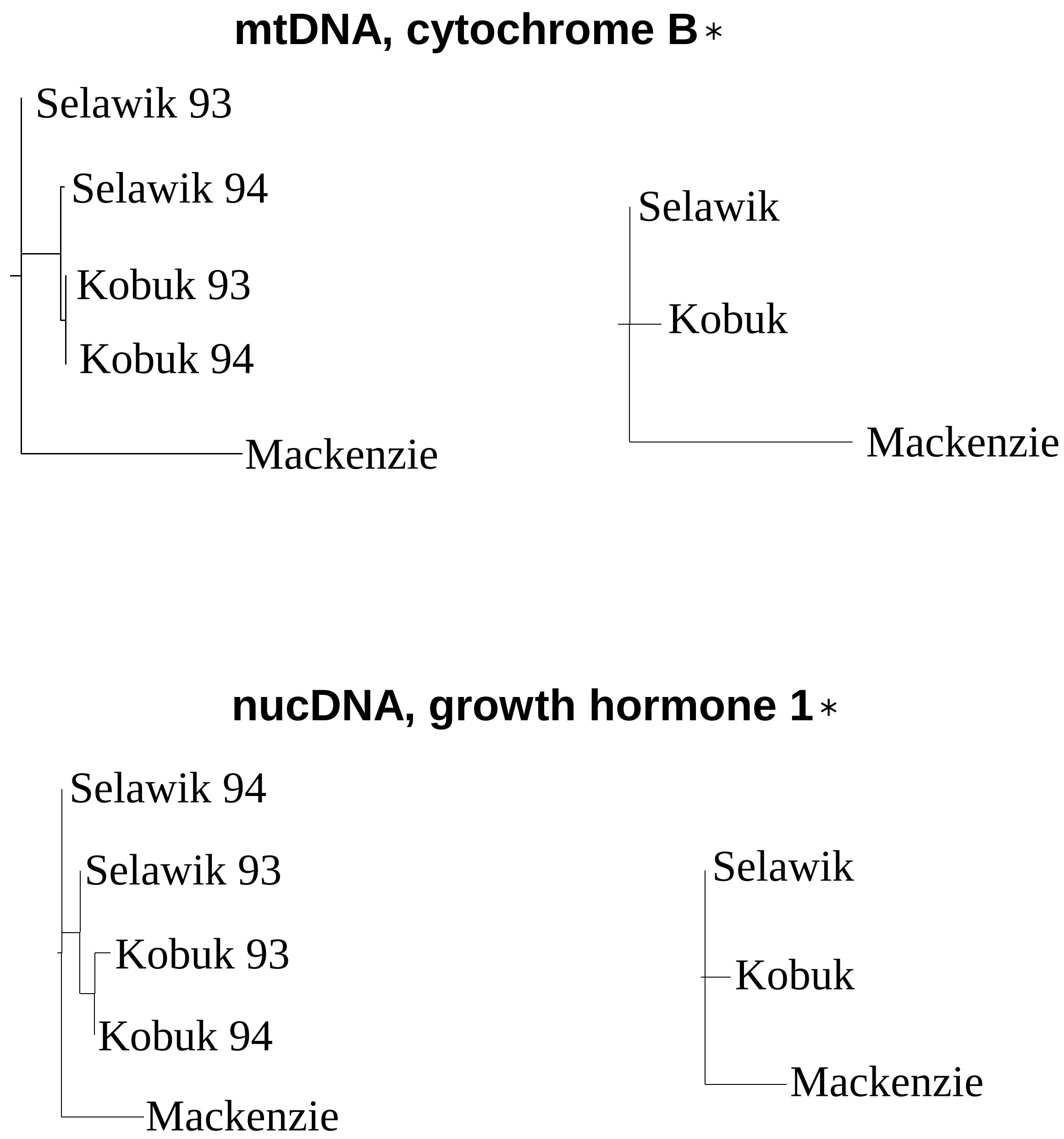


Figure 2. Neighbor–joining (Saitou and Nei 1987) trees of genetic distances (Cavalli–Sforza and Edwards 1967 chord distance) for cytochrome B* and growth hormone 1* showing the results with the collections unpooled and pooled by river.

the Selawik and Kobuk collections. Frequencies for allele A were consistently higher in the Selawik than the Kobuk collections. If no population structure existed and the inconnu of those rivers represented a single stock, we would expect all four collections to have similar genetic profiles for both *GH1* * and *cyt*B *. Unexpected were the different *GH1* * allele frequencies between the 1993 and 1994 Kobuk collections, while *cyt*B * genotype frequencies remained similar between years. *GH1* * allele frequencies for the 1993 and 1994 Selawik collections were similar with each other. These results suggested that there may be other factors associated with inconnu life history that we did not account for in our sampling design. For example, the Kobuk R. is a large drainage that may support multiple stocks that have different genetic profiles; hence, our 1993 collection may have represented a different stock mixture than the 1994 collection. The tests of Hardy-Weinberg equilibrium indicated that the Kobuk collections represented single stocks; however, that test is not sensitive enough to detect low level deviations from equilibrium. The genetic differences we observed between the Selawik and Kobuk drainages were large enough to imply that some degree of fidelity to spawning grounds exists which would restrict gene flow between drainages.

Our genetic results were supported by results from recent tagging studies. In 1993-1996, Underwood et al. (1998) tagged 1,314 spawning inconnu in the Selawik River. In 1994-1995, Taube et al. (1996) tagged 1,995 fish in the Kobuk River. Tagged fish from one river were not caught in the other river indicating that fish return to the river where they had previously spawned. In contrast, fish from both rivers have been caught in the sloughs, lakes and bays of Hotham Inlet outside of spawning season (Taube 1996). This means that inconnu from the two rivers appear to mix during a part of the year, yet segregate for spawning.

Why weren't the genetic differences greater? The relative similarities between the Selawik and Kobuk collections, in contrast to their differences from the Mackenzie fish, could reflect recent common ancestry, suggesting that the rivers were colonized from a common source. That could explain similarities such as shared genotypes and alleles. Current gene flow between drainages, such as Selawik origin fish successfully reproducing with Kobuk origin fish, may be keeping the stocks genetically similar. However, if there is gene flow it is not great enough to offset the effects of those forces that create or maintain differences, such as fidelity to natal spawning grounds.

One time historic events like the colonization of a newly available drainage can lead to genetic differentiation if the founding group is so small that it does not genetically represent the parent stock. The absence of two *cyt*B * genotypes in the Kobuk collections could indicate that the Kobuk stock originally arose from the Selawik stock as a small founder population. Due to natural sampling error, frequencies of *GH1* * alleles and *cyt*B * genotypes in the founding population could have been different from the parent population. Fidelity to natal spawning grounds (i.e., restricted gene flow) would act to maintain the original differences.

What should be done to better characterize the stock relationships? The interannual differences observed for the Kobuk collections suggest the possibility of more than one stock occurring in that drainage and warrant further investigation. Genetic comparisons of collections taken on a finer geographic scale could help to determine the number of stocks within each drainage.

Additional genetic markers (especially in the nucDNA) should be applied to improve the genetic profiles of the Selawik and Kobuk stocks. Additional markers could yield a clearer picture of the nature of the population structure in that region. Better genetic characterization of stocks would permit the application of stock composition estimation methods to address issues in fisheries and overwintering areas where the stocks intermingle.

What do the results mean to fisheries managers? The application of these genetic results can help managers formulate management plans that maximize harvests at sustainable levels and meet stock conservation goals. Managers will need to manage the inconnu of the Kobuk and Selawik rivers as separate stocks instead of a single large stock. Because different inconnu stocks occur in the Kobuk and Selawik rivers, different levels of harvest may need to be established for each stock based on its surplus production. For example, the less abundant Selawik stock would sustain smaller harvests than the more abundant Kobuk stock.

Establishing stock-specific harvests for in-river fisheries, where the stocks are geographically separate, would be relatively straightforward; however, establishing stock-specific harvests for the mixed stock fishery in Hotham Inlet area would present a greater challenge. Harvests based on the total abundance of the combined Kobuk and Selawik stocks could lead to overharvest of the less abundant Selawik stock. Because the Kobuk and Selawik rivers support different stocks instead of a single, large stock, overharvest of one stock could have long-lasting effects, including depressed production and lost genetic diversity, plus in an extreme case, extinction of the weaker stock. To help prevent overharvest, genetic assessment of the mixed stock fishery could yield valuable information regarding when and where the different stocks mix and how they contribute to the fishery.

To reach the point where genetic assessment of the mixed stock fishery is feasible, additional genetic markers would first have to be tested and applied. Then the baseline, consisting of the genetic profiles of the stocks, would be tested to determine its ability to estimate stock composition in simulated stock mixtures using computer simulations. The genetic baseline would then be ready to apply in mixed stock fishery assessments.

Until more information is available, the mixed stock fishery should be conservatively managed to avoid overharvest of the less abundant Selawik stock. Further, to ensure stocks remain viable and productive, assessments should be performed routinely to monitor genetics, abundance, age-class structure, sex ratios, recruitment, harvests, and other indicators of population well-being.

Conclusions

Tissue to support PCR–RFLP analysis can be collected using nonlethal methods, with storage and preservation requirements that are far simpler and less rigorous than those required for protein analyses.

Both the mtDNA and nucDNA can be accessed for inconnu using PCR methodology that was originally developed with rainbow trout, sockeye, coho, chum, and chinook salmon. RFLP analysis of PCR products was effective for identifying genetic variation among Kobuk, Selawik and Mackenzie river samples.

Our data indicate the Selawik and Kobuk drainages support different inconnu stocks. However, additional genetic markers should be applied to fully characterize the population structure and develop the baseline data necessary to perform mixed stock analysis of the fishery.

Recommendations

1) Apply a suite of at least 12 nucDNA markers to further characterize population structure.
2) Collect additional samples to test the hypothesis that multiple stocks occur in the Kobuk River.
3) Conduct simulations to determine the performance characteristics of the genetic baseline for application in mixed–stock assessments.
4) Extend the investigation to other drainages of interest.

Acknowledgments

J. D. Reist (Research Scientist and Section Head, Arctic Fish Ecology & Assessment Research Section, Department of Fisheries and Oceans [DFO], Canada) kindly provided the fin samples from the Arctic Red River in the Mackenzie River drainage, Northwest Territories. The staff of the U.S. Fish and Wildlife Service (USFWS) Fairbanks Fishery Resources Office was instrumental in accomplishing field collections in support of the genetics work. We appreciate the hard work of M. Millard (USFWS), R. Ramouth (USFWS), R. Simmons (USFWS), and K. Whitten (USFWS) who composed the genetics sampling crew. The cooperation of B. Armstrong (USFWS), S.E. Barr (Kobuk), E. Ward (Kobuk), and R. Ward (Kobuk) for field collections was indispensable and much appreciated. Julee Beasley (USFWS) provided crucial graphics support. The following reviewers provided valuable recommendations that improved the quality of this report: B. Armstrong (USFWS), J. Berg (USFWS), W. Buchholz (USFWS), A. Chatto (USFWS), J. Finn (U.S. Geological Survey-Biological Resources Division), C. Krueger (Cornell University), and J.D. Reist (DFO).

Literature Cited

Alt, K. T. 1987. Review of inconnu *Stenodus leucichthys* studies in Alaska. Alaska Department of Fish and Game, Fishery Manuscript Number 3, Juneau, Alaska.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Cronin, M.A., W.J. Spearman, R.L. Wilmot, J.C. Patton, and J.W. Bickham. 1993. Mitochondrial DNA variation in chinook salmon (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Canadian Journal of Fisheries and Aquatic Sciences 50:708–715.

Fobes, S., K. Knudsen, F. Allendorf. 1993. Genetic variation in DNA of coho salmon from the lower Columbia River. Final Report. Division of Biological Sciences, University of Montana, Missoula, MT 59812.

Howe, A.L., G. Fidler, and M.J. Mills. 1995. Harvest catch and participation in Alaska sport fisheries during 1994. Alaska Department of Fish and Game. Fisheries Data Series No. 95–24, Anchorage.

Lansman, R.A., R.O. Shade, J.F. Shapira, and J.C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. Journal of Molecular Evolution 17:214–226.

Lean, C.F., F.J. Bue, and T.L. Lingnau. 1993. Annual management report, 1989, 1990, 1991, Norton Sound – Port Clarence – Kotzebue. Regional Information Report No. 3A93–5, Alaska Department of Fish and Game, Division of Commercial Fish, Anchorage.

Morrow, J. E. 1980. The freshwater fishes of Alaska. Alaska Northwestern Publishing Company, Anchorage.

Patton, J. 1993. Analysis of macro– and microgeographic variation in sockeye salmon (*Oncorhynchus nerka*) using restriction site and sequence analysis of mtDNA. Report. LGL Ecological Genetics, Inc. 1410 Cavitt Street, Bryan, TX 77801.

Richardson, B. J., P. R. Baverstock, and M. Adams. 1986. Allozyme electrophoresis. A handbook of animal systematics and population studies. Academic Press, San Diego California.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biological Evolution 4:406-425.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning, a laboratory manual. 2nd ed. Cold Spring Harbor, NY.

Sokal, R. R. and R. J. Rohlf. 1981. Biometry. 2nd edition, W. H. Freeman, San Francisco, California.

Swofford, D. L., and R. B. Selander. 1989. BIOSYS–1, Release 1.7. Illinois Natural History Survey, Champaign, Illinois.

Taube, T.T. 1996. The abundance and composition of sheefish in the Kobuk River, 1994–1995. Alaska Department of Fish and Game Fisheries Manuscript No. 96–2.

Underwood, T. J., K. Whitten, and K. Secor. In press. Population Characteristics of Spawning Inconnu (sheefish) in the Selawik River, Alaska, 1993-1996, Final Report. U.S. Fish and Wildlife Service, Fairbanks Fishery Resource Office, Alaska Fisheries Technical Report 49, Fairbanks, Alaska.

Wilmot, R.L., R. Everett, W.J. Spearman, and R. Baccus. 1992. Genetic stock identification of Yukon River chum and chinook salmon 1987–1990. Alaska Fish and Wildlife Research Center, USFWS, Progress Report, 132p.

Appendix 1. List of 30 restrictions enzymes (RE) used on inconnu PCR segments categorized by absence or presence of restriction sites.

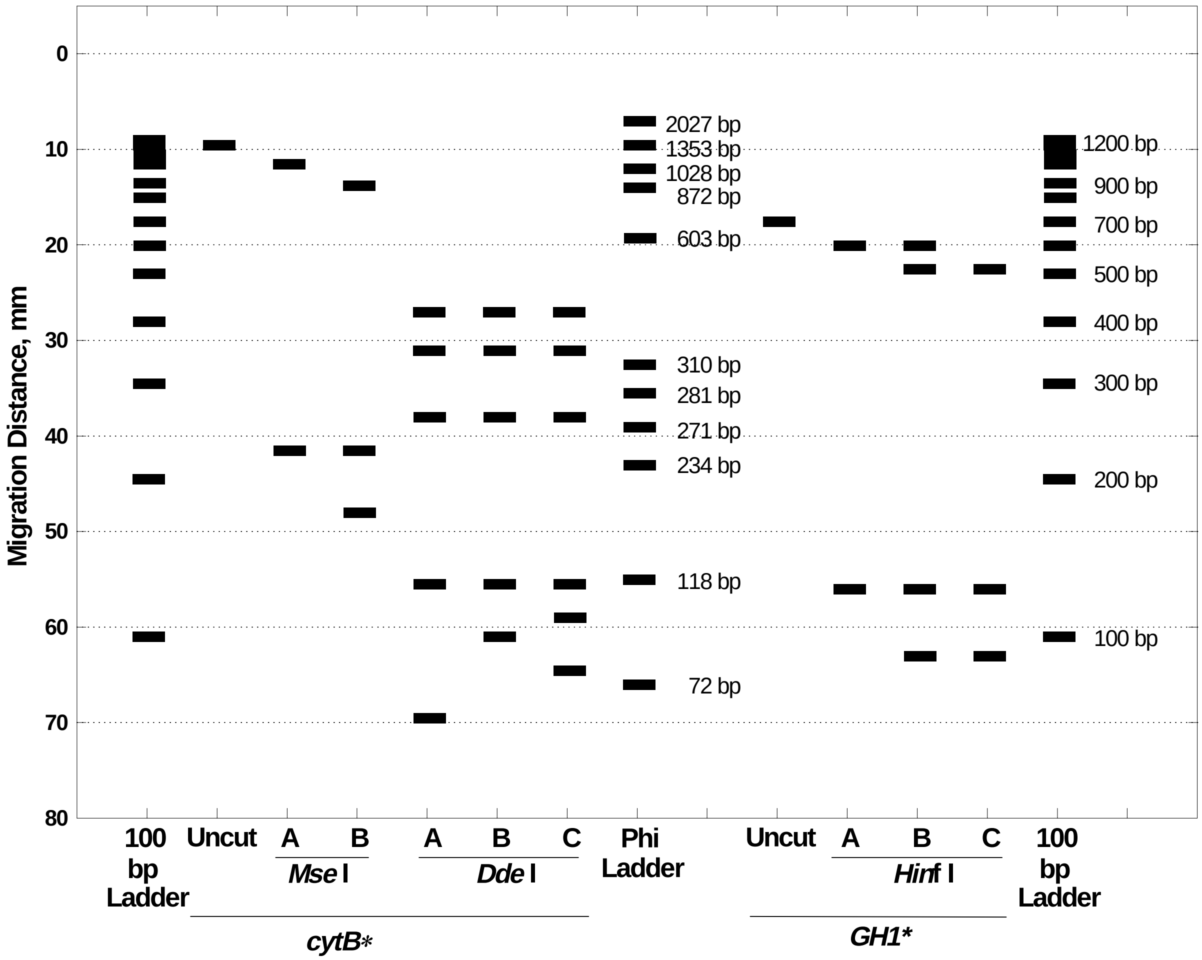
	GH1 *		*ND1* *		*cyt*B *	
All RE	Absent	Present	Absent	Present	Absent	Present
Aci I	*Aci* I	*Alu* I	*Bst*E II	*Aci* I	*Ase* I	*Aci* I
Alu I	*Ava* I	*Ase* I	*Dpn* I	*Alu* I	*Bst*U I	*Alu* I
Ase I	*Ava* II	*Bfa* I	*Dra* I	*Ase* I	*Dpn* I	*Ava* I
Ava I	*Bst*E II	*Bst*N I	*Eco*R I	*Ava* I	*Dra* I	*Ava* II
Ava II	*Bst*U I	*Dde* I	*Eco*R V	*Ava* II	*Eco*R I	*Bfa* I
Bfa I	*Dpn* I	*Hae* III		*Bfa* I	*Eco*R V	*Bst*E II
*Bst*E II	*Dpn* II	*Hin*d III		*Bst*U I	*Hin*d III	*Bst*N I
*Bst*U I	*Dra* I	*Hin*f I [1]		*Bst*N I	*Nde* II	*Dde* I1[1]
*Bst*N I	*Eco*R I	*Hpa* II		*Dde* I	*Pst* I	*Dpn* II
Dde I	*Eco*R V	*Mse* I		*Dpn* II		*Hae* III
Dpn I	*Hha* I	*Msp* I		*Hae* III		*Hha* I
Dpn II	*Nde* II	*Rsa* I		*Hha* I		*Hin*f I
Dra I	*Pst* I	*Scr*F I		*Hin*d III		*Hpa* II
*Eco*R I	*Pvu* II	*Sty* I		*Hin*f I		*Mse* I[1]
*Eco*R V	*Sau*96 I	*Taq* I		*Hpa* II		*Msp* I
Hae III				*Mse* I		*Pvu* II
Hha I				*Msp* I		*Rsa* I
*Hin*d III				*Nde* II		*Sau*96 I
*Hin*f I				*Pst* I		*Scr*F I
Hpa II				*Pvu* II		*Sty* I
Mse I				*Rsa* I		*Taq* I
Msp I				*Sau*96 I		
Nde II				*Scr*F I		
Pst I				*Sty* I		
Pvu II				*Taq* I		
Rsa I						
*Sau*96 I						
*Scr*F I						
Sty I						
Taq I						

[1]Polymorphisms were detected with these restriction enzymes (see Appendices 2 and 3).

Appendix 2. Inconnu mtDNA and nucDNA restriction fragment patterns for cytochrome B (*cyt*B *) and growth hormone 1 (*GH1* *) For example, digestion of cytochrome B * with *Mse* I yields restriction fragment patterns A and B where A has two fragments that are 913 and 190 bases long and pattern B has three fragments 766, 190, and 152 bases long.

*cyt*B *, mtDNA					*GH1* *, nucDNA				
Restriction Enzyme	Fragment Size	Pattern			Restriction Enzyme	Fragment Size	Pattern		
Dde I	481	A	B	C	*Hinf* I	593	AA[1]	AB	–
	299	A	B	C		502	–	AB	BB[1]
	218	A	B	C		120	AA[1]	AB[1]	BB[1]
	122	A	B	C		98	–	AB	BB[1]
	111	–	–	C					
	106	–	B	–					
	93	–	–	C					
	77	A	–	–					
Mse I	913	A	–	–					
	766	–	B	–					
	190	A	B	–					
	152	–	B	–					

[1]There are two fragments of the same size at this location.



Appendix 3. Schematic of a lineup gel showing the restriction fragment patterns for cytochrome B (*cyt*B*) and growth hormone 1 (*GH1* *) in inconnu. (bp=base pair)