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A SUMMARY OF STOCK IDENTIFICATION RESEARCH ON WHITE STURGEON OF THE COLUMBIA RIVER

Final Report 1985 - 1990

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A SUMMARY OF STOCK IDENTIFICATION RESEARCH ON WHITE STURGEON OF THE COLUMBIA RIVER (1985-1990)

Final Report

January 1985 - July 1991

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ABSTRACT

White sturgeon (Acipenser transmontanus) are a long-lived, primitive fish species which forage primarily along the river bottom of large river systems in the Pacific Northwest. Historically, as an anadromous species, they could distribute downstream to feed in the rich estuary or marine areas and then migrate back up the river to spawn. With the historic river becoming a series of flooded impoundments, sturgeon were denied open river access, but they appear to have been able to adapt to the altered environment. White sturgeon are found throughout the Columbia River and are thought to be successfully reproducing in some of the impoundments. In those reservoirs where little or no reproduction takes place, enhancement hatcheries may be an option for use in rebuilding isolated populations. However, the degree of stock specificity that exists in the Columbia River was unknown and precluded the use of the more abundant lower river fish as a common egg source to repropagate the upper river unless genetic similarity could be demonstrated among sturgeon throughout the river system. To resolve the issue, research was conducted to determine what level of genetic differentiation exists among sturgeon in the Columbia River system, using starch gel electrophoresis to enable a baseline of population genetic structure data to be assembled. A greater diversity in electrophoretic pattern was observed in the lower portions of the river. The bulk of the qualitative variability we noted was consistent throughout all sections of the river. Some specific quantitative differences were apparent between the areas we examined. Interpretation of the results was complicated by the fact that dam construction would tend to isolate and mix stocks by preventing the migration of fish returning upstream.

INTRODUCTION

Section 903(e) of the Fish and Wildlife Program requires research to address the current plight of white sturgeon within the Columbia River, Due to environmental impacts associated with hydroelectric development, a research program @PA project 83-3 16) was undertaken to better understand the relationship of the various subunits making up the Columbia River white sturgeon population.

Columbia River white sturgeon penser transmontanus) have a history of overcxploitation in their major freshwater river habitat. As a long-lived species weighing well over 200 pounds at fist maturation and taking as much as 12 -15 years to become reproductive, sturgeon were extremely vulnerable to the hazards of river development. As an anadromous species, they had the run of the river and could migrate a thousand miles from the headwaters to the estuary to feed, and then return upstream to spawn. Such a life history put them directly in conflict with the developing fishery for the prized chinook salmon on the lower river, and many wcrc killed and discarded as a nuisance to keep them from destroying the large nets. When markets developed for sturgeon, they wcrc subsequently targeted. Harvests reached over 5 million pounds annually in the late 1800s (Craig and Hacker, 1940), resulting in the population crash by the turn of the century. Recovery was slow and greatly thwarted by the construction of hydroelectric dams on the mainstem. Fish passage facilities for salmon weren't used by sturgeon, and populations were increasingly isolated as the dam construction progressed within the river.

During the 1970s renewed exploitation was directed at the sturgeon as the preferred sport species. Salmon fishing on the Columbia was becoming increasingly limited, and sturgeon offered an alternative with a gcncrous bag limit. By 1986 harvest rates exceeded 50,000 fish annually (Hess and King, 1987). primarily from the river below Bonneville Darn, but also from several of the population segments isolated in the reservoirs. In the face of limited access to their historic range and the pressure from the renewed fishery, serious risk of depleting the resource existed.

Although the bulk of reproductively capable fish are protected by regulations that limit harvest based on length classification, recovery of the population to historical levels is confounded by the fact that sturgeon no longer have free run of the river. Hydropower dams have partitioned the river into a series of isolated pools which have severely altered rivet-me habitat and reduced the effectiveness of nahual propagation. These circumstances may encourage management plans to include hatchery releases in areas where natural production isn't self sustaining.

Deciding what stock source to use for enhancement purposes, however, is a difficult issue. How representative present stock distribution is of the pre-dam era is unknown. Dam construction undoubtedly prevented emigrating fish from returning upstream. If genetic differences exist among the reservoir populations, it can be argued that maintenance of that variability is necessary for the health of those stocks. Selection of egg sources for stocking purposes would have to be made with that consideration. Conversely, if populations within the river system were found to demonstrate the same genetic characteristics, fish from the large population below Bonneville could be used for stocking purposes.

This study was undertaken to assess if electrophoretic or taxonomic differences arc present among population segments which would suggest genetic differentiation of stocks. Areas or target sites were chosen based on geographic location and the degree of isolation demonstrated by man-made or natural physical barriers to migration. Genetic population makeup was assembled from data collected by gel elcctrophoresis. Morphomctric characteristics selected for comparison were snout length and dorsal scute number. In the absence of clear gcnelic fixed differences, other species of closely related sturgeon have heen segregated using morphometric and mcristic information (Bailey and Cross, 1954). Snout length and scute count were selected because they were observed to show variability within populations studied. We examined in detail the enzyme systems which are polymorphic for use as evidence against the null hypothesis that all sturgeon within the Columbia River are one nonspecific gcnctic stock regardless of where they reside within the river system. Morphometric data was examined as independent or collaborative evidence that might demonstrate stock differentiation.

1985-1990 Genetic baseline evaluation of Columbia River white sturgeon

Objective: Determine if white sturgeon from various areas differ genetically.

METHODS AND MATERIALS

To assess Columbia Rover white sturgeon for the presence of genetic differences associated with geographic arcas of the river system, the basin was divided into five general regions that were identilied broad enough to allow for adequate sample collection. The regions represented are the lower Columbia River, the mid Columbia River, the upper Columbia River, the Snake River and the Kootenai River (Fig. 1). This allowed examination of the estuary and mid-river reservoirs, an upriver pool and two tributaries, respectively, and represented segments from throughout the distribution range of white sturgeon in the Columbia River.

The lower river from Bonneville to the river mouth and the resultant estuarine area represents the historical free flowing river, a habitat that was used successfully by sturgeon. The fishery in this area sustains higher annual catch rates than any other part of the river system. In this region, all samples were taken from sport-caught fish in the Ilwaco area. The mid-Columbia region has, for the purpose of this study, encompassed the river 'area from the forcbay of Bonneville Dam to the tailrace of McNary Dam. During 1985-86, sampling took place at Three-Mile Canyon, Paterson and Arlington from the Indian fishery. The upper river was identified as that area from Grand Coulee Dam to the U.S./Canada border. Grand Coulee was put into operation in 1941 and isolated sturgeon which were in this upstream area of the river at the time of flooding. Small sturgeon, approximately 14-16" long, have been caught at a few of the popular fishing areas, albeit rarely, indicating that some successful reproduction has taken place either within the lake or above since impoundment by Grand Coulee Dam. The Canadian portion of the Columbia just above the border supports a relatively small annual fishery, and a popular intense fishery exists in

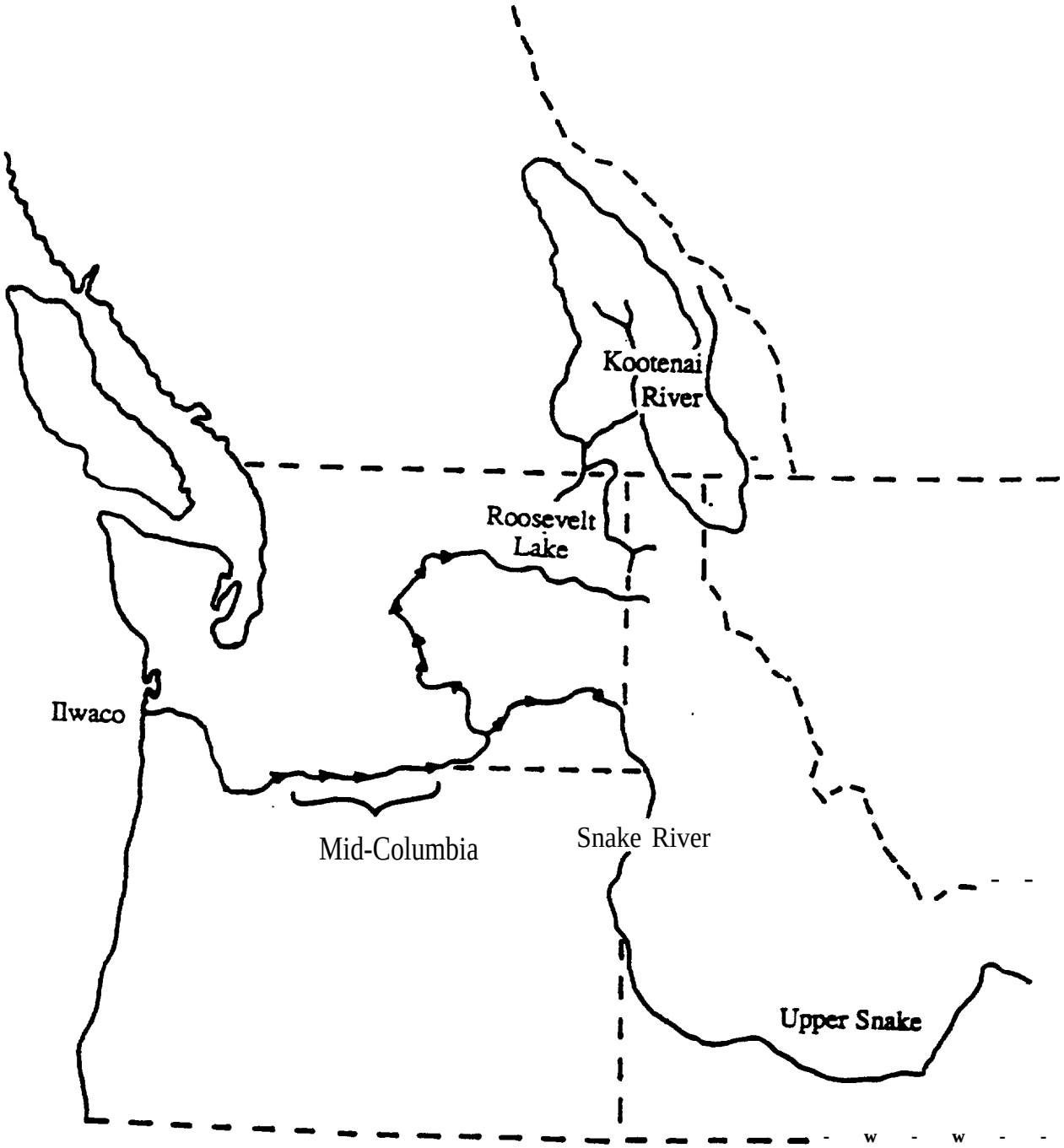


Figure 1. Sketch of Columbia River showing sampling areas.

Lake Roosevelt around Marcus at the interface of the reservoir and flowing river. Snake and Kootenai River regions were separate as river systems and were sampled with the assistance of the Idaho Department of Fish and Game.

Except for the sport and Indian fisheries, all fish were sampled by set lines. Fish caught by set lines were released after the muscle plug was removed and the wound sterilized. Set-line gear was composed of 200-foot lengths of 3/8 or 1/2inch polypropylene rope wuh gangions every 20 feet. Each gangion was between 6 and 14 inches of #42 gangion twine tied between a hook and a clip attached to a swivel. Hooks used were manufactured by Mustad and of three sizes (7/0,8/0,9/O), with the two smaller sizes being most successful in capturing fish. The smaller hooks were also most likely to open up prior to landing the fish. Circle hooks were not utilized until 1989, and three sizes (13,14,16) were then used exclusively. Circle hooks proved easier to remove from captured fish and did not break. Hook and line capture was most often performed with either a 7/O or 8/O hook.

Set line fishing is more difficult and less productive in the early spring when the water level is low because the gear cannot be as efficiently set in the fast moving water. Our efforts under these conditions produced spotty results over the entire study period. The months of May through October afforded us the greatest success in capturing white sturgeon in Lake Roosevelt. The reservoir is 75-100% of full pool elevation during this time. Water level fluctuations of a few inches to several feet overnight were common and often seemed advantageous for enticing sturgeon to take bait.

An important factor associated with catch SUCCESS was fresh bait. Fresh frozen salmon or trout was much prcfcrred over tainted or rotting bait. Bait was changed every 24 to 48 hours. Sampling was a brief and relatively stress-free procedure after the fish were captured and brought to the water surface. Smaller individuals were lifted into the boat by grasping the caudal peduncle with one hand and placing the other hand under the head. A wet towel was placed over the eyes to shield the light and hence reduce the stress-related movement. Fish length was measured, and a muscle plug was removed by inserting a steel cork borer into the area just below the dorsal ridge of scutes towards the posterior end of the fish (Fig. 2). The tissue was then placed in a ziploc bag, set on dry ice for immediate freezing, and transferred back to the laboratory. The wound was purged with a 10% nitrofurazone solution to reduce bacterial or fungal infection. A photograph documenting head shape was taken and counts of dorsal and ventral rows of scums were noted. The fish was then released and would swim down to deep water. Larger individuals wcrc taken

to shore and sampled in a similar fashion. At the laboratory, samples were stored at -85°C in a super cold freezer to prevent breakdown of tissue proteins.

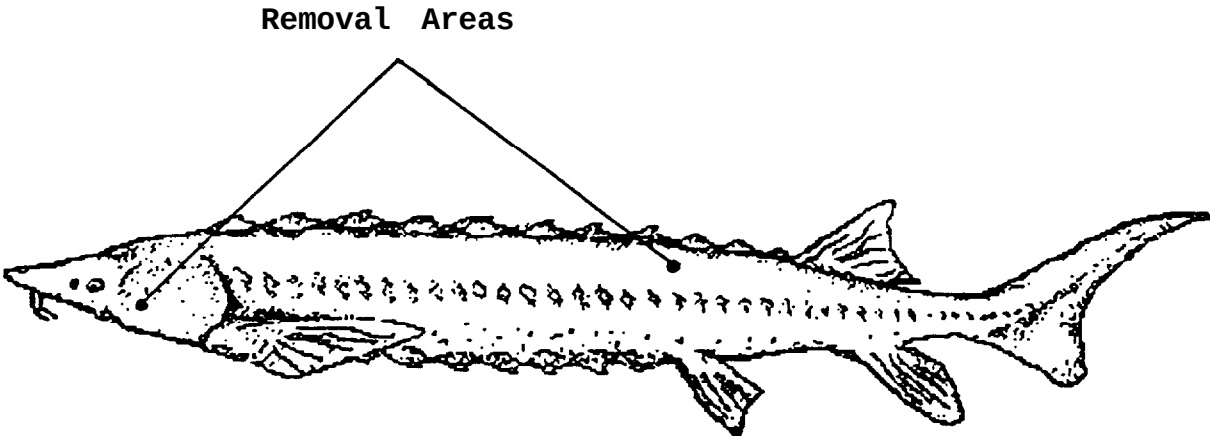


Figure 2. Muscle tissue sampling locations.

Stock Assessment

Electrophoresis

Prior to electrophoresis, each tissue was slightly thawed and a l/4" by l/8" by l/4" piece was cut off and put into a test tube. The test tube contained 0.3 ml of a tissue prepping solution (PTP; Aebersold et at., 1987) which enhances activity when some of the enzyme systems are stained. Test tubes were put into the supercold freezer (-85°C) for storage. Each tissue type was kept in a separate rack in a specific ordered sequence, and the same sequence was repeated for every tissue. Starch gels were routinely prepared the day before electrophoresis was performed. Gels were poured using Sigma starch and several buffer solutions (Table 1). Test tube racks were removed from the freezer, and tubes centrifuged at room temperature for 3 minutes to thaw the liquid. A paper wick was dipped in the test tube to absorb the protein slurry and placed across the cut face of the gel. Gels were placed on ice packs for cooling prior to placing the paper wicks against the cross-section cut in the slab. Electric current (-65 mA) was run through the gel using a Heathkit power supply for 4-6 hours. Marker dye was placed on several paper wicks so that migration of the proteins through the gel could be monitored as the electric current was applied for the appropriate length of time. The starch gel was kept refrigerated with gel ice to prevent protein breakdown.

Laboratory procedures followed standard eiectrophoresis methods (Harris and Hopkinson, 1976; May, 1980; Utter et al., 1974; Aebersold et al., 1987). Gels are sliced and covered with agar and chemicals (for specific enzyme stains) which react to produce banding patterns. Each protein has a different mobility and banding pattern representing genotypes of individual fish.

The banding patterns were recorded as genotypes and used to calculate allele frequencies. Banding patterns were scored or rated by their migration distance from the point of origin. The most common homozygote band was assigned a score of 100. Bands for homomeric proteins of other alleles were given a number representing their migration distance as a percent in relation to the common band following protocol described by Utter et al. (1974). Horizontal starch gels were run with samples from 40-50 individual fish, one tissue type at a time. Different buffers were employed to obtain the best resolution of the enzymes tested (Table 1). Once the analysis of enzyme systems

Table 1. Buffers used for sturgeon electrophoresis.

	Gel Buffer	Electrode Buffer	
1	.Tris-citrate (pH 8.7)	Lithium-borate (pH 8.0)	(Ridgway et al., 1970) (RW)
2.	Tris-borate (pH 8.7)	Tris-borate (pH 8.7)	(Aebersold et al., In Press) (TBE)
3.	Citric Acid (pH 6.5) * (pH 5.5) + (NAD added to gel and cathodal electrode tray)	Citric Acid (pH 6.5)	(Clayton and Tretiak, 1972) (AC)
4.	Tris-citrate (ph 7.0)	Tris-citrate (ph 7.0)	(Shaw and Prasad, 1970) (TC)
5.	Tris-phosphate (pH 8.2)	Tris-phosphate (pH 8.2)	(Busack et al., 1979) (TP)

began, photos were taken of the gels for later reference. Systems which were defined enough for scoring purposes are listed in Table 2, with the tissue and buffer defined.

Data were collected from each individual and analyzed using a Chi-square goodness of lit test within each area to determine if any observed genotype frequencies would not conform to Hardy Weinburg equilibrium expectations. In extrapolating to population genetic characteristics from a random sampling of individuals, genetic theory expectations are based on the assumption of Hardy Weinburg equilibrium conditions as defined in all basic genetic texts. Data collected in 1985-1989 were pooled to form a single database for analysis, so that sample sizes within most areas were sufficiently large. Genetic distance values calculated from the gene frequencies measured the closeness of the relationship between the sampling areas using the method of Nei (1978). The average heterozygosity was calculated within each sampling area to assess the degree of variation. Contingency chi-square analyses were performed on allele frequencies at each polymorphic enzyme system to determine significant diffcrcnccs between sampling areas. Twenty-nine loci were scored overall, with some not scored for all areas or all individuals within an area. Analyses were performed using the BIOSYS (Swofford and Selander, 1981) program, Minitab, and SPSSX statistical packages on the University of Washington Cyber or Vax computer. The database from tissue sampling examined 614 individual sturgeon from the various areas of the Columbia, Snake and Kootcnai rivers. The actual numbers of sturgeon examined from each area are listed below (Table 3).

Table 2. Listing of systems by tissue and buffer.

Enzyme	Buffer	Tissue
Aspartate aminoaansferase (AAT) EC. 2.6.1.1	TBE	mus, hrt*
Adenosine deaminase (ADA) E.C. 3.5.4.4	TP	mus
Aconitase hydratase (AH) E.C. 4.2.1.3	TBE	mus
Adenylate kinasc (AK) E.C. 2.7.4.3	AC	mus
Fructose biphosphate aldolase (ALD) E.C. 4.1.2.13	AC+	mus
Creatine kinase (CK) E.C. 2.7.3.2	AC+	mus, eye, hrt
Esterase (EST) E.C. 3.1.1.	AC	mus, liv
Glyccraldehyde-3-phosphate dehydrogenase (GAP) E.C. 1.2.1.12	TC	mus
Glycerate dehydrogenase (GD) E.C. 1.1 .1.29	TP	mus
Glycerol-3-phosphate dehydrogenase (GPD) E.C. l-1.1.8	TBE	mus
Glucose&phosphate isomerase (GPI) E.C. 5.3.1.9	RW	mus
Isocitric dehydrogenase (IDH) E.C. 1.1.1.42	RW	mus
Lactate dehydrogenase (LDH) E.C. 1.1.1.27	AC	mus
Malic dehydrogenase (MDH) E.C. 1.1.1.37	AC	mus
Malic Enzyme (ME) E.C. 1.1.1.40	AC	mus
A-mannosidase (a-MAN) E.C. 3.2.1.24	RW	liv
Phosphogluconate dehydrogenasc (PGD) E.C. 1.1.1.44	TBE	mus, liv
Phosphoglucomutasc (PGM) E.C. 5.4.2.2	TBE	mus
Superoxide dismutasc (SOD) E.C. 1.15.1.1	RW	mus

* muscle = mus, heart = hrt, liver = liv

Table 3. Number of individuals sampled from each area over the contract period, 19851990.

Area	#Sampled
Snake River (below Hell's Canyon dam)	12
Upper Snake River (Mountain Home to Buhl)	10
Ilwaco	360
Lk. Roosevelt	193
Mid-Columbia (below McNary to above Bonneville)	164
Kootenai River	6.5

DNA Extraction

For extraction of DNA we used frozen samples of muscle and liver tissue. Our procedure which is summarized below was taken directly from that suggested for preparation of genomic DNA from mammalian tissue (CPMB, 1988). A one gram piece of tissue was weighed and placed into a sterilized mortar and pestle which had been cooled in a -20°C freezer. The mortar and pestle were presterilized by washing in hot water with alconox, rinsing, washing in bleach and then rinsed three times with distilled water. The mortar and pestles were then UV irradiated for one half hour. Following this, they were foil wrapped and placed in a 180°C oven for 6 h. The tissue (1 g) placed in the chilled mortar and pestle was covered with liquid nitrogen and tissue crushing and grinding continued until a fine powder was obtained. The powder was then placed into a 15 ml conical bottom centrifuge tube with 12 mls of prepared digestion buffer. The digestion buffer was comprised as shown in Table 4 for each 12 ml.

This mixture was vortexed briefly using a thermolyne mixer. When all the samples had been prepared in this manner (usually 5 or 6 were done on each occasion) they were placed in a 50°C vigorously shaking incubator for 12-16 h. This step of the procedure was to lyse cells and degrade (denature) the protein that surrounds the DNA. The next series of steps was the actual extraction of DNA and RNA from the digestion solution. Saturated phenol (Amresco) was mixed (volume:volume) with the sample in a Sarstedt disposable 30 ml centrifuge tube. The tube was then spun at 4,000 rpm for 10 min at 22°C. The phenol denatures proteins and extracts water and a interface forms between the sample and the organic solvent. The top layer rests above a whitish sludge which forms between

Table 4. Tissue digestion buffer for one gram of tissue[1].

Ingredients	Quantities
distilled water	9.6 ml
1.0 M NaCl	1.2 ml
1.0 M Tris, pH 8	120 ul
0.5 M EDTA	600 ul
20% SDS	300 ul
Proteinase K *	0.4 mg

* This was added just prior to use of the buffer.

[1] Taken from CPMB, 1988.

the layers. The top layer contains the DNA and was transferred with a 1.0 ml Pasteur pipet to another 30 ml centrifuge tube labeled with the appropriate sample number. This step was repeated until no whitish sludge layer is visible after separation during centrifugation. At this point the sample is followed by two chioroform/isoamyl alcohol (24: 1) extractions. The chloroform /isoamyl is added to the sample on a volume:volume basis. Centrifugation at 4,000 rpm and 22°C for 10 min was used during all extraction steps. Once again the sample and the organic layers separate and the DNA is in the top layer. The sample is then pipetted into a clean centrifuge tube. During this last series of organic extractions, care is taken to not include any of the organic layer with the sample.

After the final extraction, the quantity of sample is measured using a ten ml pipet. The next step was to ethanol precipitate the DNA. To the tube containing the sample, a one half sample volume of 7.5 M ammonium acetate and two sample volume quantity of cold 95% ethanol was added. This mixture forces the DNA/RNA to precipitate out. The tube is place immediately in a -20°C freezer and left for l/2 - 3 h. The tube is then removed from the freezer and centrifuged for 10 min at 10,000 r-pm and 6°C. When this was completed, a DNA/RNA pellet was on the bottom of the tube. The tube was immediately inverted and the ethanol was poured off. The pellet was then rinsed with l-3 drops of 70% ethanol which was then poured or pipetted off. The pellet was then placed in a vacuum desiccator to dry and evaporate the ethanol. This took from l/2 - 2 h depending on how well the 70% ethanol could be separated from the pellet after the wash. After drying, the pellet was resuspended in sterile, distilled water. A minimal dilution at this point was the goal in order to obtain a DNA concentration in solution which

approached 1 ug/ul. In reality after getting the DNA to resuspend, the dilution was often as high as 1 ug/8 ul. The DNA/RNA was then stored at 4°C in 1.5 ml screw cap conical bottom vials and labeled with the sample number.

DNA Ouantification

The concentntion of DNA was determined using a Perkin Elmer fluorimeter. The DNA was diluted into a 1 X TNE buffer which had Hoechst dye added in the quantity of 10 ul/ 100 ml. Two ul of sample was added to 998 ul of the prepared buffer. The machine then compared the fluorimeter readings with known DNA standards to provide the value. The values were then used to judge the quantity of DNA needed for R.E. digestions.

Quantitative DNA Evaluation

Prepared DNA was examined qualitatively by loading 1-2 ug into a minigel. A 0.8 % minigel was prepared using 0.4 g agarose, 50 mls 1X TBE and 2.5 ul of ETBR (10 mg/ml). The gel was prepared and run similarly to that described below for running out digested DNA. The minigel can be run quickly, usually about 1 l/2 h at 85 V because the tray sets into a precooled gel holder. The gel is then viewed on a UV transilluminator and photographed. The DNA appears as a smear or a single band depending on the extent of fragmentation that has occurred during the extraction process or the concentration examined.

RE Digestion

In order to examine the DNA for RF'LP polymorphisms (Restriction fragment length polymorphisms) it is necessary to use restriction endonucleases to cleave the DNA into various fragments. Restriction endonucleases are enzymes that cleave DNA strands at specific nucleic acid base sequences via recognition of the sequence. Subjecting DNA to the action of a particular restriction enzyme for a specified length of time and specific temperature is henceforth referred to as RE digestion. Each restriction enzyme has an optimal buffer salt concentration and temperature for maximum activity. Most of the enzymes prefer 37°C but salt concentrations are either no salt, low,

medium or high salt. The effectiveness of the restriction enzyme cleavage on the DNA is directly influenced by these two very important variables. Restriction enzymes are sold as units with the underlying principle that 1 unit will break up 1 ug of DNA in 1 h. Digestions were usually underway for 2 l/2 - 3 h. The digested samples were stored frozen until preparation of loading onto the gel. Prior to loading into wells of the prepared agarose gel, each sample had loading buffer and Rnase added. The amount of loading buffer and Rnase depended on the volume of the digestion. Two ul of 10X loading buffer and 1 ul of Rnase (10 mg/ml) were added for each 10 ul of the digestion volume. The mixture was briefly centrifuged before loading onto the gel. The loading buffer is used both as a dye front indicator and to keep the DNA in the wells. If it is added immediately following digestion, it also ceases the activity of the restriction enzyme. The Rnase is an enzyme added to degrade the RNA which was extracted together with the DNA.

Anarose Gel Elecuonhoresis

Electrophoresis was performed using 0.8% agarose gels in Hoeffer submarine systems with 1X TBE buffer (running buffer and gel buffer were identical). Electrophoresis physical parameters were usually 15-25 V for 12-16 h. The number of wells varied based on the quantity of sample to be run. A lane of DNA standard was run for molecular wt determinations and human DNA served as a control for the enzymes and probes used.

Electrophoresis thru the agarose gel medium was used to sepamte the DNA fragments that resulted from digestion with a restriction enzyme. After running a gel long enough to separate the fragments, it was removed, photographed and viewed on the UV transilluminator. Excess gel was trimmed to minimize the size of the transfer membrane.

The gel was then placed in a Pyrex baking dish and subjected to washes prior to southern blotting. The first two washes were with a denaturing buffer (1.5 M NaCl, 0.5 M NaOH) for 15 min each at room temperature (RT). These were followed by two brief washes with distilled water. Two more washes followed these using a neutralizing buffer (1.0 M Tris; 1.5 M NaCl, pH 7.5). After cessation of washes, a southern blot was assembled to transblot the DNA from the gel to a nylon membrane. This was done using 20X SSC as the wicking buffer. DNA was transferred from gel to membrane via capillary action over a 12-16 h period.

Set up for the Southern blot followed standard procedures described by CPMB (Sec. 2.9.7) (1988). A wicking piece of filter paper was placed below the gel with both ends in the 20X buffer reservoir below. The gel was placed face down over this wick with a piece of nylon filter (same dimensions as gel) placed directly on the gel. A thin piece of wicking filter paper was then placed atop the nylon filter after being wetted in 20X SSC. Three more pieces of blotting filter paper were stacked next. Paper towels about 4 inches thick were layered above with a weight on top to enhance the blotting process. The nylon membrane, filter paper and paper towels used were cut to the exact dimensions of the gel. After 12-16 h the stack was disassembled and the membrane was rinsed in 2X SSC. The membrane was then crosslinked with a 254 nm UV light source.

Prehybridization and Hybridization of Membranes

A prehybridization interval of 6 h at 37°C was routinely performed with 100 mls of prepared solution. This 100 ml solution contained 50% formamide, 5X SSC, 5% blocking reagent, 0.1% NaSarc, and 0.02% SDS. This mixture is heated while stirring to dissolve the blocking reagent. Once dissolved the total volume is brought to 100 mls by the addition of distilled water. The solution is poured over the filter in a seal a meal bag and heat sealed. Incubation follows immediately with moderate shaking. Prehybridization serves to block the filter from excess background and leave only the areas with DNA unbound for the subsequent hybridization step. This is performed for 12-16 h also at 37°C with shaking. The next phase is the stringency washes to remove excess probe from the filter. Four subsequent washes were routinely used as follows:

2 washes at RT for 5 min with 2X SSC; 0.1% SDS

1 wash at 55°C for 15 min with 2X SSC; 0.1% SDS

1 wash at 55°C for 15 min with 1X SSC; 0.1% SDS

Washes were done in a pyrex baking dish on either a shaking platform (RT) or in an incubator shaker (55°C).

The procedure for detection of the bands follows those recommended for the Genius kit (Boetuinger Mannheim). The detection of DNA fragments homologous to the probe utilizes the antigen-antibody binding complex. The label incorporated within the complementary DNA probe (digoxigenin UTP) binds with an antibody (polyclonal sheep antidigoxigenin) conjugated to alkaline phosphatase. The alkaline phosphatase reacts with color

reagents to form a visible precipitate. The resultant bands are interpreted for variations between individuals examined

Probe

The probes which we utilized for detection of ribosomal DNA variation were for the 18s and 28s gene and originally isolated from a mouse, M. musculus (Amheim, 1979). These probes were made available for use with this project by Dr. H. Wichman, who obtained them from Dr. R. Van den Busche. Van den Busche had used these probes to detect intergeneric variation in bats for use in cladistic analysis. Dallas et al. (1988) had also used these probes to examine and detect interracial variation in the grasshopper. Because we were seeking fixed differences between geographic regions, we were grateful for getting access to this probe for our preliminary examination of white sturgeon of the Columbia River.

Probe labeling

After some preliminary trials using P^{32} as a label for the probe, we decided that the use of the nonradioactive DNA labeling kit would better fit our experimental needs. This kit utilizes the random primed method for labeling denatured DNA (Feinburg and Vogelstein, 1983). The methodology described below follows instructions included with the kit. A typical labeling reaction was undertaken as follows: 13 ul of distilled water and 2 ul of probe (200 ng) were placed in a 0.5 ul microfuge tube and boiled for 10 min to denature the DNA. This tube was placed into an ice/salt bath for 3 min. Two microliters of a hexanucleotide and 2 ul of a dNTP labeling mixture were added to the microfuge tube while still on ice. One microliter of Klenow enzyme (2 units/ul) was then added. The mixture was pulse microfuged and placed in a 37°C incubator for 20 h to generate approximately 260 ng of label incorporated complementary DNA. This mixture was stored at -20°C until needed.

Prior to use the labeled probe was further purified, to remove unincorporated nucleotides from the mixture. Spun column chromatography with Sephadex G-50 beads were used for this procedure (Sambrook et al., 1989). Half of the labeled probe was used for hybridization with a 10 cm x 10 cm filter. Solution was recycled after use and

stored in a -20°C freezer. Rrior to reuse, the labeled probe in hybridization solution was boiled for 10 min to dissolve any precipitate that might have formed. The mixture was commonly reused 4-5 times with good hybridization results.

Immunoloeical Detection

Due to the nature of the reactions which form the backbone of this procedure, the detection of banding or fragment patterns is referred to as immunological detection. Sufficient blocking of the filter (membrane) and binding of the antibody are critical steps for good detection of small quantities of DNA. The following series of steps follows immediately after the post hybridization stringency washes (Boehringer Mannheim, Genius kit).

1. Brief wash in buffer 1.
2. 30 min wash in buffer 2. This serves to block the membrane and prevent nonspecific adhesion of antibody in subsequent steps.
3. Brief wash in buffer 1.
4. 30 min wash in antibody conjugate (1:5000) dilution of supplied antibody.
5. Two - 15 min washes in buffer 1.
6. One 2 min wash in buffer 3 to neutralize membrane in preparation for the color reaction.
7. Color reaction - Reagents are added to membrane and placed flat in a dark 37°C incubator. This can be done at RT but produces bands faster at 37°C.
8. After development of bands on filter, it is stored in buffer 4.
9. The filter is photographed and the fragments are examined for polymorphism.

Restriction Enzymes used for Digestion

The restriction enzymes used for the digestion of white sturgeon DNA are listed alphabetically below with their base sequence recognition site.

BAMH-I	G/GATCC
ECOR-I	G/AA-l--K
HINC-II	GT(C or T)/(A or G)AC
SAL-I	G/TCGAC
SAU3A-I	/GATC

Single sample digests of the following enzymes were done and run out on a 0.8% gel to examine for the presence of satellite DNA bands. No strong bands could be found for elution and subsequent cloning. The purpose of this was to isolate a sequence which we could use as an additional probe. The restriction enzymes used were APA-I, BGL-II, HINC-II. HIND-III, KPN-1, PST-l, SCA-I, STU-I (6 base cutters), ALU-I, CFO-I, HPA-I, MSP-I, RSA-I, Sau3A-I, HAE-III, TAQ-I (4 base cutters). In many mammalian species, these bands are readily noted and have been used for differentiating falcon spp by Longmire (1990).

Morohometrics and Meristics

In an effort to further evaluate potential stock differences, morphometric and meristic information was collected at the time of tissue sampling so that any apparent difference in physical traits could be noted. It was hypothesized that differences would correlate with the genetic data collected through electrophoresis.

Snout shape was evaluated by multivariate statistical analysis of 13 measurements taken from photographs. Fish were placed on a white background and the head region photographed from above. A metric ruler was included in each photograph for a size reference (Fig. 3). The positions of seven landmarks (Fig. 4) were digitized from the photographs on an x-y grid using the technique of Winans (1984). Landmark 1 was tip of the snout, landmarks 4 and 5 were positions of the eyes along the body outline. Landmarks 2,3,6 and 7 were calculated. To calculate these landmarks, line 4-5 was drawn on the photograph. Then a line perpendicular to 4-5 that intersected landmark 1 was drawn. The length of this line is "x". Two lines perpendicular to this line were drawn at distances 0.25x and 0.50x from the snout, as indicated in Figure 4. The points of intersection of these two lines and the body outline

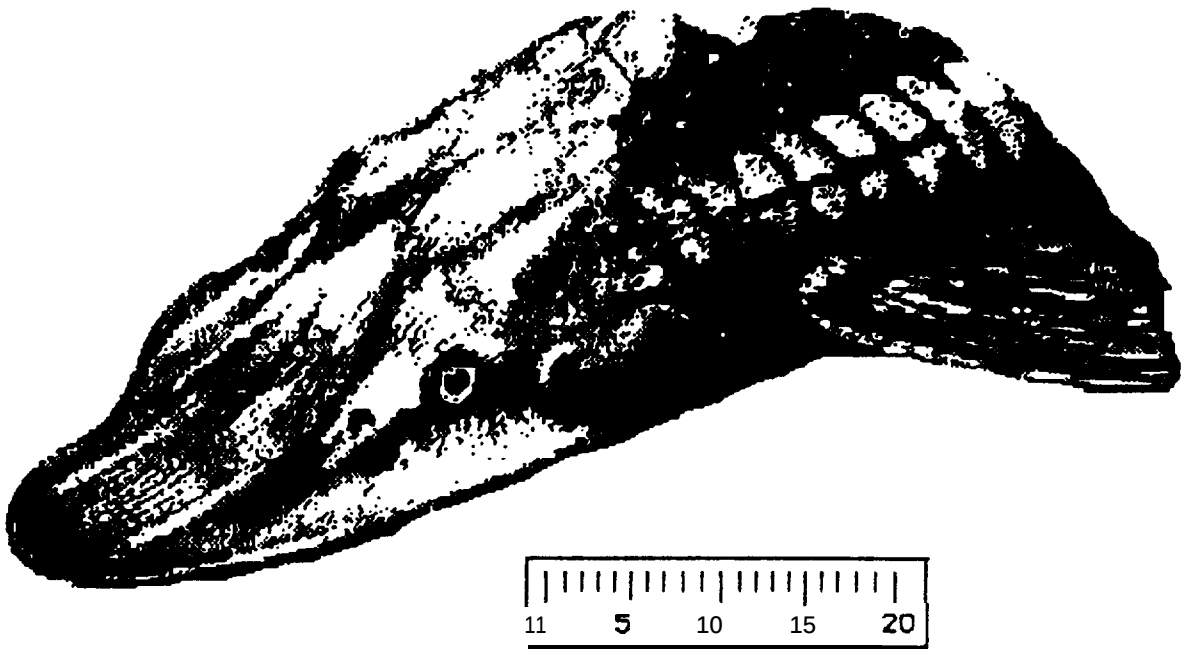


Figure 3. Position of photo taken of snout shape.

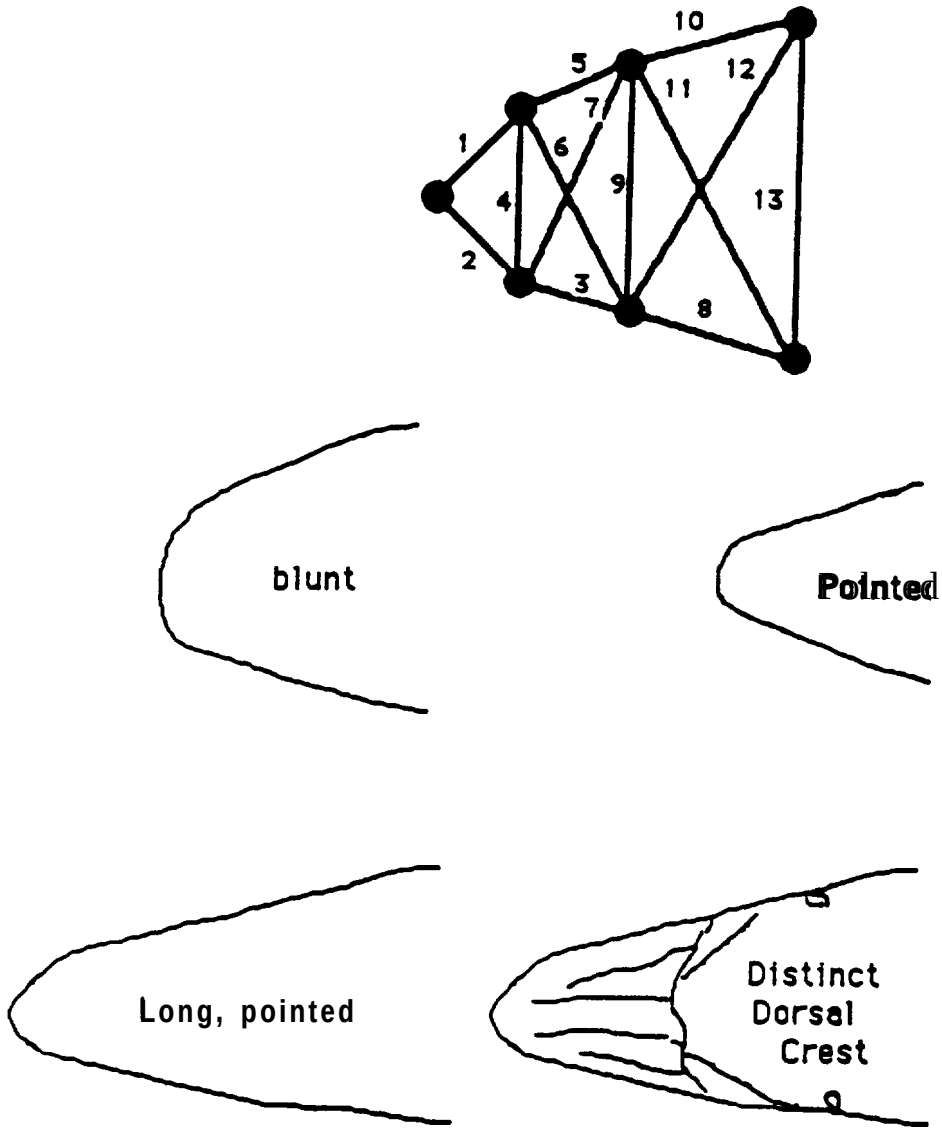


Figure 4. Landmarks used on head and outlines of observed snout shape differences.

constituted landmarks 2 and 7 (at 0.25x) and landmarks 3 and 6 (at 0.50x). We assumed that these landmarks were homologous from specimen to specimen.

Dorsal scutes are the plates which lie along the dorsal crest of the fish. Because casual observations during 1985 showed variation in the total count among fish, dorsal scute counts were included as part of the sample routine in 1986 - 1987. Lengths of fish were not routinely noted and were thought to be of no influence on the total number of scutes observed. (Laboratory sturgeon 5 - 12 cm in length have shown the full range of scute counts, personal observation.) Scute counts were analyzed between areas using a one way analysis of variance. Data were then tested against the snout data, and the electrophoretic data for any correlation. Since the electrophoretic data was recorded as genotypes, each genotype was converted to a numeric value. Head shapes were rated visually and assigned either a 1 (pointed), a 2 (long, narrow), or a 3 (blunt) based on the photograph. Data were assembled so that the three character sets collected from each individual fish created a single row of values. Each enzyme system, the head shape values, and scute counts formed the columns which were then compared. The correlation analyses were performed using SPSSX statistical program. Sample size of both the scute count and head shape data were smaller than the electrophoresis sample size. This was primarily due to the logistics of collecting data on fish during processing, and the tissue samples had priority.

RESULTS

Stock Assessment

From electrophoretic analysis of samples taken in the present study, a total of twenty-nine loci showed banding patterns which could be scored (Appendix Table 2). Twenty-three of the loci scored from the areas examined showed some variation, seventeen of which were considered polymorphic at the 0.95 level. The remaining six systems showed variation in lower frequencies and were considered rare alleles. Enzyme systems which were evaluated at the six areas studied are listed below with a brief structural description. While the banding pattern for these enzyme systems based on their molecular structure has been defined (Harris and Hopkinson, 1976), the position of the loci and the number of loci are specific for white sturgeon. Each description is followed by a drawing showing the banding patterns obtained from the electrophoretic technique. The drawings show the most common allele found at each locus labeled as 100, and alternate alleles labeled according to their relative position. Data arc reported for these systems from each individual fish by interpreting the banding patterns. The interpretation of tbe banding patterns into genotype descriptors is referred to as scoring.

Descrintion of loci

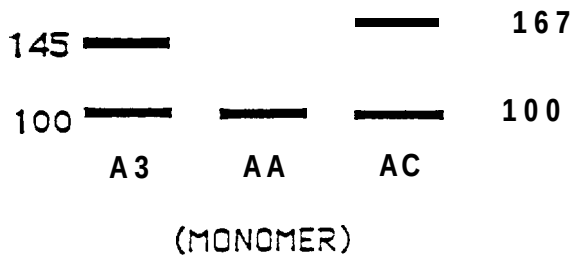
Aspartate aminotransferase (AAT) showed a cathodal locus which was scored in muscle. A fairly common slow allele at this locus was observed in all areas (Fig. 5).

Aconitase hydratase (AH) was scored using a single locus model having two fast alleles. This system is polymorphic but was difficult to score. There was a superfast allele which has been seen only in Roosevelt Lake, but even there it appears to be somewhat rare (Fig. 6).

Adenvlate kinase (AK) had one locus. A fast variant out of this locus (AK-l) was found only in the Ilwaco samples (Fig. 7).

Figure 5. Diagram of AAT banding pattern and allele frequencies.

AH



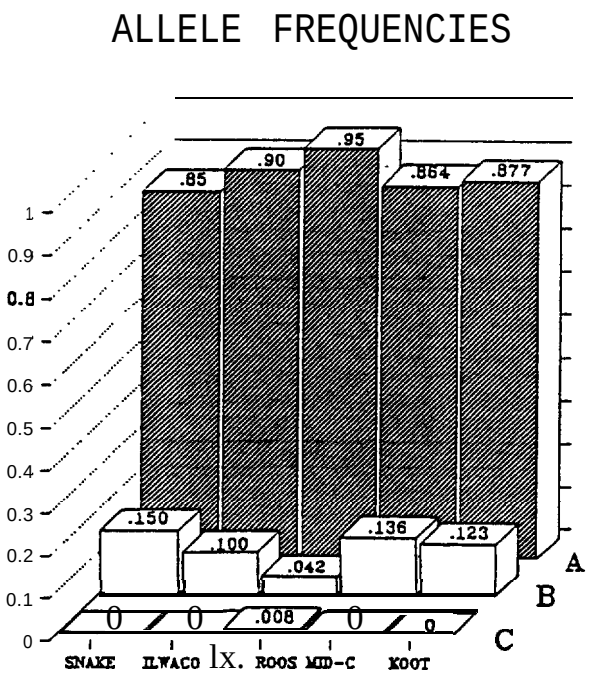
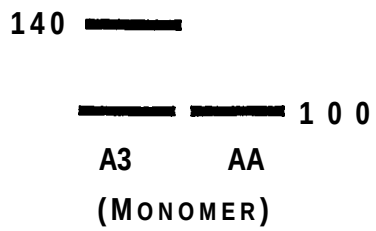


Figure 6. Diagram of AH banding pattern and allele frequencies.

AK



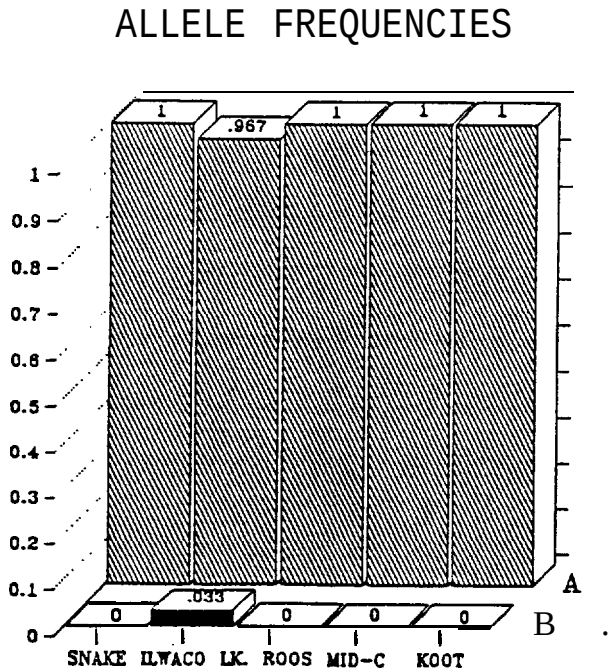


Figure 7. Diagram of AK banding pattern and allele frequencies.

Fructose biohosphate aldolase (ALD) showed one locus in muscle which migrated anodally, and there was a fast variant at this locus (Fig. 8). There appears to be tissue specific isozymes which have different mobilities but show the same variation in this system. Observed banding patterns did not conform to expected structure, most likely due to the close affinity of their relative electrical charge.

Creatine kinase (CK) had three loci of which CK-1 and CK-2 were both monomorphic. CK-3 was storable in eye and heart, and was polymorphic. A fast allele was observed in all areas scored, but a slow allele was found only in Roosevelt Lake (Fig. 9).

Esterase (EST-l) was monomorphic in all areas. EST-2 was polymorphic in Roosevelt Lake and the mid-Columbia areas in liver tissue, but liver was not available for testing from other locations.

Glvcerate dehvdrogenase (GD) migrated anodally, and showed a fast variant that was observed only at Ilwaco (Fig. 10).

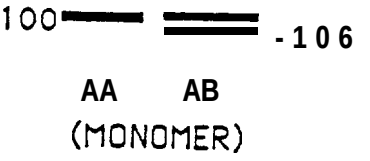
Glvcerol-3phosphate dehvdroeenase (GPD) migrated anodally, and had a slow variant. The variant was seen in all areas (Fig. 11).

Glucose nhosphate isomerase (GPI) was scored as being coded for by two loci with an interaction band (Fig. 12, 13). The first locus was near the origin and had a slow variant observed only in Ilwaco samples that migrated cathodally. The second locus was anodal and also had a slow variant observed in all areas. The large number of shadow bands could not be reduced or eliminated by treatment with either mercaptoethanol or reduced glut&none as thiol reagents.

Lactate dehvdronenase (LDH) had one locus which was scored in muscle. The common allele was on the origin and a variant migrated cathodally (Fig. 14). A high percentage of heterozygotes were seen in samples from most areas which approached deviation from the expected Hardy-Weinburg proportion. The structure of LDH and MDH in Russian sturgeon has been described by Slynko (1976) and for other fish species by (Markert et al. 1975; Markert and Aulhaber,1965), a simple model was used for scoring. White sturgeon in the Columbia River appear to have other loci specific to heart and eye.

Malate dehydrogenase (MDH) showed two loci anodally, with both MDH-1 and MDH-2 being polymorphic, MDH-1 had a fast variant allele which fell on the heteropolymeric band between the two loci, and was found in all areas scored. There was also a slow allele that showed itself out of MDH-2 in the Ilwaco area (Fig. 15).

ALD



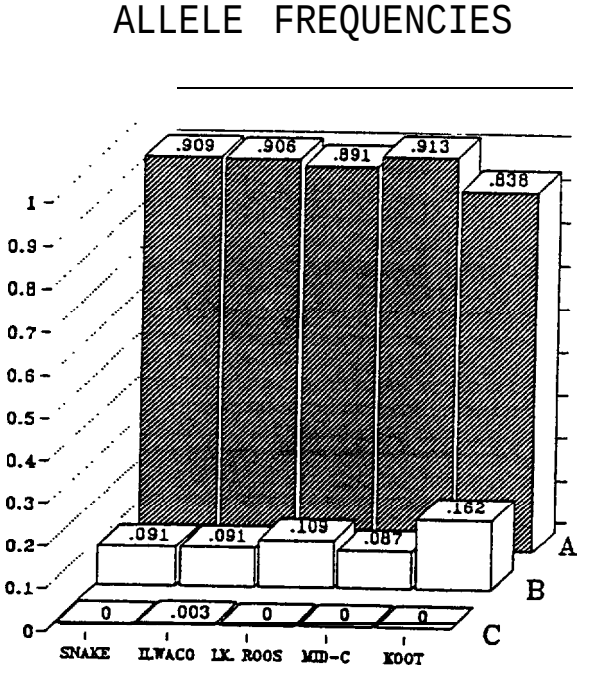
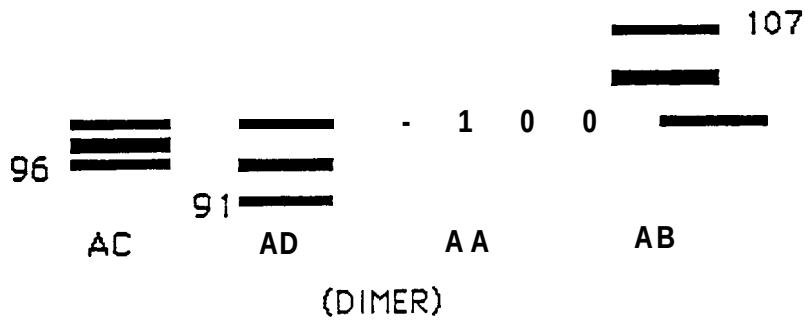


Figure 8. Diagram of ALD banding pattern and allele frequencies.

CK-3



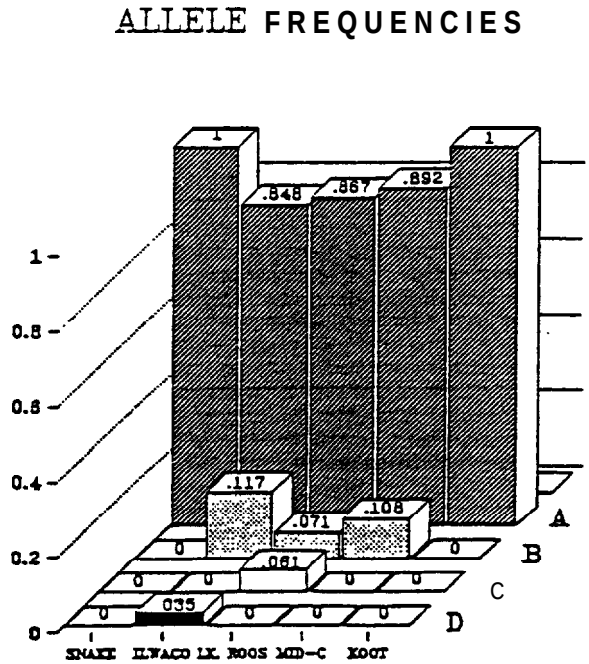
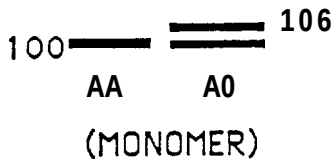


Figure 9. Diagram of CK-3 banding pattern and allele frequencies.

GD



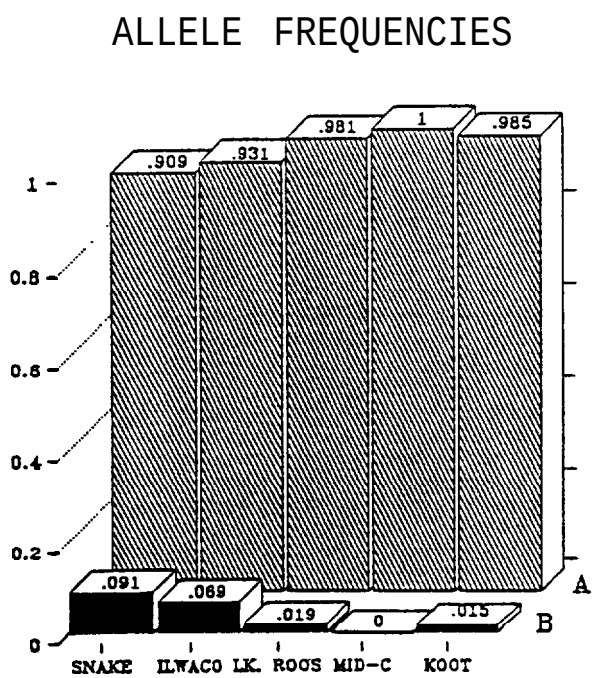
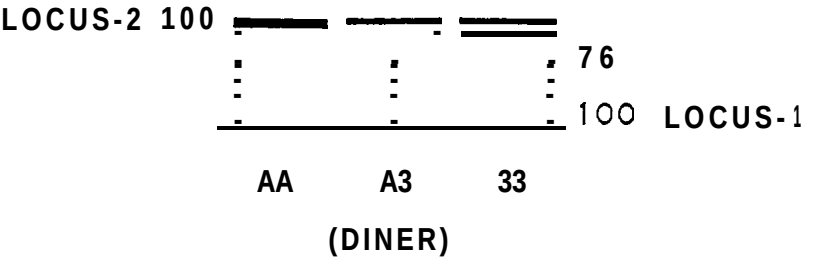


Figure 10. Diagram of GD banding pattern and allele frequencies.

Figure 11. Diagram of GPD banding pattern and allele frequencies.

GPI-1



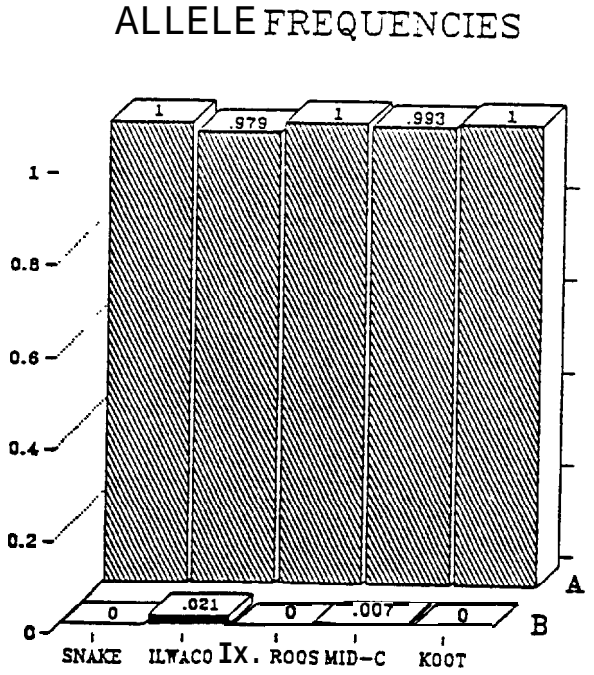
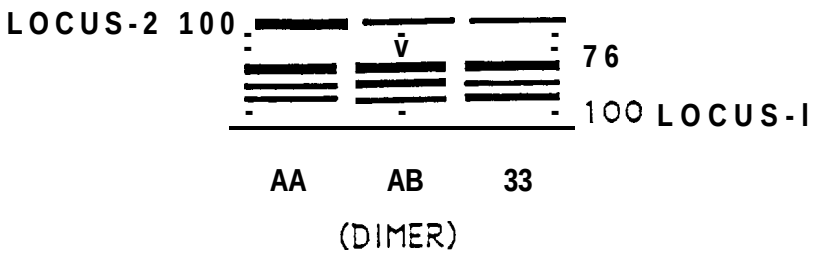


Figure 12. Diagram of GPI-1 banding pattern and allele frequencies.

GPI-2



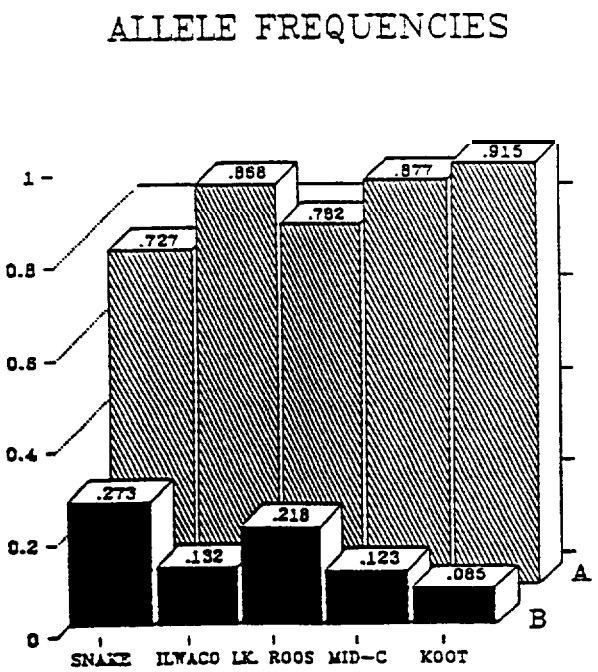


Figure 13. Diagram of GPI-2 banding pattern and allele frequencies.

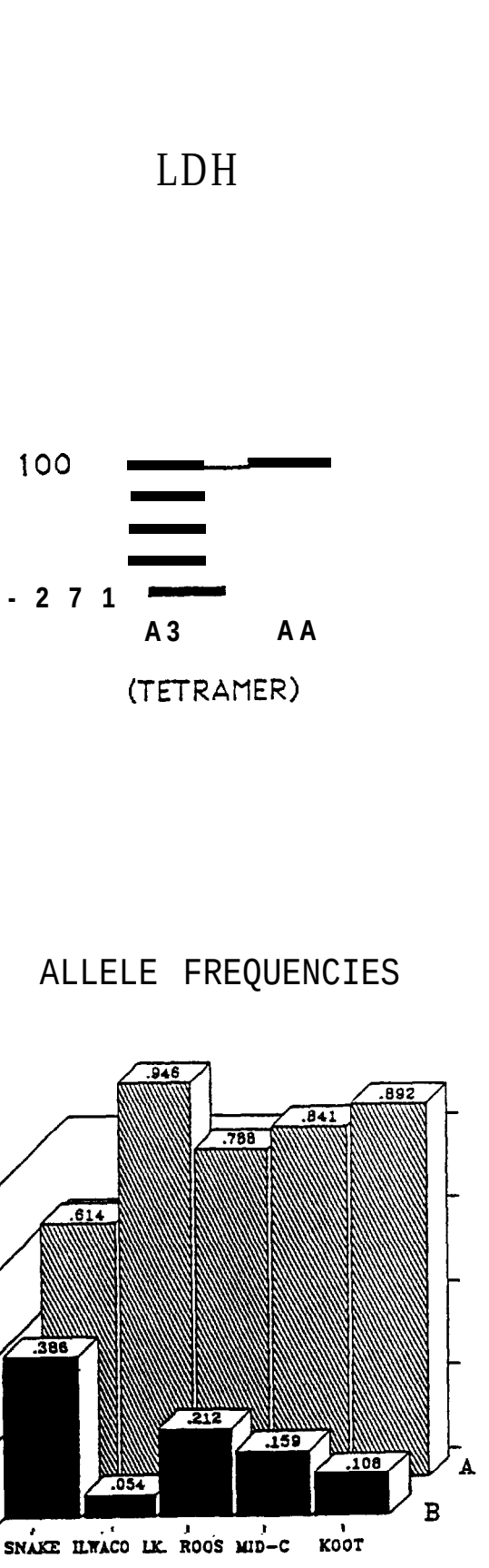
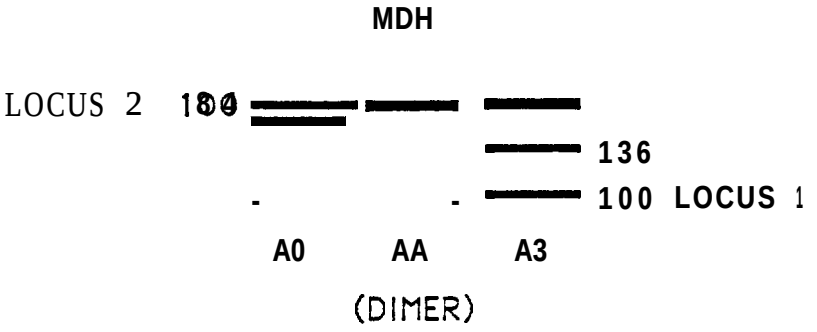


Figure 14. Diagram of LDH banding pattern and allele frequencies.

MDH-1



ALLELE FREQUENCIES

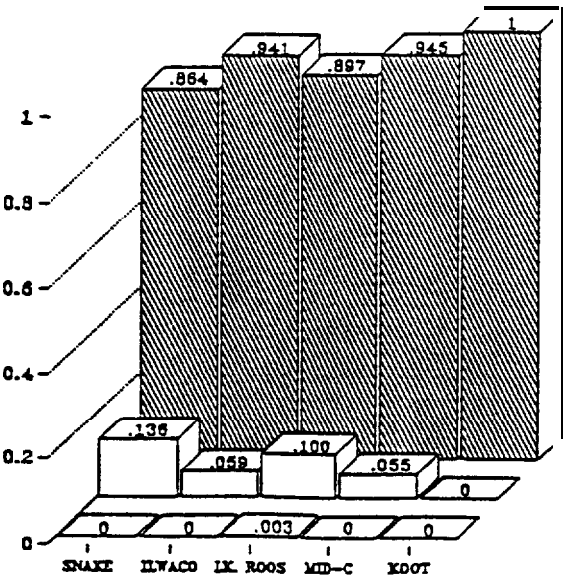


Figure 15. Diagram of MDH-1 banding pattern and allele frequencies.

Malate dehydrogenase I-NADP] (ME) had one locus which was polymorphic. ME-l migrated cathodally and had a slow variant seen only in Ilwaco samples (Fig. 16).

Peptidase (PEP) showed three loci and two were polymorphic. PEP was scored from the peptide leucyl tyrosine (JT) which revealed three loci, two of which were scored. There was a slow variant out of LT-3 and LT-1. No variation was observed in Roosevelt Lake or the Snake River for LT-1. LT-2 was fainter and less discemable, and no data were collected. (Fig. 17).

Phosuholrlucomutase (PGM-1+2) was scored in muscle tissue for two loci. Variation was seen in PGM-1. Them was a rare fast allele seen in Roosevelt Lake and a slow allele seen frequently throughout all arcas. PGM-2 was only variable in Roosevelt Lake (Fig. l&19).

Phosphogluconate dehydrogenase (PGD) was polymorphic at Ilwaco and Roosevelt Lake, and thcrc was a diffcrcncc in allele frequency observed. One Fast variant was observed.

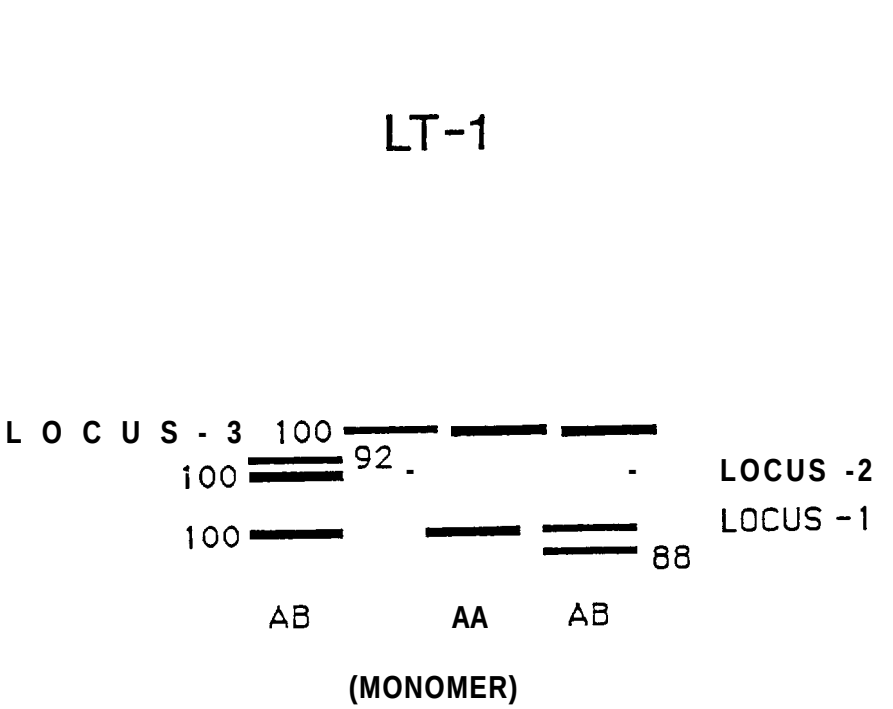
Adenosine deaminase (ADA-1+2), Glyceraldehyde-3-phosphate dehydrogenase (GAP), A-Mannose (A-MAN), (CK-1+2), Superoxide dismutase (SOD), were all monomorphic.

Polymorphic Loci

Polymorphic loci have been described above and displayed with drawings. The relative mobility of the common allele and alternate alleles for each enzyme system are compiled and shown in Appendix Table 1. The allclc frequencies are listed by system and area (Appendix Table 2) and were used for contingency chi-square evaluations between areas (Appendix Table 3). The chi-square statistic is given with the degrees of freedom and p-values for each variable locus. The within area comparisons tested with the chi-square goodness of fit for Hardy Weinburg equilibrium utilized genotypic data.

In one enzyme system (LDI-I), the observed frequencies of heterozygotes were much greater than expcctcd from an area with a stable sturgeon population conforming to Hardy-Weinburg equilibrium conditions. Another

Figure 16. Diagram of ME banding pattern and allele frequencies.



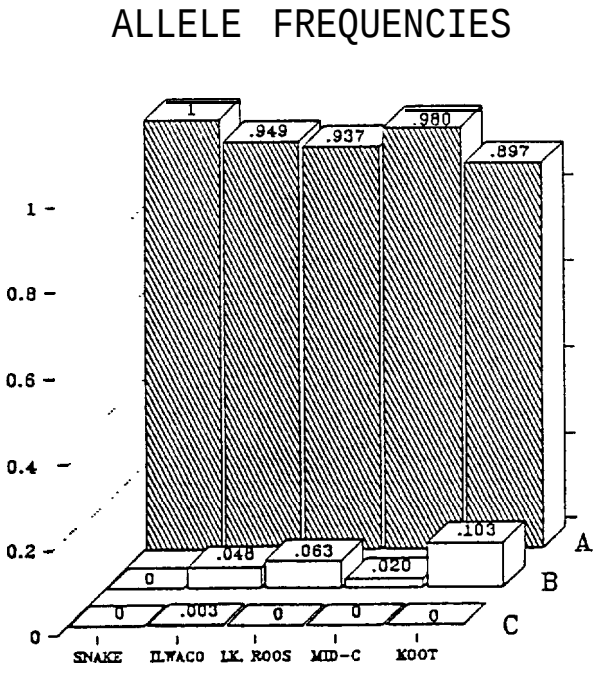
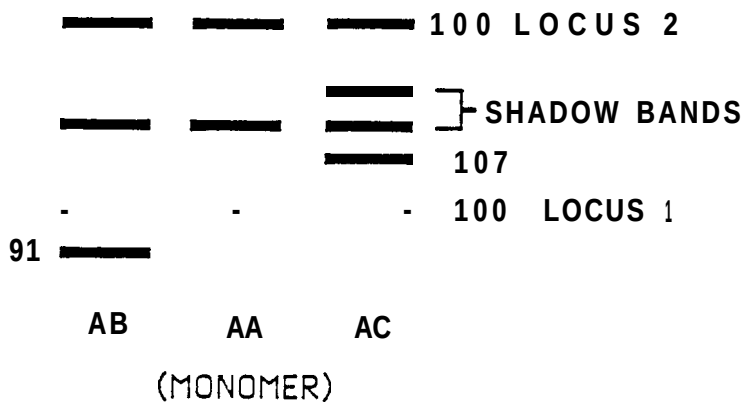


Figure 17. Diagram of LT-1 banding pattern and allele frequencies.

PGM-1



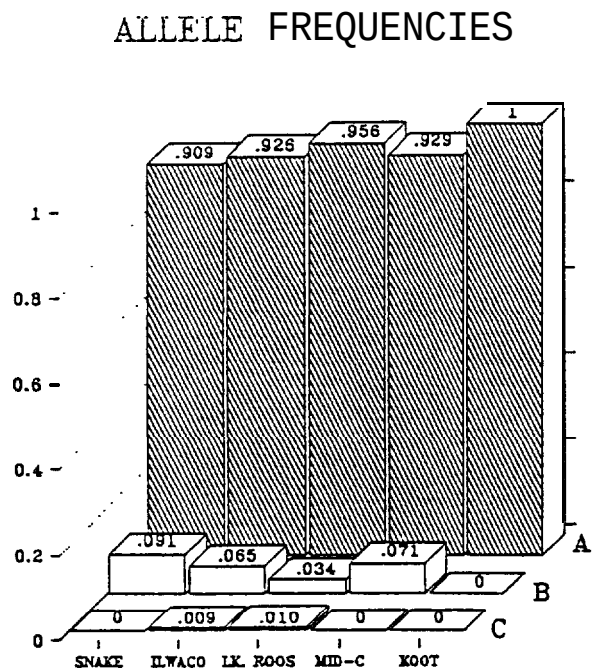
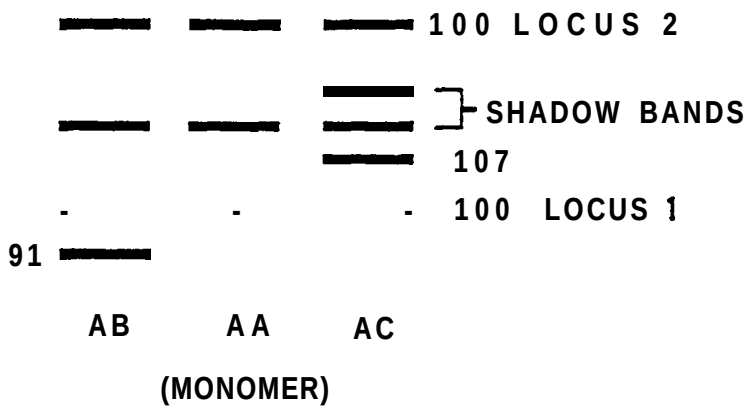


Figure 18. Diagram of PGM-1 banding pattern and allele frequencies.

PGM-2



ALLELE FREQUENCIES

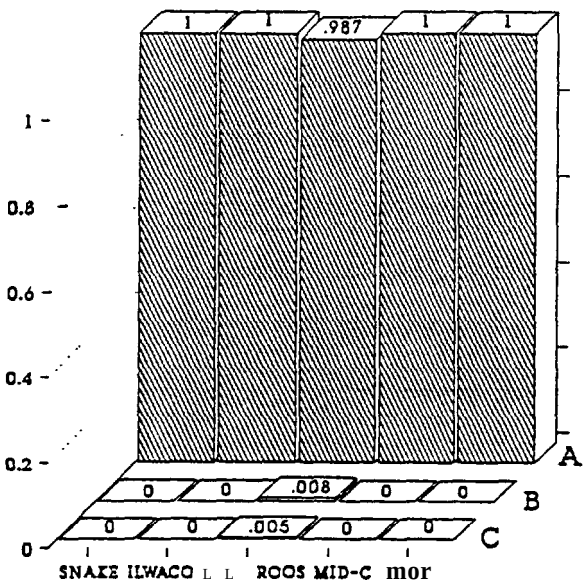


Figure 19. Diagram of PGM-2 banding pattern and allele frequencies.

enzyme system, GD, had a greater than expected number of alternate homozygotes. Both of these circumstances raised questions as to the scoring models used. With LDH that was observed from muscle, a high number of heterozygotes were apparent from several areas.

This possible expression of isoloci could make it impossible to differentiate between heterozygotes and alternate alleles (Utter et al., 1987). When isoloci occur, one locus is electrophoretically similar to the region whcrc a subunit or allele of another loci would appear on a stained gel slice. Because of the presence of a second locus in an identical position, the banding pattern intensity for both heterozygotes and alternate alleles from either locus may be indistinguishable when only one tissue is available or if the loci are similarly expressed in the tissues sampled.

Interesting systems (those polymorphic) are found throughout the river, but two others that were particularly perplexing occurred in the upriver samples. Genotypic variation existed in the AH and enzyme systems, but the model for the number of loci was unable to be verified, so the simplest model was assumed. AH may have two loci with the same mobility (Fig. 6). This was not clarified by the use of fresh samples or the USC of thiol reagents suggested by Harris and Hopkinson (1976). The single locus model used for scoring this enzyme system is apparent by noting genotypes shown in Figures 6. ALD suggests gene duplication because while each tissue seems to have a specific locus, the scoring appears the same at each locus. The hetcrozygote for both ALD (Fig. 8) and ME (Fig. 16) did not show the full banding that would be expected of tetmmeric enzymes, so the exact position or the alternate was unclear.

The average heterozygosity by area was calculated as an index of the amount of variation (Selander and Johnson, 1973). Values ranged from 0.023 to 0.096. The percentage of loci which are heterozygous in an average individual is referred to as H (Hard, 1980). There was little difference in the individual heterozygosity observed between areas (Table 5) except for in the Kootenai where much less variation was apparent.

Overall, sturgeon samples showed H = 0.082. This is slightly higher than the average value of 0.0M8 seen in other fish (Nevo et al., 1984). The average heterozygosity for green sturgeon (Acipenser medirostris) is

Table 5. Average heterozygosity by area.

GENETIC VARIABILITY AT 29 LOCI IN ALL POPULATIONS

(STANDARD ERRORS IN PARENTHESES)

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY DIRECT-COUNT	MEAN HETEROZYGOSITY HDYWBG EXPECTED**
1. SNAKE	14.0 (1.4)	1.3 (.1)	31.0	.082 (.031)	.073 (.024)
2. ILWACO	264.3 (19.2)	2.0 (.1)	72.4	.080 (.015)	.089 (.017)
3. LAKE ROOSEVELT	151.2 (11.5)	1.8 (.1)	55.2	.081 (.021)	.085 (.021)
4. MID-COLUMBIA	125.3 (8.8)	1. (.2)	44.8	.069 (.018)	.068 (.017)
5. KOOTENAI	51.9 (4.3)	1.3 (.1)	27.6	.043 (.016)	.043 (.015)

* A LOCUS IS CONSIDERED POLYMORPHIC IF MORE THAN ONE ALLELE WAS DETECTED

** UNBIASED ESTIMATE (SEE NEI, 1978)

calculated at H=O.085 (Setter, unpublished data). As a species then, they cannot be considered highly polymorphic. This presents an immediate concern for inbreeding within the landlocked groups which are isolated, have a small population size and are confined to limited habitat. The individuals examined from the Kootenai river showed the lowest average heterozygosity and have been geographically isolated for the longest period of time. Without the aid of earlier electrophoretic data, the genetic makeup of the original founders can only be hypothesized, but most likely approached the higher average variability seen for the species as a whole within the Columbia River.

The observed and expected allele frequency values according to Hardy-Weinburg equilibrium were tested by loci within each of the six areas using a chi-square analysis. The results of this analysis are shown in Table 6. The Chi-square statistic did not reject the null hypothesis that observed and expected genotypic values by loci wcrc the same within a sampling area. Contingency Chi-square table evaluations provided evidence of differentiation bctwecn sampling areas. In enzyme systems where an allele did not occur in all Lucas or where allele frequencies varied, the test statistics were significant (Appendix Table 3). Further electrophoretic comparisons, such as those begun by Bartley (1987) between Acipenserid species are needed to verify the banding pattern interpretation. This data would either substantiate or disprove this study's interpretation of phenotypes observed and how they were defined. The possibility that the banding patterns in several enzyme systems are just being inaccurately estimated by error in genotype assignment does exist. However, if the population is out of Hardy-Weinberg equilibrium, it would have implications on the population changes that are going on within the reservoir environments.

Sample size was small in the Snake rivers (n=22) for good statistical validation of the genetic differences observed. Still, it was large enough to yield a heterozygosity estimate which would probably fall within 1% of an estimate obtained from a large sample of fish since many loci were sampled (German and Renzi, 1979). The number of loci tested is limited by the number of tissues available and the number of substrates which are being used to bring up the various stains. If samples are k?ken from a catch and release situation, a small muscle plug does not allow a broad range of testing. For instance, LDH can be scored in muscle, but there is another locus in heart, and probably another in eye (Bartley et al., 1985) which could be scored if the samples were attainable and the

Table 6. Chi-square values within each area.

POPULATION: SNAKE

LOCUS	OBSERVED CLASS	EXPECTED FREQUENCY	CHI-SQUARE FREQUENCY	SQUARE	DF	P
AAT-1						
	A-A	19	19.102			
	A-B	3	2.795			
	B-B	0	.102			
				.118	1	.731
AH- 1						
	A-A	14	14.450			
	A-B	6	5.100			
	B-3	0	.450			
				.623	1	.430
ALD-1						
	A-A	9	9.091			
	A-B	2	1.818			
	B-3	0	.091			
				.110	1	.740
GD- 1						
	A-A	18	18.182			
	A-B	4	3.636			
	B-B	0	.182			
				.220	1	.639
GPI-2						
	A-A	12	11.636			
	A-3	8	8.727			
	B-B	2	1.636			
				.153	1	.696
LDH-1						
	A-A	5	8.284			
	A-B	17	10.432			
	B-B	0	3.284			
				8.722	1	.003
LGG-1						
	A-A	13	13.018			
	A-B	1	.964			
	B-B	0	.018			
				.019	1	.890
MDH-1						
	A-A	8	8.205			
	A-B	3	2.591			
	B-B	0	.205			
				.274	1	.601
PGM-1						
	A-A	19	18.182			
	A-B	2	3.636			
	B-B	1	.182			
				4.455	1	.035

Table 6. Chi-square values within each area (continued).

POPULATION: ILWACO

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	234	231.441			
	A-B	56	61.483			
	A-C	3	2.635			
	B-B	7	4.083			
	B-C	0	.350			
	c-c	0	.008			
				3.009	3	.390
AH- 1						
	A-A	211	213.660			
	A-B	53	47.680			
	B-B	0	2.660			
				3.287	1	.070
AK-1						
	A-A	300	299.345			
	A-B	19	20.311			
	B-B	1	.345			
				1.333	1	.248
ALD-1						
	A-A	237	235.540			
	A-B	46	47.108			
	A-C	0	1.812			
	B-B	3	2.355			
	B-C	0	.181			
	c-c	1	.003			
				287.208	3	0.000
CK-1						
	A-A	319	319.001			
	A-%	1	.998			
	B-B	0	.001			
				.001	1	.978
CK- 3						
	A-A	171	172.551			
	A-B	so	47.483			
	A-D	15	14.415			
	B-B	3	3.267			
	B-D	O	1.983			
	D-D	1	.301			
				3.799	3	.284
EST-2						
	A-A	26	24.174			
	A-B	S	9.014			
	A-C	2	1.639			
	B-B	3	.840			
	B-C	0	.306			
	c-c	0	.028			
				7.889	3	.048

Table 6. Chi-square values within each area (continued).

GD- 1						
	A-A	235	232.277			
	A-B	29	34.446			
	B-B	4	1.277			
				6.699	1	.010
GPD-1						
	A-A	325	325.107			
	A-B	12	11.786			
	B-B	0	.107			
				.111	1	.739
GPI-1						
	A-A	345	345.156			
	A-B	15	14.688			
	B-B	0	.156			
				.163	1	.686
GPI-2						
	A-A	276	271.267			
	A-B	73	82.465			
	B-B	11	6.267			
				4.743	1	.029
IDH-2						
	A-A	26	25.138			
	A-B	2	3.724			
	B-B	1	.138			
				6.216	1	.013
LDH-1						
	A-A	321	322.056			
	A-B	39	36.888			
	B-B	0	1.056			
				1.181	1	.277
LT- 3						
	A-A	216	216.427			
	A-B	35	34.221			
	A-C	1	.925			
	B-B	1	1.353			
	B-C	0	.073			
	c-c	0	.001			
				.191	3	.979
LT- 1						
	A-A	324	320.910			
	A-B	28	32.281			
	A-C	0	1.899			
	B-B	3	.812			
	B-C	0	.096			
	c-c	1	.003			
				362.493	3	0.000
LGG-1						
	A-A	124	122.704			
	A-B	15	17.662			
	A-C	1	.930			
	B-B	2	.636			
	B-C	0	.067			
	c-c	0	.002			
				3.418	3	.332

Table 6. Chi-square values within each area (continued).

MDH-1						
	A-A	283	283.128			
	A-B	36	35.744			
	B-B	1	1.128			
				.016	1	,898
MDH-2						
	A-A	283	283.014			
	A-B	4	3.972			
	B-B	0	.014			
				. 014	1	. 905
ME- 1						
	A-A	350	350.056			
	A-B	6	5.925			
	A-C	3	2.962			
	B-B	0	. 025			
	B-C	0	. 025			
	c-c	0	.006			
				.058	3	. 996
PGD-1						
	A-A	144	144.081			
	A-B	7	6.838			
	B-B	0	. 081			
				.085	1	.771
PGM-1						
	A-A	303	301.920			
	A-B	40	42.602			
	A-C	6	5.557			
	B-B	3	1.503			
	B-C	0	.392			
	c-c	0	. 026			
				2.107	3	.550
PGM-2						
	A-A	187	187.033			
	A-B	3	2.961			
	A-C	2	1.974			
	B-B	0	. 012			
	B-C	0	.016			
	c-c	0	. 005			
				.033	3	. 998

Table 6. Chi-square values within each area (continued).

POPULATION: LAKE ROOSEVELT

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	151	150.521			
	A-B	38	38.958			
	B-B	3	2.521			
				.116	1	.733
AH- 1						
	A-A	171	171.475			
	A-B	16	15.200			
	A-C	3	2.850			
	B-B	0	.337			
	B-C	0	.126			
	c-c	0	.012			
				.526	3	.913
ALD-1						
	A-A	153	153.285			
	A-B	38	37.430			
	B-B	2	2.285			
				.045	1	.832
cx- 3						
	A-A	38	36.862			
	A-B	3	6.071			
	A-C	6	5.204			
	B-B	2	.250			
	B-C	0	.429			
	c-c	0	.184			
				14.573	3	.002
EST-2						
	A-A	23	22.917			
	A-B	7	7.500			
	A-C	2	1.667			
	B-B	1	.614			
	B-C	0	.273			
	c-c	0	.030			
				.647	3	.886
GAP-l						
	A-A	185	184.083			
	A-B	6	7.833			
	B-B	1	.083			
				10.517	1	.001
GD- 1						
	A-A	181	179.066			
	A-B	3	6.868			
	B-B	2	.066			
				59.000	1	0.000

Table 6. Chi-square values within each area (continued).

GPD-1						
	A-A	175	175.135			
	A-B	10	9.730			
	B-B	0	.135			
				.143	1	.706
GPI-2						
	A-A	119	114.941			
	A-B	56	64.117			
	B-B	13	8.941			
				3.013	1	.083
LDH-1						
	A-A	112	118.588			
	A-B	77	63.825			
	B-B	2	8.588			
				8.139	1	.004
LT- 1						
	A-A	162	160.723			
	A-B	19	21.555			
	B-B	2	.723			
				2.571	1	.109
LGG-1						
	A-A	110	110.035			
	A-B	4	3.930			
	B-B	0	.035			
				.036	1	.849
MDH-1						
	A-A	136	136.801			
	A-B	32	30.500			
	A-C	1	.897			
	B-B	1	1.700			
	B-C	0	.100			
	c-c	0	.001			
				.480	3	.923
PGD-1						
	A-A	102	101.075			
	A-B	19	20.850			
	B-B	2	1.075			
				.968	1	.325
PGM-1						
	A-A	177	176.374			
	A-B	11	12.427			
	A-C	4	3.824			
	B-B	1	.219			
	B-C	0	.135			
	c-c	0	.021			
				3.117	3	.374
PGM-2						
	A-A	187	187.033			
	A-B	3	2.961			
	A-C	2	1.974			
	B-B	0	.012			
	B-C	0	.016			
	c-c	0	.005			
				.033	3	.998

Table 6. Chi-square values within each area (continued).

POPULATION: MID-COLUMBIA

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	111	111.910			
	A-B	22	20.180			
	B-B	0	.910			
				1.081	1	.298
AH-l						
	A-A	57	57.432			
	A-B	19	18.136			
	B-B	1	1.432			
				.175	1	.676
ALD-1						
	A-A	125	125.127			
	A-B	24	23.747			
	B-B	1	1.127			
				.017	1	.896
CK- 3						
	A-A	83	81.186			
	A-B	16	19.627			
	B-B	3	1.186			
				3.484	1	.062
EST-2						
	A-A	36	36.000			
	A-B	9	9.429			
	A-C	3	2.571			
	B-B	1	.617			
	B-C	0	.337			
	c-c	0	.046			
				.711	3	.871
GPD-1						
	A-A	154	153.184			
	A-B	9	10.631			
	B-B	1	.184			
				3.861	1	.049
GPI-1						
	A-A	132	132.007			
	A-B	2	1.985			
	B-B	0	.007			
				.008	1	.931

Table 6. Chi-square values within each area (continued).

GPI-2						
	A-A	**104**	**103.032**			
	A-B	**27**	**28.937**			
	B-B	**3**	**2.032**			
				.600	**1**	**.439**
LDH-1						
	A-A	**112**	**116.122**			
	A-B	**52**	**43.756**			
	B-B	**0**	**4.122**			
				5.821	**1**	**.016**
LT- 1						
	A-A	**143**	**143.060**			
	A-B	**6**	**5.879**			
	B-B	**0**	**.060**			
				.063	**1**	**.802**
LGG-1						
	A-A	**61**	**61.352**			
	A-B	**10**	**9.296**			
	B-B	**0**	**.352**			
				.407	**1**	**.523**
MDH-1						
	A-A	**146**	**146.494**			
	A-B	**18**	**17.012**			
	B-B	**0**	**.494**			
				.553	**1**	**.457**
PGM-1						
	A-A	**115**	**115.674**			
	A-B	**19**	**17.653**			
	B-B	**0**	**.674**			
				.780	**1**	**.377**

Table 6. Chi-square values within each area (continued).

POPULATION: KOOTENAI

LOCUS	CLASS	FREQUENCY	FREQUENCY	SQUARE	DF	P
AH-l	A-A	44	43.860			
	A-B	12	12.281			
	B-B	1	.860			
				.030	1	.863
ALD-1						
	A-A	45	45.696			
	A-B	19	17.608			
	B-B	1	1.696			
				.406	1	.524
GAP-l						
	A-A	58	58.141			
	A-B	6	5.719			
	B-B	0	.141			
				.155	1	.694
GD- 1						
	A-A	63	63.015			
	A-B	2	1.969			
	B-B	0	.015			
				.016	1	.900
GPD-1						
	A-A	54	54.150			
	A-B	6	5.700			
	B-B	0	.150			
				.166	1	.684
GPI-2						
	A-A	56	54.465			
	A-B	7	10.069			
	B-B	2	.465			
				6.039	1	.014
LDH-1						
	A-A	51	51.754			
	A-B	14	12.492			
	B-B	0	.754			
				.947	1	.331
LT- 1						
	A-A	46	46.621			
	A-B	12	10.759			
	B-B	0	.621			
				.772	1	.380

models were precise. Due to the small sample sizes within these three areas, they were not included in either the chi-square goodness of fit or the chi-square contingency table evaluations.

Genetic distance estimates were made from allele frequencies using an unbiased procedure (Nci, 1978). This method showed most areas to be very similar, which was not surprising since it is most useful in finding intcrspecies gene differences (Table 7). However, this method was executed to see if any trends of divergence were apparent genetically between the arcas examined. Genetic distance is sometimes related linearly within a species to geographic distance or arca (Nei, 1972). The calculation for genetic distance used the allele frequency data shown in Appendix Table 2. Kootenai and Snake River white sturgeon produced the higher values, suggesting that geographic isolation has made fish from these regions distinguishable electrophorctically from other Columbia River sturgeon. Mean values for stock differences within a species arc usually within the range of 0.02 - 0.6. Genetic distance values between species usually fall within the range of 0.3 - 0.9. The values obtained suggest that some differences exist between white sturgeon of the Columbia, Snake, and Kootenai rivers, but not enough genetic distance to base a strong argument for consideration as separate stocks.

The sample size of 12 and 10 individuals from the two arcas of the Snake river was considcrcd too small for accurate genetic population structure estimation. Nothing was found, however, that would indicate genotypic diffcrcnces exist between the fish of the Snake River and those in the Columbia below the confluence of the two rivers.

The 65 sturgeon sampled from the Kootcnai were distinguishable genetically by their low level of genetic variation in the enzyme systems examined with electrophoresis. The continued existence of white sturgeon in this completely frcshwatcr environment further supports their broad adaptability. This area is thought to have been isolated from interaction with the Columbia River system since the last glacial age (Alden, 1953), allowing adequate time for the population to evolve a successful non-anadromous niche.

Table 7. Nei (1978) unbiased genetic distance between areas.

POPULATION	1	2	3	4	5
					m_e
1 SNAKE					
2 ILWACO	.006				
3 LAKE ROOSEVELT	.003	,002			
4 MID-COLUMBIA	.003	.001	.001		
5 KOOTENAI	.005	,003	.003	.002	

RFLP examination

The detection of genetic variation within the Columbia River white sturgeon population was possible using southern blots prepared from genomic DNA digests of five restriction enzymes. Our main objective, to look for fixed differences between individuals of different geographic areas was not met. Our findings were able to detect frequency differences in several of the enzymes after hybridization with the 2% probe. The white sturgeon fragment patterns produced a larger quantity than expected. Due to the large number of bands detected, skepticism over the completeness of DNA digestion became the focus of our investigation for several months. We tried using a variation of post hybridization stringency washes by varying temperature and salt concentration. This did not reduce the number of bands noted. We tried a number of different enzyme unit concentrations and also set up test experiments varying the length of the incubation time for the digests. The length of time which gave consistently full digestions was 2 l/2 h although in many instances 1 h was satisfactory. Twenty h was the maximum time interval examined and nothing was observed that varied from the shorter digests. For this reason, 3 h became the standard. The quantity of enzyme used was varied from 5 units to 40 units. The quantity of DNA was kept consistent at approximately 5 ug. Ten units of enzyme was adopted as a standard. A higher frequency of partial digestions did occur when only 5 units of enzyme were used. This could have been due to enzyme quality, error in calculation of DNA concentration, time of digestion or inaccuracy of pipetting devices.

Variation within individuals was noted with the 28s probe when the DNA had been cut with HINC-II, SAU3A-I and SAC-I. BAMH-I and ECOR-I showed homologous fragment patterns among all individuals examined.

Morphometrics and Meristics

Multivariate statistical analysis of the differences in snout shape provided an objective evaluation to substantiate the visual evidence recorded. In a preliminary assessment of this approach (1986), fish were classified visually as pointed-nosed, rounded-nosed, or blunt-nosed, with 18 fish per category. Thirteen interlandmark distances from each fish were calculated between the seven landmarks illustrated. A variance-covariance matrix from log10 transformed data was subjected to a principal component analysis. Coefficients were relatively equal in size on the

first component and separated fish by overall size. This component was not considered further, but accounted for 85% of the variance. The second component (PCII) separated fish by snout shape and accounted for 11% of the variance observed from the variance-covariance matrix. The signs of coefficients in PC II can be used to interpret the multicharacter relationship described by PC II. Namely, five characters had positively-signed coefficients; the remaining characters had negatively-signed coefficients. This is simply interpreted to mean that the five characters contrast with the remaining characters. We interpreted the variation in PC II values as multivariate differences in snout elongation. Mean values of PC II per group were 0.10, 0.0, and -0.10 for the pointed, rounded, and blunt-nose groups in the 1986 preliminary analysis. An analysis of variance of these preliminary (1986) PC II scores by **group** was statistically significant (F=25, P<O.OOl).

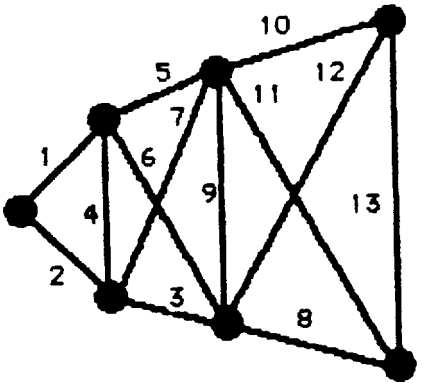
The preliminary test results indicated that snout shape was readily quantifiable by this method, and so it was used in 1987-1988 to identify observed differences more precisely. Photographs that were taken of sturgeon from **the** various **areas** were then digitized and analyzed using multivariate statistics. The data were analyzed by sampling am and tested against green sturgeon. Green sturgeon served as a reference point because all members of the species have a very pointed snout. All green sturgeon were from the mouth of the Columbia River. PC11 values obtained are given by area in Table 8. Lake Roosevelt and Upper Snake head shapes show a strong correlation with those of green sturgeon. Both Kootenai and Upper Snake river white sturgeon head shapes also showed a correlation with those from Lake Roosevelt.

These results could reflect genetic differences between populations in the Columbia, but it is possible that differences are caused environmentally. Ruban and Sokolova (1986), suggested that snout shape was influenced by rapid growth which provides an alternative explanation for the differences observed. Warmer temperatures during early development and the resultant faster growth may be instrumental in varying head shape characteristics. The estuarine area near Ilwaco is thought to provide abundant food and warmer temperatures than arcas further upstream in the Columbia. The quantity of fish harvested in this lower river stretch (over 50,000 annually; Hess and King, 1987) suggests optimal growing conditions. With lower temperatures, growth rates in the Snake River (Coon ct al., 1977) have been shown to be slower than in areas of the lower Columbia River.

Table 8. PC2 values by area.

EIGENVECTORS OF PC2 BY POPULATION
(Based on cumulative data 1986-1988)

	ILWACO	LK ROOS	KOOT	GREEN	U.SNAKE	MID-COL
1	0.198	0.135	-0.157	0.003	-0.038	-0.232
2	-0.369	0.118	0.020	-0.036	-0.173	0.488
3	-0.445	-0.452	0.433	0.354	-0.231	0.158
4	-0.264	0.407	-0.415	-0.40s	0.406	-0.341
5	0.223	-0.438	0.335	0.286	-0.204	0.264
6	0.246	0.191	-0.201	-0.227	0.268	-0.170
7	-0.221	0.228	-0.283	-0.245	0.260	-0.290
a	0.386	-0.349	0.413	0.469	-0.444	0.399
9	0.225	0.279	-0.281	-0.295	0.358	-0.330
10	0.225	-0.308	0.352	0.445	-0.446	0.304
11	0.263	0.019	0.022	-0.017	0.056	-0.042
12	0.193	0.081	-0.065	-0.020	0.076	-0.041
13	0.197	0. 127	-0.064	-0.088	0.190	-0.136



Dorsal scute counts varied throughout the river both between and within areas sampled (Table 9). Analysis of data showed no significant difference between areas. The number of dorsal scutes may vary due to water temperature or growth rate during early rearing and may indicate more about early rearing conditions than genetic variability.

Data from all three character sets were tested for any correlation. No significant correlation between genotype data and either the head shape or scute count data was found. A summary of coefficients obtained and their respective sample size is given in Appendix Table 3.

Table 9. Summary of dorsal scute count statistics.

	Kootenai	Lk.Roosevelt	Mid Columbia	Ilwaco	Snake
Mean	11.750	12.133	11.803	11.853	12.083
StDev	1.035	0.915	1.112	1.077	2.109
(N)	8	15	66	34	12

Analysis of Variance

$H_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$

$H_A = \mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5$

SOURCE	DF	SS	MS	F
FACTOR	4	2.01	0.50	0.35
ERROR	130	186.85	1.44	
TOTAL	134	188.86		

$F_{(0.25,3,123)} = 2.68$ Do not reject Ho , $(p > .25)$

DISCUSSION

Sturgeon are found from the estuary at the mouth of the river, up the Snake River, well into Canada and in the Kootenai drainage. Over 50,000 white sturgeon are harvested annually in the lower areas of the Columbia River while the annual harvest is considerably less as you move farther upstream. However, even above Grand Coulee Dam (NPS unpublished data), approximately 100 white sturgeon are reported caught annually which is probably a minimum estimate of the harvest. The problem is that geographic isolation of population segments was imposed by the hydroelectric development projects. Where fish passage facilities exist they are insufficient for sturgeon use (Bajkov, 195 1). Dams prevent the long, distant movements that were once the sturgeon's normal pattern of migration to feed and spawn (Haynes et al., 1978).

To sustain sturgeon populations in some regions of the upper Columbia system, enhancement measures ma) be necessary. Before enhancement efforts are planned for this species, however, it is recommended that managers determine the genetic makeup of fish that reside in different locations of the river, and then reinforce the population from the resident gene pool if justified. The present survey of the genetic similarity of fish distributed over the five regions selected in the Columbia River system, allowed a range of available habitats to be studied.

The identification of population genetic structure depends on the ability to locate polymorphic enzymes with significant allelic variation among areas or other genetic related morphometric and meristic variation. Phelps and Allendorf (1983) analyzed pallid and shovelnose sturgeon and found 3 polymorphic loci, but no statistically significant allele frequency differences between these species were detected at any of the variable loci. Bartley et al. (1985) found 7 polymorphic loci in white sturgeon from four different river systems in the Pacific Northwest, but were limited by sample size to distinguish major differences.

Evidence for intraspecific groups of sturgeon has been noted in the Delaware River (Dean, 1894) and the southern rivers of the USSR (Gerbilskii, 195 1). Both papers make reference to several peaks of migration and the condition of the gonads at the time of upstream migration. The early fish had immature gonads and were headed for the furthest upstream spawning sites. There was also variation related to upper lethal temperature in the developing eggs of the fish from the various segments of a run (Systina et al., 1985). This shows that family Acipenseridae has adapted to specific environmental conditions by varying timing of their upstream migration. It seems not unrealistic that

that historically a similar type of biological race structure could have existed amongst white sturgeon in the Columbia River.

White sturgeon in the Columbia River were naturally anadromous. The fishery data from the late 1890'~ (Craig and Hacker, 1940) shows consistent large catches from specific areas, suggesting a non-random dispersal pattern. If different subgroups migrated different distances upstream to reach ancestral habitat, dam construction could have trapped them in various reservoirs along the river, and a mixture of upriver subgroups may now be represented within the various reservoirs, and even below Bonneville.

Electrophoresis has been used to identify some differential variation among sturgeon from different areas. While this technique can detect only a percentage of the existing protein variation, electrophoretic analysis has made it possible to differentiate fish populations by both species and stock (Allendorf et al., 1987). The allelic frequency data from Columbia River sturgeon in Appendix Table 2 shows the enzyme systems and the degree of variability in genotype by area. Five systems where variation is different ($p < .05$) from other areas sampled are found in Lake Roosevelt. AH, CK and PGM each have an allele seen only in this area. GPI-1 showed no variation here, while GPI-2 showed higher variability than other areas (Az.768, B=.232). Specific variation was also evident at Ilwaco in enzyme systems: AK, CK, GD, ME, and MDH-2. Gene frequency at LDH (A=.945, B=.055) was different than from the other areas sampled. PGD variation was not seen in the Mid-Columbia samples. Low frequencies are assumed to depict rare alleles because the alternate genotype is not seen in more than 95% of the individuals from a given area.

Hardy Weinburg calculations demonstrated how well the observed sample data fit the classic genetic population equilibrium model. Several systems did not meet model expectations at p=.O5 due to either a high number of heterozygotes (LDH, ALD, GAP) or alternate homozygotes (GD-1, GPI-2) among the individuals observed. Non-conformance to Hardy Weinburg equilibrium frequencies may imply that selective mortality is occurring amongst certain genotypes. Although it is possible that dams have imposed such a change on sturgeon populations in the Columbia, it is more likely that interpretation of the banding patterns for those systems was in error. Because the systems only slightly deviated ($.05 > p > .Ol$) from expectations, we have not placed much significance on the results from the Hardy Weinburg analysis.

Genetic distance values were not of significant magnitude to suggest substantial divergence. The Snake River values compared with Kootenai or Ilwaco were the largest, indicating that geographic area may become an important variable in distinguishing subpopulations. Intuitively, it appeared that fish from the separate areas would prove to be very similar due to up river migrants intermixing in the lower river after dam construction, and in fact they were because no fixed variation occurred between any of the areas sampled.

The broadest diversity of genotypes is found in the samples taken from Ilwaco. This is not surprising since sturgeon from the entire river system are thought to have historically migrated downstream to at least the estuary for the abundant food resources that exist. When hydroelectric development began limiting the migratory ascents and descents by sturgeon in the river, the population trapped below a dam became more diverse due to the accumulation of a mixture of different stocks originating from further upstream. Similarly, as the river was further developed white sturgeon population structure was masked by the mixture of upriver stocks captured in restrictive habitats. Today except for possible passage through boat locks, migration is limited to downstream movement only and in conjunction with straying from other river systems helps to account for the diversity seen at the mouth of the Columbia.

Support of the theory for geographically differential genetic population structure was evident with the samples obtained from the Kootenai River. Electrophoresis showed these fish to lack the quantity of variation found in aU other areas examined. Because these fish are thought to have been isolated since the last glacial age over 10,000 years ago, they would have evolved a relatively fixed genotype.

Therefore, from the fact that genotypic variation decreased from the higher diversity in the main stem to the lower variation in the Kootenai and Snake rivers, we suggest that either a racial structure may have existed historically or that a bottleneck due to the erosion of the quantity of genetic variation within a small population with no new gene flow is now apparent. The lack of identifiably different genotypes between sample areas is interpreted simply to be the result of the hydroelectric developments preventing the segregation of individuals that would have distributed upstream.

While evolution in a long-lived species like white sturgeon progresses quite slowly, all adaptations to environmental changes serve to incorporate important traits into future generations. Data collected through electrophoresis showed little specific variation between the sampling areas of the mainstem Columbia River or the

Snake River. While the quantity of genetic variation between these sampling locations was low, the presence of differential variation cannot be overlooked. Lake Roosevelt, the mid-Columbia and Ilwaco all had alleles not found in other areas in several enzyme systems. By maintaining at least these three groups as distinct reproductive units it will allow the associated variation to remain specific to those regions of the river. Sturgeon can now be viewed as isolated populations residing within a number of different habitats. Each habitat offers slightly varied food resources, substrates, water quality and water velocity, all of which influence adaptive strategy. We feel that the present population structure within each impoundment represents the best gene pool for producing and maintaining future successful generations, This data was grouped for the region below McNary dam to above Bonneville dam and therefore cannot be used to distinguish if any genetic differentiation exists among the present stocks in these three reservoirs.

We conclude sturgeon from the Columbia River, even though they have encountered massive habitat alteration, provide the best genetic stock for enhancement within the river. Genetic changes are expected to occur slowly as the environmental factors to which evolutionary changes respond become altered. If the environmental change is too rapid, the genetic response may not be fast enough and the population may be adversely affected. In the present situation, each isolated group will be responding in a different degree depending on the magnitude of environmental alteration. The low annual catch of sturgeon in certain areas of the Columbia are believed the result of altered environmental conditions which have reduced both spawning success and food availability. While whim sturgeon are available from other river systems such as the Sacramento, environmental conditions are different between rivers and most likely genetic disposition also varies as suggested by Bartley et al. (1985). We think that sturgeon in other isolated systems like the Kootenai R. cannot be represented by a gene pool more suitable than the one which currently exists, primarily because of an inherent adaptability to changing environmental conditions. When distinct phenotypic characteristics exist for genotypically similar individuals, these apparent physical features can be researched and interpreted, and assumed an important adaptation of the stock.

The construction of hydroelectric darns promoted a change in existing habitat and life history strategies of anadromous and resident fish of the Columbia River. In most of the river, sturgeon now reside totally in freshwater and in time unique adaptations to this altered life cycle will evolve. For instance, morphological variation could occur within different areas due to the availability of different prey items. This is particularly important if you

compare the run of the river reservoirs with the storage reservoirs. The longer retention time of water in storage reservoirs favors different food chain ecosystems than a river system. Lake Roosevelt is distinctly different from even Lake Umatilla, the other storage reservoir. Lake Umatilla has a maximum retention time of seven days while Lake Roosevelt at maximum water level may have a retention time up to 45 days (Stober et al., 1979). Sturgeon in pools near fast velocities will need to utilize a different strategy for capturing fish and other prey items than sturgeon residing in deep, slow moving backwater pools. The quicker prey species in clear, non-turbulent water will provide a particular challenge. Miller (1987) has shown sturgeon to be quite ineffective capturing young salmonids under these conditions. Because Acipenserid subspecies have been shown to differ in snout length, mean dorsal and ventral scute counts and gill raker counts between three river systems (Artyukhin and Zarkua, 1986) and within a species (Usynin, 1980). meristic and morphometric traits may be easily affected by variable environments. Hybrids have exhibited intermediate morphological and meristic characters with crosses made between the beluga (Huso huso) and the sevryuga (Apenser stellatus) sturgeon. Crass and Gray (1982) have documented that morphological variation in snout shape exists among white sturgeon in the Columbia, but how that might relate to their life history strategies is uncertain. A genetic basis for the observed snout shape dimorphism could not be proven with the data obtained from the present study. However, the fact remains that differences do exist between the upper and lower river sturgeon and that may suggest a racial segregation of the Columbia River population.

The additional morphometic and meristic data sets collected and interpreted strengthen the argument of rejecting the hypothesis that all fish within the Columbia River are similar and display no differential variation. The morphological characteristic of snout shape varies within the Columbia River. Snout dimorphism of white sturgeon has been reported by Crass and Gray (1980) and Brannon et al (1986) in the Columbia River. Stock specific differences in snout shape and length have been observed in the Siberian sturgeon and also the sterlet (Sokolov et al., 1986), suggesting this may be common for sturgeon species. Carlson et al. (1985) have noted differences in morphometric and meristic characters of pallid and shovelnose sturgeon using a technique similar to the one employed for this study, principal component analysis. While principal component analysis can detect differences, this data would be most beneficial to white sturgeon population management in the Columbia River if a link to genetic or environmental conditions was shown.

Two distinct head shapes were noticed both within the river and between the Columbia and Sacramento rivers. Blunt nosed sturgeon are found primarily in the mid and lower reaches of the Columbia, while long, pointed snouts predominate upriver. White sturgeon in the Sacramento River are usually blunt-nosed resembling the fish of the lower reach of the Columbia River (Ken Beer, personal communication). This seems to lend credence to the theory that warmer water temperature and a faster growth rate may influence snout length as suggested by Ruban and Sokolov (1986). This area of research will be pursued further when a larger selection of research fish are available to learn more about the geographic and ontogenetic elements of snout differentiation.

Meristic data may also be an important area of pursuit as further documentation of phenotypic characteristics in white sturgeon. Scute counts are quite variable throughout the river but exhibit no obvious geographic pattern. Data collected for this study did not show that variability in scute counts was significant between the areas sampled. ConsequentIy, there is no indication that scute counts could be used as an indice for determining what area of the Columbia River a sturgeon resided, Evidence that a warm water culture environment causes a reduction in the total number of scutes and fin rays has been documented for two Russian Acipenser species (Ruban and Sokolov, 1986) but has not yet been evaluated for white sturgeon.

The DNA evaluation with the ribosomal probes 18s and 28s disclosed no information relevant to the task undertaken. We were searching for a sensitive tool to discriminate fish between the geographic areas. The two DNA probes used provided an examination of individual fish differences, but no markers were evident between geographic locations. This work suggests that fingerprinting probes would be more successful in providing information on population differences.

In our study, protein electrophoresus was the more definitive tool for use in population separation. Should a need arise for enhancement efforts to sustain or rebuild the sturgeon population in the Columbia River, the information provided here from protein electrophoresis can be used to substantiate that some between area variation exists, suggesting that subpopulations of sturgeon inhabited the historical river system. While snout shape was variable between regions and could be quantified, no relationship to confirm a stock structure has yet been shown. Further in-depth investigation of remote locations such as the Kootenai River will provide a broader view of all characteristics that may be representative of any white sturgeon population over time. Our recommendation is that

any genetic stock used in enhancement programs should, of course, closely mimic the existing gene pool of the area being considered and the best representation will be obtained from the resident fish.

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APPENDIX

Appendix Table 1

Appendix Table 1. List by system showing mobility of alternate alleles.

System	Allele 1	Allele 2	Allele 3	Allele 4
AAT-1	-100	-62	-175	
AH-1	100	145	167	
AK- 1	100	140		
ALD-1	-100	-106	-93	
CK-1	100	-244		
CK-3	100	107	96	91
EST-2	100	138	8.5	
GD- 1	100	106		
GAP-1	100	112		
GPD- 1	100	94		
GPI- 1	100	-127		
GPI-2	100	76		
IDH-2	100	79		
LDH- 1	-100	-27 1		
LGG- 1	100	78		
LT- 1	100	88	108	
LT-3	100	92		
MDH-1	100	136		
MDH-2	100	84		
ME-1	100	92	105	
PGD	100	114		
PGM- 1	100	91	107	
PGM-2	100	88		

**The common allele is designated as 100 and alternate alleles are a measure of their percent migration distance in proportion with the common.

Appendix Table 2

ALLELE FREQUENCIES

	POPULATION				
LOCUS	SNAKE	ILWACO	LK. ROOS	MID-C	KOOT
AAT-1					
(N)	22	300	192	133	65
A	.932	.878	.885	.917	1.000
B	.068	.117	.115	.083	0.000
C	0.000	.005	0.000	0.000	0.000
ADA-l					
(N)	13	91	150	84	65
A	1.000	1.000	1.000	1.000	1.000
ADA-2					
(N)	15	92	150	84	65
A	1.000	1.000	1.000	1.000	1.000
AH- 1					
(N)	20	264	190	77	57
A	.850	.900	.950	.864	.877
B	.150	.100	.042	.136	.123
C	0.000	0.000	.008	0.000	0.000
AK- 1					
(N)	11	320	188	164	62
A	1.000	.967	1.000	1.000	1.000
B	0.000	.033	0.000	0.000	0.000
ALD-1					
(N)	11	287	193	150	65
A	.909	.906	.891	.913	.838
B	.091	.091	.109	.087	.162
C	0.000	.003	0.000	0.000	0.000
CK- 1					
(N)	11	320	194	164	65
A	1.000	.998	1.000	1.000	1.000
B	0.000	.002	0.000	0.000	0.000
CK- 2					
(N)	4	320	174	164	57
A	1.000	1.000	1.000	1.000	1.000
CK- 3					
(N)	2	240	49	102	1
A	1.000	.848	.867	.892	1.000
B	0.000	117	.071	.108	0.000
C	0.000	0: 000	.061	0.000	0.000
D	0.000	.035	0.000	0.000	0.000
EST-1					
(N)	15	360	192	164	65
A	1.000	1.000	1.000	1.000	1.000

EST-2					
(N)	2	36	33	49	1
A	1.000	.819	.833	.857	1.000
B	0.000	.153	.136	.112	0.000
C	0.000	.028	.030	.031	0.000

GAP-l					
(N)	22	330	192	111	64
A	1.000	1.000	.979	1.000	.953
B	0.000	0.000	.021	0.000	.047

GD- 1					
(N)	22	268	186	50	65
A	.909	.931	.981	1.000	.985
B	.091	.069	.019	0.000	.015

GPD-1					
(N)	22	337	185	164	60
A	1.000	.982	.973	.966	.950
B	0.000	.018	.027	.034	.050

GPI-1					
(N)	22	360	196	134	65
A	1.000	.979	1.000	.993	1.000
B	0.000	.021	0.000	.007	0.000

GPI-2					
(N)	22	360	188	134	65
A	.727	.868	,782	.877	.915
B	.273	.132	.218	.123	.085

IDH-1					
(N)	15	213	149	164	57
A	1.000	1.000	1.000	1.000	1.000

IDH-2					
(N)	2	29	1	1	1
A	1.000	.931	1.000	1.000	1.000
B	0.000	.069	0.000	0.000	0.000

LDH-1					
(N)	22	360	191	164	65
A	.614	.946	.788	.841	.892
B	.386	.054	.212	.159	.108

LT- 3					
(N)	15	253	1	50	57
A	1.000	.925	1.000	1.000	1.000
B	0.000	.073	0.000	0.000	0.000
C	0.000	.002	0.000	0.000	0.000

LT- 1					
(N)	13	356	183	149	58
A	1.000	.949	.937	.980	.897
B	0.000	.048	.063	020	103
C	0.000	.003	0.000	0: 000	0: 000

LGG-1					
(N)	14	142	114	71	1
A	.964	.930	.982	.930	1.000
B	036	.067	.018	.070	0.000
C	0: 000	.004	0.000	0.000	0.000

MAN-l					
(N)	4	199	52	164	57
A	1.000	1.000	1.000	1.000	1.000

MDH-1					
(N)	11	320	170	164	65
A	.864	.941	.897	.945	1.000
B	.136	.059	.100	.055	0.000
C	0.000	0.000	.003	0.000	0.000

MDH-2					
(N)	4	287	170	164	65
A	1.000	.993	1.000	1.000	1.000
B	0.000	.007	0.000	0.000	0.000

ME- 1					
(N)	22	359	193	152	56
A	1.000	.987	1.000	1.000	1.000
B	0.000	.008	0.000	0.000	0.000
C	0.000	.004	0.000	0.000	0.000

PGD-1					
(N)	4	151	123	164	63
A	1.000	.977	[illegible]	1.000	1.000
B	0.000	.023	[illegible]	0.000	0.000

PGM-1					
(N)	22	352	193	134	65
A	.909	.926	.956	.929	1.000
B	.091	.065	.034	.071	0.000
C	0.000	.009	.010	0.000	0.000

PGM-2					
(N)	22	360	192	164	9
A	1.000	1.000	.987	1.000	1.000
B	0.000	0.000	.008	0.000	0.000
C	0.000	0.000	.005	0.000	0.000

Appendix Table 3

CONTINGENCY CHI-SQUARE TABLE

LOCUS: AAT-1

POPULATION		ALLELE A	ALLELE B	ALLELE C
SNAKE	OBS (0)	41.000	3.000	0.000
	EXP (E)	39.612	4.295	.093
	(O-E)**2 / E	.049	.390	.093
ILWACO	OBS (0)	527.000	70.000	3.000
	EXP (E)	540.169	58.567	1.264
	(O-E)**2 / E	.321	2.232	2.384
LAKE ROOSEVELT	OBS (0)	340.000	44.000	0.000
	EXP (E)	345.708	37.483	.809
	(O-E)**2 / E	.094	1.133	.809
MID-COLUMBIA	OBS (0)	244.000	22.000	0.000
	EXP (E)	239.475	25.965	.560
	(O-E)**2 / E	.086	.605	.560
KOOTENAI	OBS (0)	130.000	0.000	0.000
	EXP (E)	117.037	12.690	.274
	(O-E)**2 / E	1.436	12.690	.274

CHI-SQUARE = 23.155
D.F. = 8
P = .00317

CONTINGENCY CHI-SQUARE TABLE

LOCUS: AH- 1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	34.000	6.000	0.000
	EXP (E)	36.283	3.618	. 099
	(O-E)**2 / E	. 144	1.568	. 099
ILWACO	OBS (0)	475.000	53.000	0.000
	EXP (E)	478.934	47.763	1.303
	(O-E)**2 / E	. 032	.574	1.303
LAKE ROOSEVELT	OBS (0)	361.000	16.000	3.000
	EXP (E)	344.688	34.375	. 937
	(O-E)**2 / E	.772	9.822	4.538
MID-COLUMBIA	OBS (0)	133.000	21.000	0.000
	EXP (E)	139.689	13.931	. 380
	(O-E)**2 / E	. 320	3.587	. 380
KOOTENAI	OBS (0)	100.000	14.000	0.000
	EXP (E)	103.406	10.313	.281
	(O-E)**2 / E	. 112	1.319	.281

CHI-SQUARE = 24.850
D.F. = 8
P = .00165

CONTINGENCY CHI-SQUARE TABLE

LOCUS: AK- 1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	22.000	0.000
	EXP (E)	21.690	. 310
	(O-E)**2 / E	. 004	. 310
ILWACO	OBS (0)	619.000	21.000
	EXP (E)	630.980	9. 0.20
	(O-E)**2 / E	.227	15.911
LAKE ROOSEVELT	OBS (0)	376.000	0.000
	EXP (E)	370.701	5.299
	(O-E)**2 / E	. 076	5.299
MID-COLUMBIA	OBS (0)	328.000	0.000
	EXP (E)	323.377	4.623
	(O-E)**2 / E	. 066	4.623
KOOTENAI	OBS (0)	124.000	0.000
	EXP (E)	122.252	1.748
	(O-E)**2 / E	.025	1.748

CHI-SQUARE = 28.289
D.F. = 4
P = . 00001

CONTINGENCY CHI-SQUARE TABLE

LOCUS: ALD-1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	20.000	2.000	0.000
	EXP (E)	19.741	2.228	. 031
	(O-E)**2 / E	. 003	. 023	. 031
ILWACO	OBS (0)	520.000	52.000	2.000
	EXP (E)	515.055	58.132	.813
	(O-E)**2 / E	. 047	. 647	1.733
LAKE ROOSEVELT	OBS (0)	344.000	42.000	0.000
	EXP (E)	346.361	39.092	.547
	(O-E)**2 / E	. 016	. 216	. 547
MID-COLUMBIA	OBS (0)	274.000	26.000	0.000
	EXP (E)	269.193	30.382	,425
	(O-E)**2 / E	. 086	. 632	. 425
KOOTENAI OBS	(0)	109.000	21.000	0.000
	EXP (E)	116.650	13.166 .	184
	(O-E)**2 / E	. 502	4.662 .	184

CHI-SQUARE = 9.755
D.F. = 8
P = .28266

CONTINGENCY CHI-SQUARE TABLE

LOCUS: CK- 1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	22.000	0.000
	EXP (E)	21.985	.015
	(O-E)**2 / E	.000	.015
ILWACO	OBS (0)	639.000	1.000
	EXP (E)	639.576	,424
	(O-E)**2 / E	.001	.781
LAKE ROOSEVELT	OBS (0)	388.000	0.000
	EXP (E)	387.743	.257
	(O-E)**2 / E	.000	.257
MID-COLUMBIA	OBS (0)	328.000	0.000
	EXP (E)	327.782	.218
	(O-E)**2 / E	.000	.218
KOOTENAI	OBS (0)	130.000	0.000
	EXP (E)	129.914	.086
	(O-E)**2 / E	.000	.086

CHI-SQUARE = 1.357
D.F. = 4
P = .85161

CONTINGENCY CHI-SQUARE TABLE

LOCUS: CK- 3

POPULATION		ALLELE A	B	C	D
SNAKE	OBS (0)	4.000	0.000	0.000	0.000
	EXP (E)	3.452	.431	.030	.086
	(O-E)**2 / E	.087	.431	.030	.086
ILWACO	OBS (0)	407.000	56.000	0.000	17.000
	EXP (E)	414.213	51.777	3.655	10.355
	(O-E)**2 / E	.126	.344	3.655	4.264
LAKE ROOSEVELT	OBS (0)	85.000	7.000	6.000	0.000
	EXP (E)	84.569	10.571	.746	2.114
	(O-E)**2 / E	.002	1.206	36.991	2.114
MID-COLUMBIA	OBS (0)	182.000	22.000	0.000	0.000
	EXP (E)	176.041	22.005	1.553	4.401
	(O-E)**2 / E	.202	.000	1.553	4.401
KOOTENAI	OBS (0)	2.000	0.000	0.000	0.000
	EXP (E)	1.726	.216	.015	,043
	(O-E)**2 / E	.044	.216	.015	.043

CHI-SQUARE = 55.811
D.F. = 12
P = .00000

CONTINGENCY CHI-SQUARE TABLE

LOCUS: EST-2

POPULATION		ALLELE A	ALLELE B	ALLELE C
SNAKE	OBS (0)	4.000	0.000	0.000
	EXP (E)	3.372	.512	. 116
	(O-E)**2 / E	. 117	.512	. 116
ILWACO	OBS (0)	59.000	11.000	2.000
	EXP (E)	60.694	9.223	2.083
	(O-E)**2 / E	. 047	. 342	.003
LAKE ROOSEVELT	OBS (0)	55.000	9.000	2.000
	EXP (E)	55.636	8.455	1.909
	(O-E)**2 / E	.007	. 035	. 004
MID-COLUMBIA	OBS (0)	84.000	11.000	3.000
	EXP (E)	82.612	12.554	2.835
	(O-E)**2 / E	. 023	. 192	. 010
KOOTENAI	OBS (0)	2.000	0.000	0.000
	EXP (E)	1.686	. 256	.058
	(O-E)**2 / E	. 058	,256	.058

CHI-SQUARE = 1.783
D.F. = 8
P = .98697

CONTINGENCY CHI-SQUARE TABLE

LOCUS: GAP-l

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	44.000	0.000
	EXP (E)	43.572	.428
	(O-E)**2 / E	. 004	. 428
ILWACO	OBS (0)	660.000	0.000
	EXP (E)	653.574	6.426
	(O-E)**2 / E	.063	6.426
LAKE ROOSEVELT	OBS (0)	376.000	8.000
	EXP (E)	380.261	3.739
	(O-E)**2 / E	. 048	4.858
MID-COLUMBIA	OBS (0)	222.000	0.000
	EXP (E)	219.839	2.161
	(O-E)**2 / E	. 021	2.161
KOOTENAI	OBS (0)	122.000	6.000
	EXP (E)	126.754	1.246
	(O-E)**2 / E	. 178	18.135

CHI-SQUARE = 32.322
D.F. = 4
P = .00000

CONTINGENCY CHI-SQUARE TABLE

LOCUS: GD- 1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	40.000	4.000
	EXP (E)	42.139	1.861
	(O-E)**2 / E	.109	2.458
ILWACO	OBS (0)	499.000	37.000
	EXP (E)	513.327	22.673
	(O-E)**2 / E	.400	9.052
LAKE ROOSEVELT	OBS (0)	365.000	7.000
	EXP (E)	356.264	15.736
	(O-E)**2 / E	.214	4.850
MID-COLUMBIA	OBS (0)	100.000	0.000
	EXP (E)	95.770	4.230
	(O-E)**2 / E	.187	4.230
KOOTENAI	OBS (0)	128.000	2.000
	EXP (E)	124.501	5.499
	(O-E)**2 / E	.098	2.227

CHI-SQUARE = 23.824
D.F. = 4
P = .00009

CONTINGENCY CHI-SQUARE TABLE

LOCUS: GPD-1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	44.000	0.000
	EXP (E)	42.883	1.117
	(O-E)**2 / E	.029	1.117
ILWACO	OBS (0)	662.000	12.000
	EXP (E)	656.887	17.113
	(O-E)**2 / E	.040	1.528
LAKE ROOSEVELT	OBS (0)	360.000	10.000
	EXP (E)	360.605	9.395
	(O-E)**2 / E	.001	.039
MID-COLUMBIA	OBS (0)	317.000	11.000
	EXP (E)	319.672	8.328
	(O-E)**2 / E	.022	.857
KOOTENAI	OBS (0)	114.000	6.000
	EXP (E)	116.953	3.047
	(O-E)**2 / E	.075	2.862

CHI-SQUARE = 6.570
D.F. = 4
P = .16041

CONTINGENCY CHI-SQUARE TABLE

LOCUS: GPI-1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	44.000	0.000
	EXP (E)	43.519	.481
	(O-E)**2 / E	. 005	.481
ILWACO	OBS (0)	705.000	15.000
	EXP (E)	712.124	7.876
	(O-E)**2 / E	. 071	6.443
LAKE ROOSEVELT	OBS (0)	392.000	0.000
	EXP (E)	387.712	4.288
	(O-E)**2 / E	.047	4.288
MID-COLUMBIA	OBS (0)	266.000	2.000
	EXP (E)	265.068	2.932
	(O-E)**2 / E	. 003	,296
KOOTENAI	OBS (0)	130.000	0.000
	EXP (E)	128.578	1.422
	(O-E)**2 / E	. 016	1.422

CHI-SQUARE = 13.074
D.F. = 4
P = .01092

CONTINGENCY CHI-SQUARE TABLE

LOCUS: GPI-2

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	32.000	12.000
	EXP (E)	37.334	6.666
	(O-E)**2 / E	,762	4.269
ILWACO	OBS (0)	625.000	95.000
	EXP (E)	610.923	109.077
	(O-E)**2 / E	.324	1.817
LAKE ROOSEVELT	OBS (0)	294.000	82.000
	EXP (E)	319.038	56.962
	(O-E)**2 / E	1.965	11.005
MID-COLUMBIA	OBS (0)	235.000	33.000
	EXP (E)	227.399	40.601
	(O-E)**2 / E	.254	1.423
KOOTENAI	OBS (0)	119.000	11.000
	EXP (E)	110.306	19.694
	(O-E)**2 / E	.685	3.838

CHI-SQUARE = 26.343
D.F. = 4
P = .00003

CONTINGENCY CHI-SQUARE TABLE

LOCUS: LDH-1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	27.000	17.000
	EXP (E)	38.431	5.569
	(O-E)**2 / E	3.400	23.467
ILWACO	OBS (0)	681.000	39.000
	EXP (E)	628.878	91.122
	(O-E)**2 / E	4.320	29.814
LAKE ROOSEVELT	OBS (0)	301.000	81.000
	EXP (E)	333.655	48.345
	(O-E)**2 / E	3.196	22.056
MID-COLUMBIA	OBS (0)	276.000	52.000
	EXP (E)	286.489	41.511
	(O-E)**2 / E	. 384	2.650
KOOTENAI	OBS (0)	116.000	14.000
	EXP (E)	113.547	16.453
	(O-E)**2 / E	. 053	. 366

CHI-SQUARE = 89.706
D.F. = 4
P = 0.00000

CONTINGENCY CHI-SQUARE TABLE

LOCUS: LT- 3

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	30.000	0.000	0.000
	EXP (E)	28.484	1.476	,040
	(O-E)**2 / E	. 081	1.476	. 040
ILWACO	OBS (0)	468.000	37.000	1.000
	EXP (E)	480.431	24.896	. 673
	(O-E)**2 / E	. 322	5.884	. 159
LAKE ROOSEVELT	OBS (0)	2.000	0.000	0.000
	EXP (E)	1.899	. 098	. 003
	(O-E)**2 / E	.005	. 098	.003
MID-COLUMBIA	OBS (0)	100.000	0.000	0.000
	EXP (E)	94.947	4.920	. 133
	(O-E)**2 / E	. 269	4.920	. 133
KOOTENAI	OBS (0)	114.000	0.000	0.000
	EXP (E)	108.239	5.609	.152
	(O-E)**2 / E	.307	5.609	.152

CHI-SQUARE = 19.458
D.F. = 8
P = .01260

CONTINGENCY CHI-SQUARE TABLE

LOCUS: LT- 1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	26.000	0.000	0.000
	EXP (E)	24.681	1.285	. 034
	(O-E)**2 / E	. 070	1.285	. 034
ILWACO	OBS (0)	676.000	34.000	2.000
	EXP (E)	675.884	35.178	. 938
	(O-E)**2 / E	. 000	. 039	1.202
LAKE ROOSEVELT	OBS (0)	343.000	23.000	0.000
	EXP (E)	347.435	18.083	.482
	(O-E)**2 / E	. 057	1.337	. 482
MID-COLUMBIA	OBS (0)	292.000	6.000	0.000
	EXP (E)	282.884	14.723	. 393
	(O-E)**2 / E	. 294	5.168	. 393
KOOTENAI	OBS (0)	104.000	12.000	0.000
	EXP (E)	110.116	5.731	. 153
	(O-E)**2 / E	. 340	6.857	. 153

CHI-SQUARE = 17.711
D.F. = 8
P = .02350

CONTINGENCY CHI-SQUARE TABLE

LOCUS: LGG-1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	27.000	1.000	0.000
	EXP (E)	26.567	1.392	.041
	(O-E)**2 / E	.007	.110	.041
ILWACO	OBS (0)	264.000	19.000	1.000
	EXP (E)	269.468	14.117	.415
	(O-E)**2 / E	.111	1.689	.824
LAKE ROOSEVELT	OBS (0)	224.000	4.000	0.000
	EXP (E)	216.333	11.333	.333
	(O-E)**2 / E	.272	4.745	.333
MID-COLUMBIA	OBS (0)	132.000	10.000	0.000
	EXP (E)	134.734	7.058	.208
	(O-E)**2 / E	.055	1.226	.208
KOOTENAI	OBS (0)	2.000	0.000	0.000
	EXP (E)	1.898	.099	.003
	(O-E)**2 / E	.006	.099	.003

CHI-SQUARE = 9.729
D.F. = 8
P = .28458

CONTINGENCY CHI-SQUARE TABLE

LOCUS: MDH-1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	19.000	3.000	0.000
	EXP (E)	20.584	1.401	.015
	(O-E)**2 / E	.122	1.824	.015
ILWACO	OBS (0)	602.000	38.000	0.000
	EXP (E)	598.795	40.767	.438
	(O-E)**2 / E	.017	.188	.438
LAKE ROOSEVELT	OBS (0)	305.000	34.000	1.000
	EXP (E)	318.110	21.658	.233
	(O-E)**2 / E	.540	7.034	2.527
MID-COLUMBIA	OBS (0)	310.000	18.000	0.000
	EXP (E)	306.882	20.893	.225
	(O-E)**2 / E	.032	.401	.225
KOOTENAI	OBS (0)	130.000	0.000	0.000
	EXP (E)	121.630	8.281	.089
	(O-E)**2 / E	.576	8.281	.089

CHI-SQUARE = 22.308
D.F. = 8
P = .00438

CONTINGENCY CHI-SQUARE TABLE

LOCUS: MDH-2

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	8.000	0.000
	EXP (E)	7.977	. 023
	(O-E)**2 / E	. 000	. 023
ILWACO	OBS (0)	570.000	4.000
	EXP (E)	572.336	1.664
	(O-E)**2 / E	. 010	3.280
LAKE ROOSEVELT	OBS (0)	340.000	0.000
	EXP (E)	339.014	.986
	(O-E)**2 / E	. 003	. 986
MID-COLUMBIA	OBS (0)	328.000	0.000
	EXP (E)	327.049	. 951
	(O-E)**2 / E	. 003	. 951
KOOTENAI	OBS (0)	130.000	0.000
	EXP (E)	129.623	. 377
	(O-E)**2 / E	. 001	. 377

CHI-SQUARE = 5.633
D.F. = 4
P = . 22828

CONTINGENCY CHI-SQUARE TABLE

LOCUS: ME- 1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	44.000	0.000	0.000
	EXP (E)	43.747	. 169	. 084
	(O-E)**2 / E	. 001	. 169	. 084
ILWACO	OBS (0)	709.000	6.000	3.000
	EXP (E)	713.868	2.754	1.377
	(O-E)**2 / E	. 033	3.824	1.912
LAKE ROOSEVELT	OBS (0)	386.000	0.000	0.000
	EXP (E)	383.779	1.481	. 740
	(O-E)**2 / E	. 013	1.481	. 740
MID-COLUMBIA	OBS (0)	304.000	0.000	0.000
	EXP (E)	302.251	1.166	.583
	(O-E)**2 / E	. 010	1.166	.583
KOOTENAI	OBS (0)	112.000	0.000	0.000
	EXP (E)	111.355	.430	.215
	(O-E)**2 / E	.004	.430	.215

CHI-SQUARE = 10.666
D.F. = 8
P = .22136

CONTINGENCY CHI-SQUARE TABLE

LOCUS: PGD-1

POPULATION		ALLELE A	ALLELE B
SNAKE	OBS (0)	8.000	0.000.
	EXP (E)	7.762	. 238
	(O-E)**2 / E	. 007	.238
ILWACO	OBS (0)	295.000	7.000
	EXP (E)	293.030	8.970
	(O-E)**2 / E	. 013	.433
LAKE ROOSEVELT	OBS (0)	223.000	23.000
	EXP (E)	238.693	7.307
	(O-E)**2 / E	1.032	33.704
MID-COLUMBIA	OBS (0)	328.000	0.000
	EXP (E)	318.257	9.743
	(O-E)**2 / E	.298	9.743
KOOTENAI	OBS (0)	126.000	0.000
	EXP (E)	122.257	3.743
	(O-E)**2 / E	. 115	3.743

CHI-SQUARE = 49.325
D.F. = 4
P = .00000

CONTINGENCY CHI-SQUARE TABLE

LOCUS: PGM-1

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	40.000	4.000	0.000
	EXP (E)	41.358	2.355	.287
	(O-E)**2 / E	. 045	1.149	,287
ILWACO	OBS (0)	652.000	46.000	6.000
	EXP (E)	661.723	37.681	4.595
	(O-E)**2 / E	. 143	1.836	. 429
LAKE ROOSEVELT	OBS (0)	369.000	13.000	4.000
	EXP (E)	362.820	20.661	2.520
	(O-E)**2 / E	.105	2.840	. 870
MID-COLUMBIA	OBS (0)	249.000	19.000	0.000
	EXP (E)	251.906	14.345	1,749
	(O-E)**2 / E	. 034	1.511	1.749
KOOTENAI	OBS (0)	130.000	0.000	0.000
	EXP (E)	122.193	6.958	.849
	(O-E)**2 / E	.499	6.958	.849

CHI-SQUARE = 19.304
D.F. = 8
P = .01332

CONTINGENCY CHI-SQUARE TABLE

LOCUS: PGM-2

POPULATION		ALLELE A	B	C
SNAKE	OBS (0)	44.000	0.000	0.000
	EXP (E)	43.853	. 088	. 059
	(O-E)**2 / E	. 000	. 088	. 059
ILWACO	OBS (0)	720.000	0.000	0.000
	EXP (E)	717.590	1.446	.964
	(O-E)**2 / E	. 008	1.446	.964
LAKE ROOSEVELT	OBS (0)	379.000	3.000	2.000
	EXP (E)	382.715	.771	.514
	(O-E)**2 / E	. 036	6.443	4.295
MID-COLUMBIA	OBS (0)	328.000	0.000	0.000
	EXP (E)	326.902	. 659	. 439
	(O-E)**2 / E	. 004	. 659	.439
KOOTENAI	OBS (0)	18.000	0.000	0.000
	EXP (E)	17.940	. 036	. 024
	(O-E)**2 / E	. 000	. 036	. 024

CHI-SQUARE = 14.502
D.F. = 8
P = .06959