Columbia River White Sturgeon (Acipenser transmontanus)

Population Genetics and Early Life History Study

Final Report
1986

DOE/BP-18952-2

December 1986

This Document should be cited as follows:

Brannon, E., G. Winans, L. Carperter, S. Brewer, F. Utter, W. Hershberger, A. Setter, M. Miller, "Columbia River White Sturgeon (Acipenser transmontanus)", Project No. 1983-31600, 64 electronic pages, (BPA Report DOE/BP-18952-2)

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P.O. Box 3621
Portland, Oregon 97208

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

COLUMBIA RIVER WHITE STURGEON

(Acipenser transmontanus)

POPULATION GENETICS and EARLY LIFE HISTORY STUDY

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Final Report

January 1, 1986 to December 31, 1986

to

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Project 83-316
Contract No. DE-AI79-84BP18952

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Acknowledgements

The success of our field efforts to obtain tissue samples for electrophoresis was due to many individuals and agencies. We wish to thank Mr. Paul Aebersold for sharing his expertise on the use and analysis of electrophoretic data for genetic identification of populations. Many helpful suggestions for good sampling areas and help obtaining samples were given by individuals with the Washington Dept. Fisheries and from Oregon Fish & Wildlife. The Kettle Falls National Park Service rangers collected valuable samples from Lake Roosevelt. Cynthia and Charlene at the Lakeside Grocery in Marcus stored samples in their freezers and made helpful inquiries among fishermen suggesting they donate samples. Jerry Marco, Colville Indian Tribe biologist for his voluntary effort in the sample collection.

Countless sport fishermen along the entire river were generous enough to allow us to sample their fish. Uncle Bobs in Ilwaco was again extremely accomodating in allowing us to sample during their processing.

Particular appreciation goes to Percy and Dan Brigham, commercial Umatilla Indian fishermen who offered much practical knowledge of sturgeon life history, and allowed us to sample their catch as an effort to contribute more knowledge for the management of the sturgeon in the Columbia river.

Thanks to "The Fishery" at Coverts landing for providing laboratory animals for use in the early life history work. Dr. Serge Doroshov was instrumental in making sure we obtained fish for the laboratory work.

Abstract

The 1986 Columbia River white sturgeon investigations continued to assess genetic varibility of sturgeon populations isolated in various areas of the Columbia River, and to examine environmental factors in the habitat that may affect early life history success. Baseline data have been collected for three character sets. Twenty-eight loci have been analyzed for differences using electrophoresis, snout shapes were assessed for multivariate distinction, and scute counts have been examined as an index of variability. Fish that reside in the mid-Columbia and lower river have been sufficiently characterized by electrophoresis to compare with up-river areas. To date, few electrophoretic differences have been identified. However, Lake Roosevelt sturgeon sample size will be increased to determine if some of the observed differences from lower river fish are significant. Snout shape has been shown to be easily quantifiable using the digitizing technique. Scute count data initially indicate that variability exists within as well as between areas. Patterns of differentiation of one or more of these data sets may be used to formulate stock transplant guidelines essential for proper management or enhancement of this species.

The historical habitat available to sturgeon in the Columbia River has changed through the development of hydroelectric projects. Dams have reduced the velocity and turbulance, and increased light penetration in the water column from less silt. These changes have affected the ability of sturgeon to feed and have made them more vulnerable to predation, which appear to have altered the ability of populations isolated in the reservoirs to sustain themselves. Present studies support the theory that both the biological and physical habitat characteristics of the Columbia River are responsible for reduced sturgeon survival, and justify consideration of enhancement initatives above Bonneville to improve sturgeon reproductive success.

INTRODUCTION

Columbia River White Sturgeon (Acipenser transmontanus) Early Life History addressed sections 804 (b) (1) (c) and 804 (e) (8) of the Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program. The specific research conducted on Columbia River white sturgeon has addressed early life history and stock identification needs which were recognized by the White Sturgeon Research Needs Workshop held at Seattle in November, 1983 (Fickeisen et al. 1984) as high priority for this species.

Differentiating stocks of white sturgeon in the Columbia River system, and applying such information in management of the species, continues to be a challenge. Historically, white sturgeon made good use of anadromy in spawning and feeding migrations between the estuary and upper river. Thousands of sturgeon were able to feed in the rich marine environment adjacent to the river mouth and coastal areas, and return to the river for spawning and early rearing. Free river access changed with the advent of hydroelectric development. Although white sturgeon are still distributed throughout the river system, they are now isolated and their movement limited within the series of impoundments that make up most of the river. They reproduce in some of the impoundments, but how well they have adapted to environmental changes and what their long-range status will be is uncertain.

Columbia River white sturgeon have become one of the more important species in the Columbia. Sport and commercial fishermen harvested nearly 60,000 sturgeon in 1985. The fishery below Bonneville accounts for most of the harv est and has been able to sustain an annual catch of from 30,000 to 56,000 fish since 1979 (Ring, 1983). The status of the species above Bonneville is largely unknown and in some cases populations may not not be able to sustain any harvest. To manage the populations, the species life history and underlying genetic population structure must be understood. Enhancement and harvest measures can then be developed that apply to the needs of the populations distributed throughout the system.

The present report is the third in a research series examining the early life history and genetics of Columbia River white sturgeon. The first study investigated spawning and incubation, larvae and fry distribution behavior, and fry feeding responses. In 1986, the life history studies were extended, and stock identification by electrophoretic examination of allelic frequencies between populations was initiated. This most recent phase has enhanced the genetic database and identified habitat characteristics that encourage success of early life history stages. **The objectives** of the study were to:

1. Examine genetic variability and degree of isolation of white sturgeon populations in the Columbia River.

2. Examine the effect of light and cover in simulated river flows on feeding success and predator avoidance of young white sturgeon.

Investigations involving field samples were made possible by the cooperative assistance of the Umatilla Indian Tribe, Washington Department of Fisheries, Oregon Department of Fish and Wildlife, National Marine Fisheries Service, National Park Service and "The Fishery" at Coverts Landing.

TASK I

Genetic variability of Columbia River white sturgeon populations.

STATEMENT OF PROBLEM

Large numbers of white sturgeon are harvested annually in the lower areas of the Columbia River while the annual harvest is considerably less in upstream areas (Kreitman, personal communication: preliminary WDF data). Population isolation has been forced on the fish to some degree by the hydroelectric development projects that have taken place in the system. Dams prevent long, distant movements that have been the sturgeon's normal pattern of feeding and spawning (Haynes 1978), and also interrupt the normal maturation cycle which can result in infertility of the spawn (Gerbilsky 1959; Votinov and Kas'yanov 1979). To sustain sturgeon populations in the Columbia, enhancement measures are believed necessary in some regions of the upper system. Before enhancement efforts can be planned for this species, however, it is extremely important to know 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. Since the fish are long lived, the genetic makeup of populations presently inhabiting the Columbia River is believed to represent the same gene pool that existed in predam years. The present field sampling program is a survey of the genetic similarity of fish distributed over the Columbia River system.

Objective 1: Examine genetic variability and degree of isolation of white **sturgeon** populations on the Columbia River.

Null Hypothesis: The genetic composition of the Columbia River white sturgeon is the same over its entire range.

DESCRIPTION OF STUDY AREA

The mouth of the Columbia and the resultant estuarine habitat provide an area which apparently is extremely suitable for white sturgeon. The fishery in this **area** shows no sign of decline, and sustains a high annual catch rate. For the past two years no difficulty has been experienced at IIwaco, WA obtaining fish for tissue samples. Fishing seems to be best in the early summer months.

The area we identify as the mid-Columbia region for sampling purposes encompassed the river from the forebay of Bonneville dam to the tailrace of McNary dam. Sixty-five samples were taken in 1986 from fish caught in the commercial fishery at Three-Mile Canyon and Arlington. Samples were were taken from fish that ranged in length from 48 - 72 inches. This portion of river supports an increasing commercial fishery, largely in Lake Umatilla.

The area identified as the upper river was exclusively Lake Roosevelt. Grand Coulee was flooded in 1941 and confined the sturgeon population above that point to Lake Roosevelt and the river area upstream. Sport fishing is heavy during the water drawdown in the spring,

Appendix Table 3. Chi-square contingency table statistics for entire river.

Locus	No. of alleles	Chi-square	D.F.
AAT-1	2	1.02	3
AH-l	3	5.69	3
AK-l	2	6.88	3
ALDl	2	0.74	3
CK-3	3	0.81	2
EST-2	3	1.07	2
GD-1	2	5.24	3
GPD-1	2	0.99	2
GPI- 1	2	0.38	1
GPI-2	2	19.14 **	3
LDH- 1	2	25.42 **	1
LT-1	2	3.30	2
LT-3	2	2.91	2
MDH-1	2	4.17	2
MDH-2	2	5.83	3
ME-1	2	2.82	3
PGM-1	3	1.81	3

Appendix Table 5. Systems where ANOVA indicated a significant difference between areas. In all cases except LDH and GPI-2 an allelic differentiation was apparent. In LDH and GPI-2, several areas had an excess of heterozygotes.

Enzyme system	F stat	Table value @ (.05)
AH-l	2.783	> 2.40
AK-1	3.474	> 3.02
CK-3	8.555	> 3.03
GPD- 1	7.290	> 2.39
GPI-2	4.756	> 2.39
LDH- 1	11.324	> 2.39
MDH-2	2.930	> 2.63

mainly because with the lower water level the river channel velocity is increased and fish are concentrated in fewer deep pools. Sturgeon are known to be late spring spawners in lower areas of the Columbia River (Stockley, 1981). In the upper river spawning may be later to coincide with increasing water levels and possibly reduced flow in the storage reservoir. Sturgeon approximately 14-16" long have been caught at a few of the popular fishing areas, indicating that some reproduction is taking place in the lake or river above. One sportsman mentioned having caught a fish apparently tagged in British Columbia, which indicates that some movement occurs downstream from areas in Canada.

Muscle samples for electrophoretic analysis were taken in the field from Lake Roosevelt, the mid-Columbia and below Bonneville Dam representing the overall distribution range and the three major areas of the Columbia River: the upriver pool, the mid-river reservoirs, and the estuary. Samples from several other tissues were obtained from the sport and commercial harvest along the river and from fish packing facilities. Set lines were fished in Lake Roosevelt, and the fish were released after muscle tissue was sampled Tissue samples were collected from March through October (Table 1). with emphasis placed on the upper pool. No sampling effort was attempted on the Snake River in 1986. Snout photographs and scute counts were taken whenever possible. These additional data were collected for both independent analysis and for a relationship with electrophoretic data.

Table 1. Area and time of sampling in 1986.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ckt	Nov	Dec
Lk. Roosevelt		x	x		x		xx		x	x		
Mid- Columbia			x		x							
Ilwaco						x						
Below Bonneville					x							

METHOD AND MATERIALS

Four major tissues were collected for electrophoretic analysis from individual fish. When it was possible to sample from fresh dead specimens the eye, heart., liver, and muscle tissue were removed. In those instances when the fish would not be sacrificed, a muscle plug was taken by inserting a steel cork borer into the area just below the dorsal ridge of scutes towards the posterior end of the fish (Fig 1). The tissue was then placed in a ziploc bag, set on dry ice for immediate freezing, and transferred back to the University of Washington. At the laboratory samples were stored at **-85°C** in a super cold freezer to prevent breakdown of tissue proteins.

Prior to electrophoresis, each tissue was slightly thawed and a l/4" by l/8" piece was cut off and put into a test tube. The test tube contained 0.3 ml of a tissue prepping solution (called PTP; Aebersold et al. , In Press) which enhances activity when some of the enzyme systems are stained. Test tubes were put into the superfreezer (-85°C) for storage. Each tissue type was kept in a separate rack in a specific ordered sequence, and repeated for all tissues. Tissues obtained from all individuals in each sample area were stored in the same test tube rack.

Starch gels were routinely prepared the day before electrophoresis was performed. Gels were poured using Sigma starch and the buffer solutions shown in Table 2. Test tube racks were removed from the freezer, and tubes centrifuged 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 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.

Laboratory procedures followed standard electrophoresis methods (Harris and Hopkinson 1976; May 1980; Utter et al. 1974; Aebersold et al. In Press). 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 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 40-50 individuals of one tissue type at a time. Gels were run utilizing tissue from muscle, liver, eye, and heart when available. Different buffers were employed to obtain the best resolution of the enzymes tested (Table 2). Once the analysis of enzyme systems began, photos were taken of the gels for later reference. Enzyme recipes were tried again using other buffer systems (Table 2) if resolution was not storable on the first run. Systems which were defined enough for scoring purposes are listed in Table 3, with the tissue and buffer defined. Data were collected from each individual and analyzed between and within each area. Data from 1985 and 1986 was pooled to from a single database for analysis. Twenty-eight loci were scored overall, with some not scored for all areas or all individuals within an area. Analyses were performed

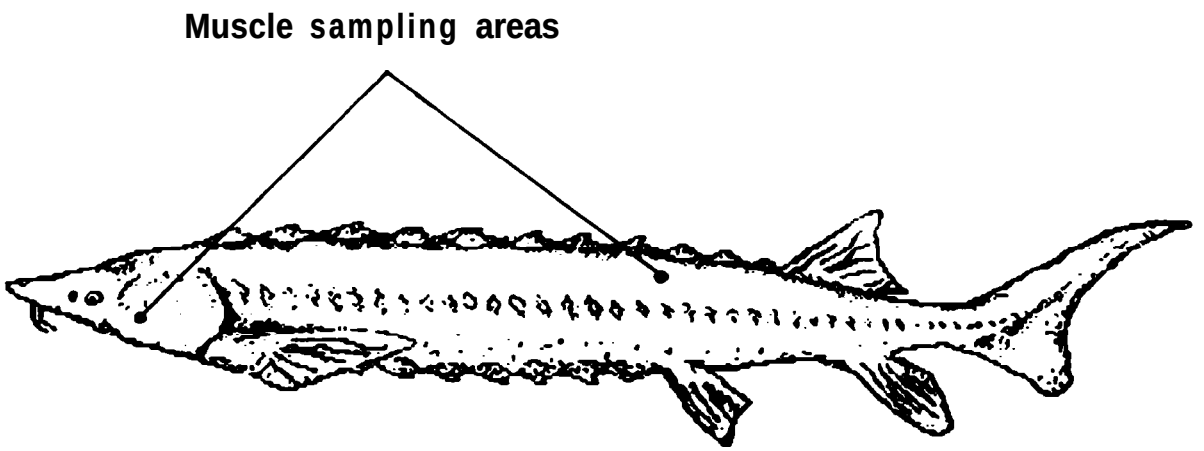

Fig. 1 Muscle tissue sampling locations on sturgeon

Table 2. 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)	Citric Acid (pH 6.5)	(Clayton and Tretiak, 1972) (AC)
	+ (NAD added to gel and cathodal electrode tray)		
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)

Table 3. Listing of systems by tissue and buffer.

Enzyme	Buffer	Tissue
Aspartate aminotransferase (AAT) E.C. 2.6.1.1	TBE	mus, hrt*
Adenosine deaminase (ADA) E.C. 3.5.4.4	TP	mus
Aconitase hydratase (AH) EC. 4.2.1.3	TBE	mus
Adenylate kinase (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
Glyceraldehyde-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. 1.1.1.8	TBE	mus
Glucose-6-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 dehydrogenase (PGD) E.C. 1.1.1.44	TBE	mus, liv
Phosphoglucomutase (PGM) EC. 5.4.2.2	TBE	mus
Superoxide dismutase (SOD) E.C. 1.15.1.1	RW	mus

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

using BIOSYS (Swofford and Selander, 1981) program on the University of Washington Cyber computer.

Starch gel electrophoresis techniques have been applied to tissue samples taken from regions of the Columbia River system in an effort to identify and analyze the genetic population structure of white sturgeon. The estimate of sturgeon population genetic structure in the Columbia depends on the abilty to identify polymorphic enzymes with significant allelic variation between areas. Allendorf and Phelps (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 (1987) found 7 polymorphic loci in white sturgeon from four different river systems in the Pacific Northwest, but were limited by sample size to distinquish any differences. Carlson et al. (1982) found low genetic variability and slight suggestion of differentiation between geographically isolated populations of paddlefish. We also collected morphometric and meristic information at the time of the tissue sampling. Differences in snout shape among sturgeon have been observed in the Columbia (Crass and Gray 1982) and were assessed as a morphometric characteristic. The number of dorsal scutes on sturgeon is known to vary between 11 and 14 (Scott and Crossman, 1975). and was included as the meristic characteristic.

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 2). The positions of seven landmarks (Fig 3) 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 3. The points of intersection of these two lines and the body outline 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, it was included as part of the sample routine in 1986 to examine for comparative purposes. 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 the scute counts.) Data were then entered on a computer and tested against the snout data, and the electrophoretic data for any correlation.

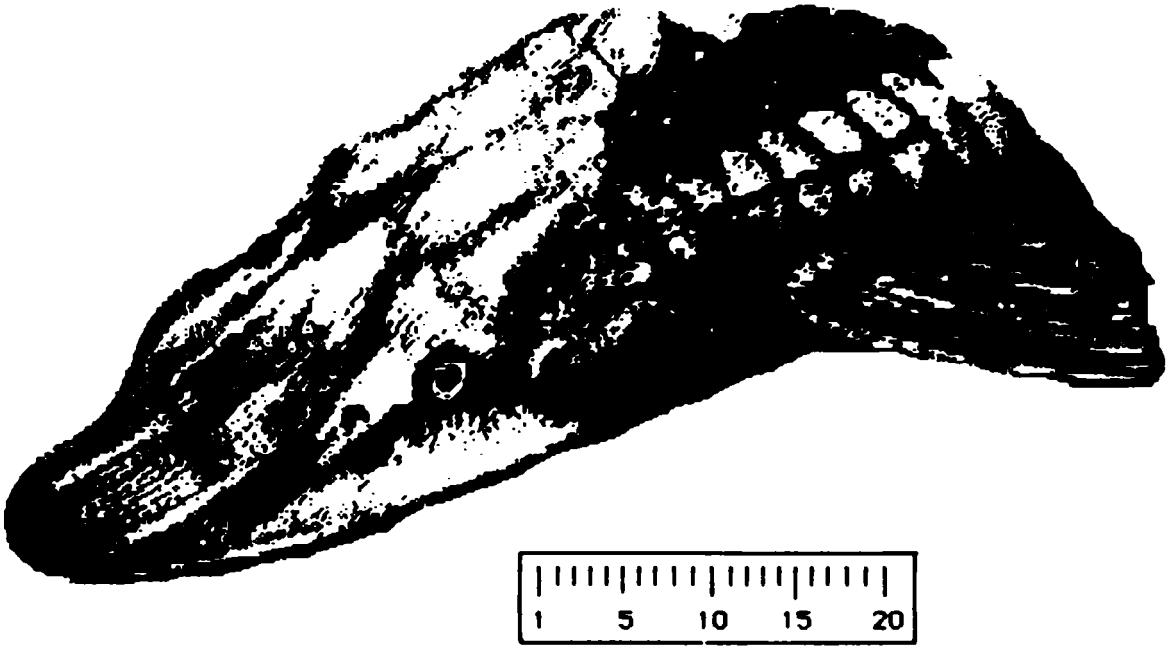

Fig. 2 Posrtion of photo **taken of snout shape**

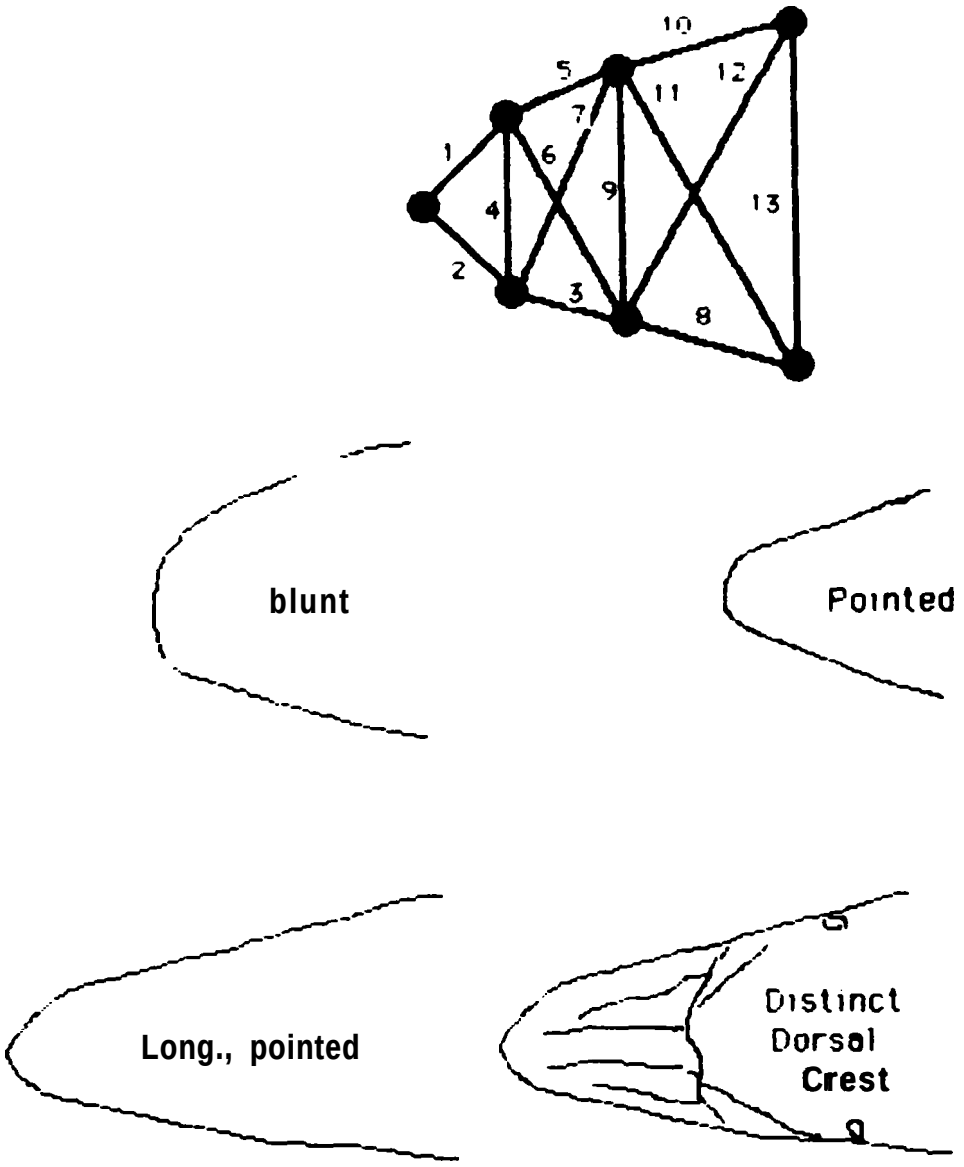

Fig. 3 Landmarks used for head, and outlines of observed snout shape differences

RESULTS

In the overall electrophcnetic analysis of the present study, a total of twenty-eight loci showed banding patterns which could be scored (Table 3). Eighteen of the loci scored from the areas examined showed some variation, but only eleven were considered polymorphic ($p<.95$). The remaining seven systems were assumed to have shown a somewhat rare allelic variation.

Description of loci

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 it appears to be somewhat rare.

Aspartate aminotransferase **(AAT)** showed a cathodal locus which was scored in both muscle and heart We observed a fairly common slow allele at this locus.

Adenylate kinase (AK) had one locus. There was a fast variant out of this locus (AK-l).

Fructose biphosphate aldolase (ALD) showed one locus in muscle which migrated aanodally. There was a fast variant from this locus. There appears to be tissue specific isozymes which have different mobilities but show the same variation in this system.

Creatine kinase (CK) had three loci of which CK-1 and CK-2 were both monomorphic. CK-3 was scorable in eye and heart and was polymorphic. We saw a fast and slow allele.

Esterase (EST-l) was monomorphic in all areas. EST-2 was polymorphic in Roosevelt Lake and the mid-Columbia areas in liver tissue. Liver was not available at other locations.

Glycerol-3-phosphate dehydrogenase (GPD) migrated anodally and had a slow variant.

Glycerate dehydrogenase (GD) migrated anodally and showed a fast variant.

Glucose phosphate isomerase (GPI) was scored as having two loci with interaction bands. The first locus was near the origin with a variant that migrated cathodally. The second locus was anodal and also had a slow variant The number of shadow bands couldn't be reduced or eliminated by treatment with either mercaptoethanol or reduced glutathione as thiol reagents.

Lactate dehydrogenase (LDH) had one locus which was scored in muscle. The common allele was on the origin and a variant migrated cathodally. A large percentage of heterozygotes were seen in samples from most areas. The structure of LDH and MDH in Russian sturgeon has been described by Slynko (1976) and for other fish species by (Market-t et al. 1975; Marken and Faulhaber,l965), a simple model was used for scoring. White sturgeon (A. transmontanus) in the Columbii River appear to have other loci specific to heart and eye. The heart loci banding pattern seems to be expressed by muscle tissue better in older individuals when the muscle locus is no longer discernable (D. Bartley, personal communication).

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. There was also a slow allele that showed itself out of MDH-2.
Malate dehydrogenase -NADP (ME) had one locus which was polymorphic. ME-l migrated cathodally and had a slow variant seen only in one area.

Peptidase (PEP) showed three loci and two were polymorphic. PEP was scored from the peptide leucyl tyrosine (LT) which revealed three loci, two of which were scored. There was a slow variant out of LT-3 and a fast variant from LT- 1 LT-2 was fainter and less discemable, and no data were collected.

Phosphoglucomutase (PGM-1+2) was scored in muscle tissue for two loci. Variation was seen in PGM- 1. There was a rare fast allele and a slow more frequently seen allele.

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

Polymorphic loci are demonstrated by drawings (Appendix Figure 1). and their relative mobility to the common allele is listed (Appendix Table 1). The allele frequencies are listed by system and area (Appendix Table 2).

One possible explanation for the high number of heterozygotes observed in muscle LDH of white sturgeon may be an occurence of gene duplication. A duplicated gene is the result of either the presence of more than two sets of chromosones (polyploidy), or a small segment of chromosone is represented twice. With the LDH locus observed in muscle, resolution of gene duplication would improve the scoring model. Data from throughout the Columbia River has shown a high frequency of heterozygosity between the A and B subunits (Fig. 4). The ratio of these subunits revealed as a tetrameric pattern, if a cell were to produce both subunits in equal amounts, would be 1 : 4 : 6 : 4 : 1. If the A subunit were duplicated and four times more were produced than B subunits, the ratio of subunit types seen would be shifted to 256 : 256 : 94 : 16 . 1. This makes the A (4 doses) and the A(3 doses) - B (1 dose) subunit types by far the most prevalent (Ohno et al., 1967). The number of isozyme loci of LDH follows the determinations for fish made by Markert et al (1975).

Interesting systems (those polymorphic) are found throughout the river, but two that were particularly perplexing occured in the upriver samples. Variation existed in both AH and PGM, but the model for the number of loci was unclear, so a simple model was assumed. PGM-1 appears to suggest possible gene duplication with each having a **different** mobility (Fig 5a) while AH may have two loci with the same mobility (Fig 5b). This was not clarified by the use of fresh samples or the use of thiol reagents. ALD suggests gene duplication because each tissue seems to have at least one specific locus, but the scoring appears the same at each locus.

The average heterozygosity by area was calculated as an index of the amount of variation (Selander and Johnson, 1973). Values ranged from 0.043 to 0.079. Overall sturgeon samples H = 0.075 , this is very close to average values seen in fish (Nevo et al., 1984). There was

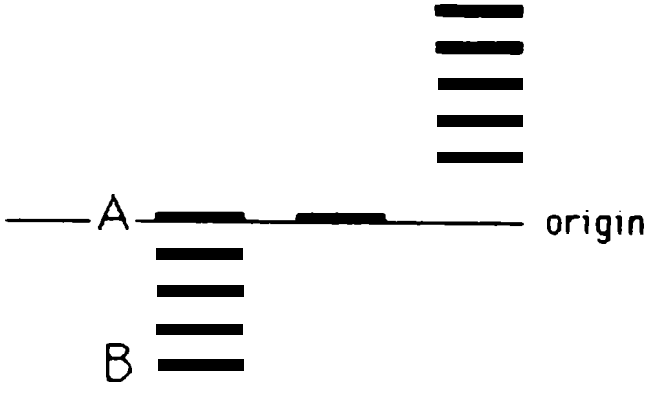

Fig. 4. Drawing of banding pattern produced **by LCH**

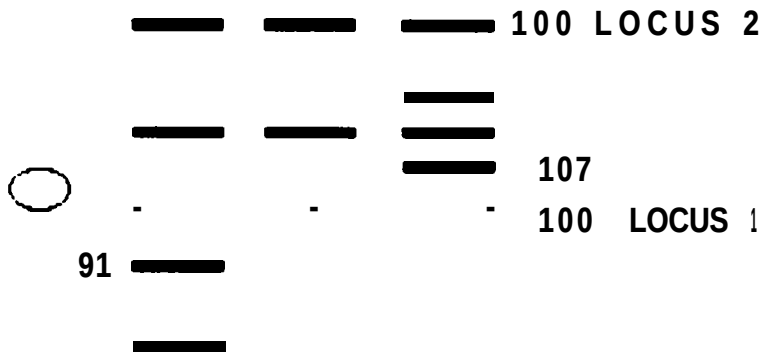

Fig. 5a Drawing of banding patterns produced by PGM (5a)

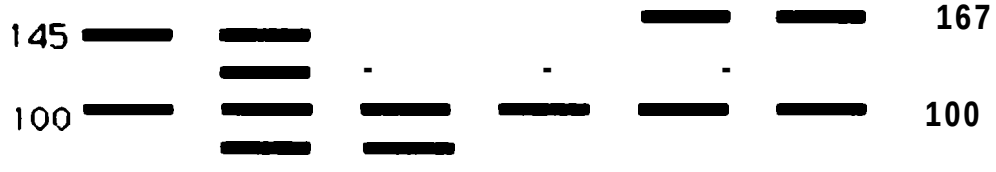

Fig. 5b Drawing of banding patterns produced by and AH(5b).

little variation in the individual heterozygosity between areas (Table 4).

The observed and expected allele frequency values according to Hardy-Weinburg equibrium were tested by loci between and within each of the five areas using a chi-square analysis. The systems where the observed frequencies did not fit Hardy-Weinburg expectations are shown with an asterisk in Appendix Table 3 by area. These are systems which have been discussed previously in relation to this result. The Chi square statistic did reject the null hypothesis that differences between observed and expected values by loci were also significant between areas in systems where an allele did not occur in all areas (Appendix Table 4) or where either an excess or deficiency of heterozygotes occurred. Determination of the cause for a rejection involved further contingency chi square evaluations. The systems which do not fit Hardy-Weinburg expectations after after additional contingency Chi Square analyses were GPI-2 and LDH- 1 (Appendix Table 4). Because of the high number of heterozygotes and the lack of alternate alleles found in some systems when scored, further sampling will be undertaken to verify that the population is in fact out of Hardy-Weinburg equilibruim and not just being inaccurately estimated because of sample size or an excess of individuals fiom a specific location.

Sample size was small in Roosevelt Lake. 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 a large number of loci were sampled (see: Gorman 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 axe taken from a catch and release situation, a small muscle plug does not allow a large variety 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, 1987) which could be scored if the samples were attainable and the models were precise.

Genetic distance estimates were made from allele frequencies using an unbiased procedure (Nei, 1978). This method showed all areas to be very similar (Table 5). Also, one way analysis of variance was used to analyze the raw data (obtained from scoring the gels) between each of the five areas (Zar, 1974). A significant difference at $p < .05$ was found in seven systems indicating some differentiation between areas (Appendix Table 5). In many of these cases an allele was found in one are-a with a very slight frequency.

Analysis of Snout Shape

The computerized approach has lent preciseness to the visual evidence for substantiating differences in snout shape. In a preliminary assessment of this approach, fish were classified as pointed, rounded, or blunt-nose, with approximately 18 fish per group. Thirteen interlandmark distances from each fish were calculated between these seven landmarks as illustrated. A variance-covariance matrix from $\log_{10}$ transformed data was subjected to a principal component analysis. The first two eigenvectors are presented in Table 6. Coefficients were relatively equal in size on the first component and separated fish by overall size. This component was not considered further. The second component (PCII) separated fish by snout shape. The signs of coefficients in PC II can be used to interpret the multicharacter relationship described by PC II. Namely, four characters had positively -signed coefficients; the remaining characters had

Table 4. Average heterozygoslty by area

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYSOSITY	
				DIRECT-COUNT	DDYWBG EXPECTED**
1. BELOW B	25.9 (3.6)	1.3 (.1)	28.6	.045 (.017)	.043 (.015)
2. ILWACO	135.4 (9.3)	1.6 (.1)	57.1	.061 (.014)	.062 (.014)
3. RDOS	46.1 1 2.1)	1.5 (.1)	35.7	.082 (.025)	.079 (.023)
4. HID-C	131.0 (6.2)	1.5 (.1)	46.4	.074 (.019)	.073 (.018)

• A LOCUS IS CONSIDERED POLYHDRPHIC IF MORE THAN ONE ALLELE WAS DETECTED

** UNBIASED ESTIMATE (SEE NEI. 1978)

Table 5. Net (1978) unbiased genetic distance between areas

POPULATION	1	2	3	4
1 BELOW 8	*****			
2 ILYACD	.002	• ⊠⊠⊠		
3 RDOS	.004	.003	• ⊠⊠⊠	
4 MID-C	.002	.OOl	.OOl	*****

Table 6. Eigenvector of principal components 1 and 2.

	PC1	PC2
X l	- 0.96	- 0.07
x 2	- 0.94	- 0.19
x 3	- 0.90	- 0.39
x 4	- 0.95	- 0.30
X 5	- 0.93	0.31
X 6	- 0.98	- 0.17
x 7	- 0.98	- 0.20
X 8	- 0.93	0.32
x 9	- 0.97	- 0.21
X10	- 0.94	0.27
x11	- 0.99	- 0.01
x12	- 0.99	- 0.08
x13	- 0.97	- 0.14

negatively-signed coefficients. This is simply interpreted to mean that the four outside characters contrast with the remaining characters. We interpret 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. An analysis of variance of the PC II scores by group was statistically significant ($F=25$, $P<0.001$).

This result indicates that snout shape can be quantified. We are pursuing this area of research to learn more about the geographic and ontogenetic elements of snout differentiation. It was curious that when we broke up our head shape photographs by area, fish from the mid Columbia River were found in all three groups, Lake Roosevelt fish fell into the pointed and long snout categories, and Ilwaco fish comprised a large part of the blunt nosed classification.

Dorsal scute counts were recorded at the time of tissue sampling, and the fish number assigned the tissue was noted for later anlysis. The scute counts varied throughout the river (Table 7). Analysis of data thus far has shown no relationship between dorsal scute count and head shape.

Our study using of growth differences and heritability of morphological characters is still underway in the laboratory. This experiment was undertaken using eggs and milt provided by "The Fishery" at Covert's Landing, OR. Gametes from one male and one female were combined and the resultant fish are presently being reared for evaluation. There is a potential for slight contamination of the milt . The syringe which was used to fertilize the eggs had previously been used for milt from another male. While the contribution might have been extremely small, the hope for accurate breeding data is unrealistic. The fish will he sampled, head shape will be noted and photographed, and a count of dorsal scutes will be made. Dorsal scute counts on fish which have died during the rearing period show variability from the parental counts. The fish now range from 2-5" and will be sacrificed shortly. It is hoped that a further replicate of this can be done in 1987 using two females and two males, with gametes fertilized one to one.

Table 7. Sturgeon dorsal scute counts from Columbia River samples.

	Lk.Roosevelt	Mid Columbia	Ilwaco
Mean	12.133	11.083	11.750
StDev	0.915	1.112	0.992
(N)	15	66	64

DISCUSSION

Elecuophoresis has been used extensively for genetic evaluations in many species of fish and shellfish (Utter et al, In Press). Genetic considerations are now being investigated by management personnel prior to any habitat alteration (Riggs, 1986). Because the genetic makeup of any stock is a result of generations of natural selection in a particular environment, the loss of stock gene pools cannot be tolerated. Fish culture provides the opportunity to enhance fish stocks following an environmental perturbation, but the embryos utilized must be as specifically adapted to one geographic area or ecosystem as possible however broad that range might be defined.

White sturgeon in the Columbia River have historically exercised anadromy as a natural function. Mixing of stocks within a drainage is therefore quite natural. The presence of dams may have substantially decreased any between-stock mixing. If we suppose that different subgroups migrated long distances upstream to reach ancestral habitat, dam construction would have trapped them in various reservoirs along the river. A mixture of upriver subgroups may now be represented in the upper reservoirs. This could promote further stock differentiation.

Changes might be expected among fish that now reside totally in freshwater, and unique adaptations are likely to evolve. For instance, morphological variation in feeding strategy could occur within different areas because of the variety of ecosystems which exist in the various reservoirs. This is particularly true if you compare the run of the river reservoirs with the storage reservoirs. 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). If sturgeon in the upper areas feed mainly on crayfish, fish and mussels, one might expect some morphological changes to occur which would make them better competitors in that specific food chain. 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). Morphological character variation within the Chulym river has been documented by Usynin (1980). We feel there is a strong probability that such morphogical variation exists among white sturgeon in the Columbii and that a genetic basis may be responsible.

Analysis was performed on fish throughout the Columbia, and there seems to be some evidence thus far of genetic differences between the upper and lower reaches of the river. In 1985 emphasis was placed on screening for enzyme systems that produced activity with good resolution. Twenty-eight loci have been isolated and the analyses suggest that some genetic differences potentially exist between areas. This potential differentiation will likely become more apparent as more morphomettic and meristic data is obtained to complement the electrophoretic data. When a locus shows different allelic patterns between areas it is a good indicator that subjective selectivity may have occurred. This situation presented itself in three systems AH, PGM-1 and CK-3 between the upper and lower areas of the river and provides evidence of differences in genetic stock characteristics among the areas sampled during both 1985 and 1986. This would be an indication that differences exist, and data that examined

variation within the lower areas suggests there will be a further opportunity to support that still more different allele patterns exist uniquely in each area.

Genetic changes are expected to occur as the environmental factors that dictate genetic characteristics change. The immediate effect of environmental change, however, will be a decrease in fitness. In the present situation, each isolated population will be responding in a different degree depending on the magnitude of environmental alteration. The low number of sturgeon in certain areas of the Columbia are believed the result of conditions severely limiting their success. Consequently, enhancement efforts to sustain or rebuild populations must take into consideration their individual limitations. For example, enhancement through stocking hatchery raised sturgeon fry will probably not occur below Bonneville because spawning success doesn't appear to be the limiting factor there. In contrast, the Roosevelt Lake population might benefit from hatchery plants since the exploitation rate appears to be high, based on observations of the sports fishery, and recruitment may be limited. The food level available to support Lake Roosevelt sturgeon, however, may be the limiting influence.

The population genetic characteristics become useful when artificial propagation is appropriate. If genetic differences are shown between the populations presently isolated in the Columbia, are those differences any longer meaningful when the environment responsible for stock characteristics has changed to such an extent? Perhaps the best way to proceed under such a situation is to assume that the present population represents the most suitable genetic base from which the stock should expand Although natural selection has been operating in the newly defined environments for a relatively short period of time, the fish that are isolated in those environments have already begun the process of adaptation, and even a little gain in that regard would be beneficial and show responsible management practice.

TASK II

Effect of light and cover in simulated river flows on feeding success and predator avoidance of young white sturgeon.

STATEMENT OF THE PROBLEM

Little is known about the habitat requirements of Columbia River white sturgeon. Previous study of the early Life history behavior of this species (Brannon, et al., 1985) has indicated that they may have very specific habitat requirements for successful feeding and predator avoidance.

Although the feeding mechanism may be best suited for benthic prey, sturgeon are not Limited to benthic organisms for food. They eat a variety of fish species (Radtke, 1966; Semakula and Larkin, 1968; Mckechnie and Fenner, 1971; Brannon, et al., 1985). and in the Sacramento-San Joaquin delta ***Neomysis awatschensis*** was the most important food item of juveniles. Next to the tube dwelling gammarid amphipod ***Corophium sp.*** mysids are probably one of the more important components in the diet of juvenile white sturgeon in the lower Columbia River and its estuary. As non-visual feeders, the ability of sturgeon to capture mobile prey types is expected to depend heavily on the environmental conditions present. Factors such as light, current and cover are believed very important in defining their feeding niche.

Similarly, the ability of sturgeon larvae and fry to avoid capture by predators must be strongly influenced by the environmental conditions present. Early life history stages of white sturgeon have distinct behavioral patterns at the larvae swim-up phase, during the subsequent hiding phase, and after fry emergence that have evolved under the historical habitat available to this species. In previous studies behavior patterns of juveniles in simulated river habitat were identified. The present study examined the potential affect that such behavior patterns will have on sturgeon survival when displayed in altered habitat, especially in regard to feeding and predator avoidance. Light and turbulance are two environmental factors associated with habitat altered by hydroelectric projects that were examined with respect to their influence on survival.

Objective: To examine the influence of light and turbulance in simulated river flows on feeding success and predator avoidance of young white sturgeon.

Null **Hypothesis** - Variation in light and turbulance have no influence on sturgeon feeding success or avoidance of predators.

MATERIALS AND METHODS

Sturgeon larvae were supplied for the study by "The Fishery" at Covert's Landing, Oregon on the Columbia River just below the Bonneville Darn Larvae, l-3 days post hatch, were transported to the laboratory at the School of Fisheries and placed in doughnut-shaped observation arenas described in Brannon et al. 1985, or in holding tanks from which they were removed for tests in other arenas. Juvenile sturgeon utilized in the study were reared at the University of Washington's hatchery facility.

Capture of Salmonid Frey

The ability of white sturgeon to capture salmonid fry under different conditions of light and water movement was assessed in two 1.5 m fiberglass circular tanks. Both tanks had water levels of 30 cm, center standpipes and an inflow of 5 L/min. dechlorinated city water. The tanks were uncovered to allow illumination of the tank or covered with black plastic to prevent light penetration. The angle of inflow was adjustable to regulate water velocity in the tank without changing the inflow rate. The amount of turbulence, or mechanosensory stimuli present, was regulated by turning on three airstones resting on the bottom of the circulars. Chinook salmon fry, *Oncorhynchus tschawytscha*, and rainbow trout fry, *Salmo gairdneri*, used in the tests were obtained from artificially spawned and reared stocks at the University of Washington's experimental hatchery in Seattle, Washington.

The first experiment was designed to determine the effect of light on the ability of sturgeon to capture prey. About 500 chinook salmon fry were held in a circular tank identical to the uncovered test tank, and were transferred as needed to the two test circulars. Two sturgeon ranging from 300-350 mm (total length) were held in each of four 73 cm diameter circular tanks with a water depth of 60 cm. A total of eight paired tests were performed in which one sturgeon was added to each test circular tank with 30 chinook salmon fry (58 mm mean length) that had acclimated to the test tank for one hour before testing. One test tank was covered with black plastic and the other was uncovered Thirty minutes after the sturgeon were introduced to the test tanks the number of salmon remaining was counted and recorded. Each sturgeon was tested twice consecutively in both light and dark test tanks, for a total of two tests per sturgeon.

Both test tanks were covered in the second experiment, and three air-stones were placed on the bottom of each tank. Only one tank received air through the stones during each test, and the tank receiving air was alternated between tests. The water inflow was vertical, minimizing water velocity in the tanks. Four sturgeon (325-375 mm) were acclimated for two days in both test tanks before testing. About 500 rainbow trout (49 mm mean length) were acclimated for two days in a circular identical to the test tanks. Before each test, 20 fry were removed from the holding tank, placed into a bucket, then gently released into each test circular with four sturgeon. The tanks were covered about 15-30 seconds after the trout were introduced Both groups of sturgeon were tested simultaneously. At 15, 30, and 45 min. after initiation of each test, the number of fry remaining in each tank was counted using a flashlight. The water and air were turned off during census. The first five of rune paired tests did not have observations at the 45 min. time interval. Tests were run on consecutive days at different times during the photoperiod.

The third experiment was designed to determine what effect current has on the predatory success of sturgeon. An identical test procedure to that of the second was used without airstones, and the angle of the inflow was set at either a 45^{0} or 90^{0} angle to regulate water velocity. Rainbow trout were held in one of the smaller circular tanks previously described,

Preliminary tests and observations with smaller sturgeon (155 to 180 mm) were conducted in the doughnut-shaped observation arenas with current velocity of about 2-3 cm/sec and a uniform substrate composition of sand and small gravel. Four resident sturgeon were in each of two doughnut arenas. One arena had a 25 watt overhead light and the other had no light. Twenty rainbow trout fry (25 mm mean length) were added to each test arena and the number remaining at 1-4 hour intervals was recorded. Identical tests were performed in which both arenas had similar lighting but one arena had cover in the form of 20 round stones of about 60 mm in diameter evenly distributed around the arena In the second set of tests, one sturgeon was introduced to various densities of resident rainbow trout fry in the doughnut arena. The number of strikes and captures made by sturgeon during the first 10 min. period of foraging under conditions of light and no light were compared.

Tests designed to observe phototaxic behavior of rainbow trout fry in response to sturgeon predators were conducted in two 75 gal. glass aquaria with uniform sand and fine gravel substrate. One side of each aquaria had an overhead light which resulted in a gradient of photointensity between the two sides. In each test, 20 trout fry were introduced to the center of the tank holding l-4 resident sturgeon. One tank had no obstruction to movement and the other had two plexiglass dividers separating the tank into three equal chambers that were connected by three 40 mm diameter holes in each partition, 60 mm from the water surface. After the fry were introduced, the number of fry in each area of the tanks was recorded over time and used to determine whether the fry preferred the light or dark side of the tank.

Capture of Mysid Prey

Mysid shrimp, ***Neomysis mercedis***, abundant in the Columbia River, were used in tests to evaluate the nature of the ability of sturgeon to capture mysids under different conditions. They were captured using an epibenthic sled with a 16 x 35 cm opening on August 8 around Puget Island, on the lower Columbia, at depths ranging from l-3 m and transported in **Zip-Lock™** plastic bags to the University of Washington the same evening. In the laboratory, the mysids were held in flow-through and static aquaria and fed a diet of chopped tubifex worms. Mysids used in tests and observations were removed from holding tanks using siphon tubes, sorted and then counted into glass beakers.

Tests were conducted in three doughnut-shaped observation arenas with identical flow and substrate as was described above for the studies utilizing salmonids. One resident sturgeon (210-230 mm length) per arena was used in each of eight paired tests and were acclimated to feeding on mysids for two days prior to testing. The tests compared the number of mysids captured by sturgeon under light and no-light conditions. Light was provided by a 25 watt bulb over the center of the arena compared to arenas covered with black plastic. Ten mysids (1 cm in length) were added to each arena at the beginning of a test and the number remaining was

counted after 30 min. Observations of the foraging behavior of the sturgeon and the responses of the mysids to the presence sturgeon were made during some of the lighted tests.

Observations of the distribution behavior of mysids were conducted in 75 gal. aquaria provided with sand and small gravel, including small stones on one side of the tank. The behavior of the mysids was observed before and after the introduction of the sturgeon to determine if there was any interaction between the mysids and the stones in the tank.

Video recordings were made of sturgeon foraging for mysids in static aquaria with bright lighting. Sturgeon were added to tanks with resident mysids and individual mysids were added to tanks with resident sturgeon. The recordings were subsequently viewed in slow motion to analyze the responses of sturgeon to the presence of mysids and the predator avoidance mechanisms of the mysids.

Predator avoidance

White sturgeon larvae (l-4 days post-hatch) were obtained from eggs spawned and incubated at Covert's Landing in Oregon. Larvae were transported to the University of Washington and held in a 1.5 m circular tank with an inflow of dechlorinated city water. Eighty larvae were introduced into each of three doughnut-shaped observation arenas. Each arena had a different amount of cover: no cover, 60 mm stones spread over the whole tank, and 60 mm stones in half the tank. Daily observations were performed in each tank until 18 days post hatch to compare distribution behavior among larvae in different habitats.

Tests to determine the vulnerability of larvae to various predators were conducted primarily in the half-cover doughnut by introducing a predator and counting the number of strikes and captures during a 10 minute period beginning with the first strike or capture. Foraging method and intensity of the predators was also noted. Eleven tests were conducted under medium light levels to allow observation of behavior and noting amount of predation. A bluegill sunfish, *Lepomis macrochiris,* a wild goldfish *Carassius auratus,* and several white sturgeon were used as predators.

Sturgeon larvae were also acclimated to a variety of aquarium habitats, with predators introduced later for the same 10 min. tests counting strikes and captures. Seven tests with different regimes of cover and light were conducted in large aquaria with the same set of predators as used in the doughnut-shaped observation arena tests. Other observations were conducted by introducing sturgeon larvae into aquaria with resident chinook and rainbow fry. Video recordings of some of the larvae and predator introductions were made to help evaluate the avoidance ability of the larvae.

RESULTS

Capture of Salmonid Prey

The number of salmon fry captured in the light and dark circular tests after 30 min was significantly different ($p(t8) < .0005$). No more than one fry was captured in any of the tests with light and as many as seven (avg. captured = 3) were captured in the dark tank. Observations of the fish in the lighted tank during tests clearly indicated that the fry are able to avoid the approaching sturgeon well before it is within striking distance.

Results of tests investigating the influence mechanical aeration has on the ability of sturgeon to capture prey showed significant differences at both the 15 and 30 min. test intervals ($p(t8) < .0005$). More fry were captured during the first interval in the tanks that had active airstones than all three periods in tanks with no airstones (Fig. 6). The average rate of capture was only higher in the tanks with airstones during the first 15 mm. interval (Table 8).

Differences in numbers of fry remaining at the 15 minute and 30 minute observations in the tests comparing the influence of current were also significant ($0.01 < p(t_4) < 0.025$) . The average number of captures in the tanks with current was higher than that for tanks with no current (Fig. 7). Table 9 shows that the average number of fry captured in the presence of a current is only slightly more than when no current exists. Only at the 15 and 30 min. observations is the rate of capture greater under current conditions.

Observations in the doughnut arenas indicated that sturgeon are able to consume large numbers of eggs and alevins. Amount and type of cover is clearly important in determining their vulnerability to capture by sturgeon. Once at the swim-up stage, salmonids can avoid capture better. In the presence of light, however, alevins are more vulnerable to capture than later stage fry. As the fry grow older, light intensity appears to be the most important factor in determining their ability to avoid capture. The presence of cover will slightly reduce the capture rate, but more testing is needed to allow proper analysis. Fry were not observed to seek cover for hiding under lighted conditions so it is possible that obstruction to movement of the sturgeon accounts for the reduced capture rate.

The avoidance responses of the earlier stage rainbow fry in the light gradient test tanks showed a clear tendency to move to those areas with high light intensity. The fry also preferred lighted areas in the doughnut arenas before and after the addition of sturgeon. The positive phototaxic responses appear to be correlated with enhanced avoidance ability of fry under lighted conditions. Rainbow fry about four months older seem to prefer the lower light. Laboratory rearing conditions and the experience of older fry, however. could be a bias when testing phototaxic responses in this manner.

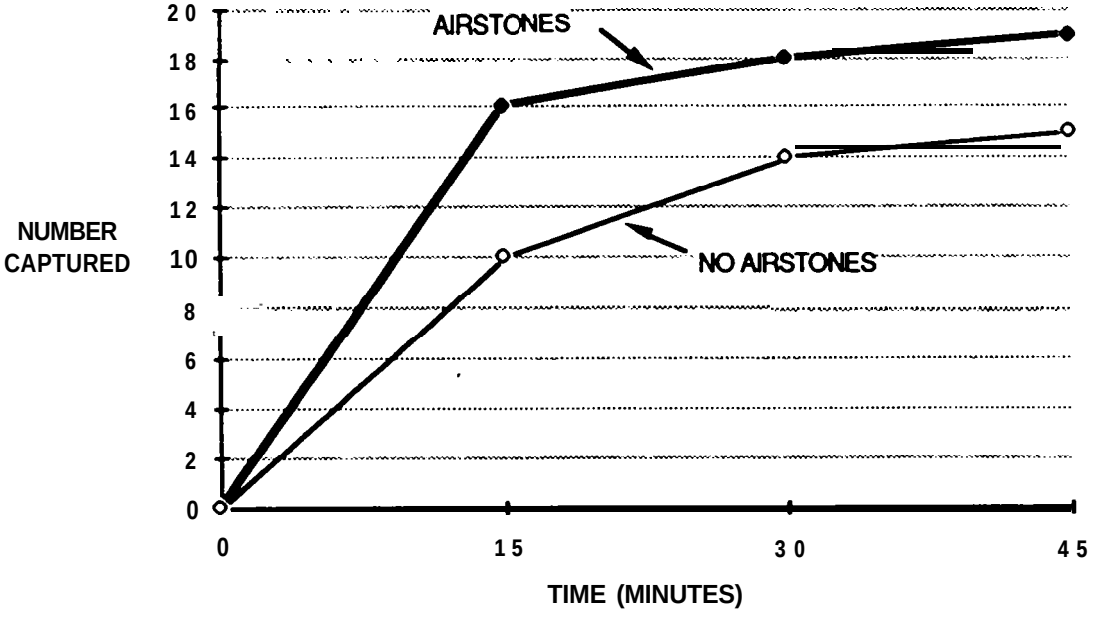

Figure 6. Cumulative average number of rainbow trout fry captured by four resident sturgeon with and without mechanical aeration observed at 15 minutes (N=9), 30 mln. (N=9), and 45 mln. (N=4) Into the test.

Table 8. The average number of rainbow trout fry captured (fry/minute) by four resident sturgeon under conditions of turbulence and no turbulence observed at fifteen minute intervals over a forty-five minute test period.

	15 MIN	30MIN	45 MIN	60 MIN
AIRSTONES	1.09	.09	.12	
NO-AIRSTONES	.67	.22	.13	

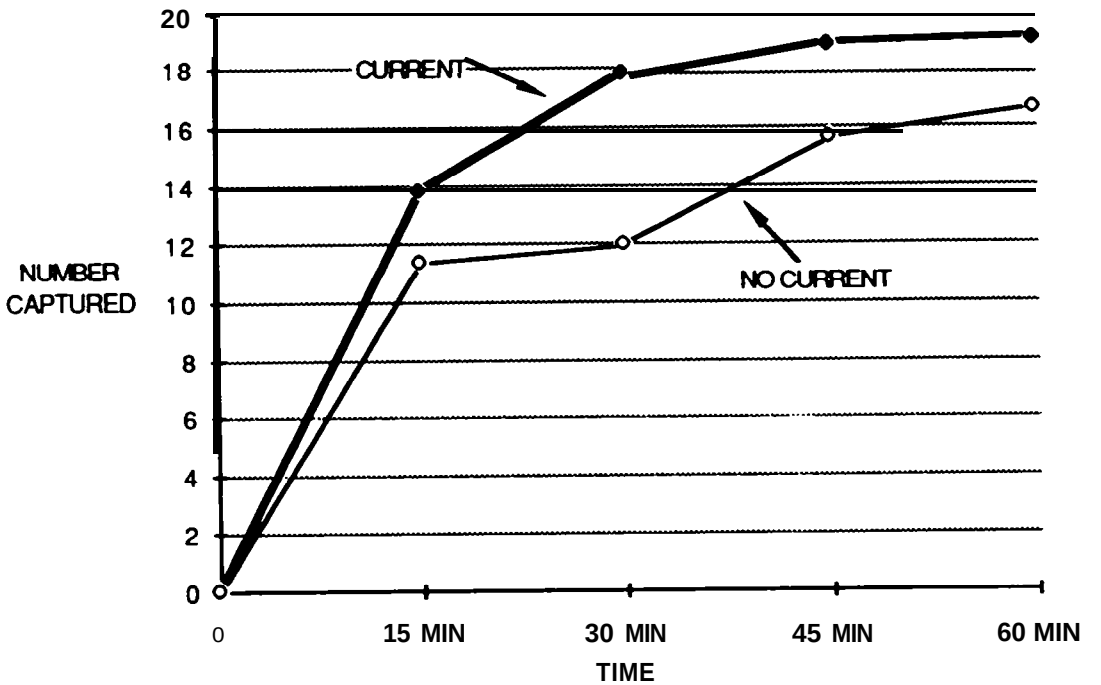

Figure 7. Cumulative average number of ralnbow trout fry captured by four resident sturgeon during testing of the influence of current upon the ability of sturgeon to capture prey (N=5).

Table 9. The average number of rainbow trout fry captured (fry/minute) by four resident sturgeon under conditions of current and no current observed at fifteen minute intervals over a forty-five minute test period.

	15 MIN	30MIN	45 MIN	60 MIN
CURRENT	.92	.27	.08	.01
NO-CURRENT	.76	.04	.24	.07

Capture of Mysid Prey

The sturgeon used in the doughnut tests acclimated to feeding on mysids very quickly and showed good foraging intensity during tests. The sturgeon appeared to respond to odors in the water that contained the mysids when added to the test arena. The results of the doughnut tests indicated that light intensity had no apparent effect on the ability of sturgeon to capture mysids. The average number of mysids remaining after 30 min under lighted conditions was 2.6 with 2.8 remaining in the dark arenas, resulting in no significant difference between the two conditions ($.375 < p(t_{10}) \leq .4$). During tests, most mysids were observed to be moving occasionally on the substrate, but there were also a few in the water column travelling with the current. Most captures appeared to be made on the substrate.

Observations in the large aquarium with cover on one side gave no indication that mysids use cover to avoid predation under these conditions. They appeared to be randomly distributed over the substrate and walls of the tank. No mysids were observed in close proximity to any of the stones before or after the addition of sturgeon.

Analysis of video recordings of foraging sturgeon showed that the mysids have a well developed avoidance response, consisting of a very rapid (l/30 sec.) jump. The jump is perpendicular relative to the surface on which the mysid is resting. The average ratio of jump distance to body length for nine measured jump responses was 1.7 and the maximum 2.2. The distance between the mysid and approaching sturgeon at initiation of the jump response varied from 0.5 to 3.5 mysid body lengths. In contrast to jumping when confronted by an immediate threat, mysids also responded to sturgeon in close proximity by swimming rapidly from the approaching sturgeon. Mysids do not always detect the sturgeon in time to use these avoidance mechanisms. This is clearly indicated by the fact that if mysids were left in the test tank with sturgeon they were captured in less than 24 hours.

Careful analysis of the behavior of sturgeon in video recordings indicated they had some ability to detect the presence of a mysid before any physical contact. Some sturgeon were observed to suddenly swing their snout towards a mysid or abruptly change their direction of swimming after passing a mysid. In most instances of apparent advance detection, the small back "legs" of the mysids are moving and might be detected by the lateral line of sturgeon. If sturgeon are not within a few centimeters of the mysid, they swim past with no indication of detection.

The sturgeon were very responsive to odors released in areas that mysids were captured. This was indicated by observations noting that well after the capturing sturgeon left the area, other sturgeon in the tank began frantically searching and striking in the same area. A significant amount of body fluids must be released during the ingestion of mysids, because in several instances the majority of the sturgeon in the tank would soon converge on the location of a capture.

Predator Avoidance

Observations in the doughnut arenas indicated that during the hiding phase from about day 5 to day 10, there are significant differences in potential vulnerability to predation. In the two

arenas with cover, most larvae were out of sight, hiding under or against stones. In the arena with half cover, all the larvae were hiding in the cover area. The larvae in the arena with no cover were evenly distributed over the substrate in small depressions or against small pebbles.

During the swim-up phase the sturgeon larvae were vulnerable to visual predators like the sunfish, which had 11 captures and 7 other strikes in a ten minute test. The goldfish had no captures in three tests and appeared to be unmotivated or unable to see the larvae in low light. Sturgeon predators were most successful in the no-cover arena with one fish having 16 captures and 35 strikes in the presence of larvae 17 days post hatch.

In the aquaria tests the goldfish was very successful at capturing larvae in high light, with as many as 15 captures and 28 strikes. Sturgeon predators are sucessful at capturing larvae provided they were not in the water column or under cover. Salmonid fry were very good at capturing sturgeon larvae introduced into their tank. They could capture larvae in the water column or pick them off the substrate under lighted conditions.

Observations of the behavior of larvae during tests and analysis of video recordings did not clearly indicate that sturgeon larvae can detect approaching predators before they strike. Once attacked, larvae show distinct avoidance response by rapidly swimming away from the predator. In some of the doughnut tests an increase in activity and water column swimming of the larvae was observed after very active sturgeon predators have been foraging for a few minutes.

DISCUSSION

Light is often one of the most important determinant factors in predator-prey interactions of fish (Cerri, 1983) and many species have light-dependent predator avoidance mechanisms such as schooling (Major, 1977; Magurran, et al., 1985; Hobson, 1986). The tests and observations of the ability of sturgeon to capture salrnonid fry clearly indicate that light was a very important factor affecting capture efficiency. The non-visual feeding mechanism of sturgeon (Sbikin, 1973; Brannon et al., 1985; Buddington and Christofferson, 1985) is ineffective at capturing all but the youngest salmonid fry under lighted conditions. Similarly, the torrent sculpin was found to feed on salmonids exclusively at night (Patten, 1975). This difficulty in capturing salmonids in water with good visibility is due to the rapid, visually mediated avoidance response of the salmonids. Sturgeon should only have an advantage over salmonids and other visually oriented fish when foraging under very poor light conditions when the visually mediated avoidance response of the prey cannot function (Miller, 1978).

Light did not appear to be an important factor in determining the ability of sturgeon to capture mysids. It is possible that mysids do not use vision to detect predators and depend more on low-visibility habitats to avoid visual predators. Their rapid jump response could be triggered by mechanical cues and appears to work quite well in total darkness. The ability of sturgeon to capture mysids could be influenced by the amount of current present to hinder detection of movement of an approaching sturgeon.

The ability of sturgeon to capture salrnonid fry in total darkness was shown to be influenced by the amount of mechanosensory stimulus (in the form of air bubbles and current) present in the tank. Salmonids have a lateral line on each side of the body and a series of canals on the head (Disler, 1971) that could be used to detect the mechanical stimuli of an approaching sturgeon. Lateral line receptors are sensitive to certain types of mechanical stimuli and can be used by fishes to capture prey (Hoedstra and Janssen, 1986) and probably avoid predation (Blaxter and Hoss, 1981). It is possible that salmonids rely primarily on mechanical stimuli to detect predators at night and in other low visibility situations. Stimulus caused by movement of water over the lateral line receptors and the general turbulence associated with current could mask the cues used by salmonids to detect sturgeon. If this hypothesis is correct, when foraging for fish, sturgeon should concentrate their effort in areas of high current.

Cover did not show a pronounced effect on feeding success as did light and water movement, except when feeding on eggs and alevins which depend entirely on the cover for inaccessability. Neither swim-up salmonid fry or mysids showed any indication of utilization of cover provided in test tanks. Only one basic cover type, however, was used in this series of tests. It was apparent that cover for prey tends to be an obstruction to the blind movement of sturgeon. Certain types of cover may inhibit sturgeon from foraging, thus providing protection for prey.

The observations of sturgeon larvae exposed to predators indicated that the larvae are potentially vulnerable to a variety of native and introduced predators found in the Columbia River. Under lighted conditions, before and after the hiding phase, sturgeon larvae appear to be

vulnerable to any visual predator. If suitable cover is available, sturgeon larvae are less vulnerable to predation during the hiding phase. Sturgeon were the only predator type tested that should be sucessful at capturing larvae in the dark.

The 1986 studies support the assumption that both the biological and physical habitat characteristics are very important in determining survival during the early life history of white sturgeon. The hydroelectric projects on the Columbia River have altered the river environment. Reservoir development has reduced velocity and turbulance, and light penetration in the water column has increased which tend to reduce the ability of sturgeon to feed on swimming prey, and to make them more vulnerable to visual predators.

SUMMARY AND CONCLUSIONS

Three major areas of the Columbia River were selected for sampling white sturgeon in 1986 continuing an investigation of the genetic population structure in the Columbia River. Sturgeon are found from the estuary at the mouth of the river, up the Snake River, and well into Canada in the Kootenai drainage. In general populations residing in the impoundments are isolated. Furthermore, the pools behind Chief Joe and Grand Coulee dam have no provision for upstream passage. While there are many miles of river which have not been sampled over the past two years, we feel that fish which reside in the mid-Columbia and lower river have been sufficiently characterized using electrophoresis to compare to up-river areas. Lake Roosevelt sturgeon require further investigation to increase sample size in order for observed differences to potentially become significant. We have collected baseline data for three character sets. Twenty-eight loci have been analyzed using electmphoresis, snout shapes were examined for multivariate distinction, and scute counts are themselves an index of variability. Patterns of differentiation of one or more of these data sets may be used to formulate stock transplant guidelines essential for proper management or enhancement of this species. To date we have shown few electrophoretic differences between areas, however Lake Roosevelt does suggest some. Snout shape has been shown to be easily quantifiable using the digitizing technique. Scute count data initially indicate that variability exists within as well as between areas.

The fact remains, however, that historical sturgeon habitat in the Columbia River has changed through the development of hydroelectric projects. Habitat formed by the reservoirs has reduced velocity and turbulance, and increased light penetration in the water column from less silt, compared to the historical habitat. These conditions reduce the ability of sturgeon to feed and make them more vulneralble to predation, which appears to have altered the ability of populations isolated in the reservoirs to sustain themselves. With regard to these habitat changes, present studies support the theory that both the biological and physical habitat characteristics of the Columbia River are responsible for reduced sturgeon survival, and justify consideration of enhancement initatives above Bonneville to improve sturgeon reproductive success.

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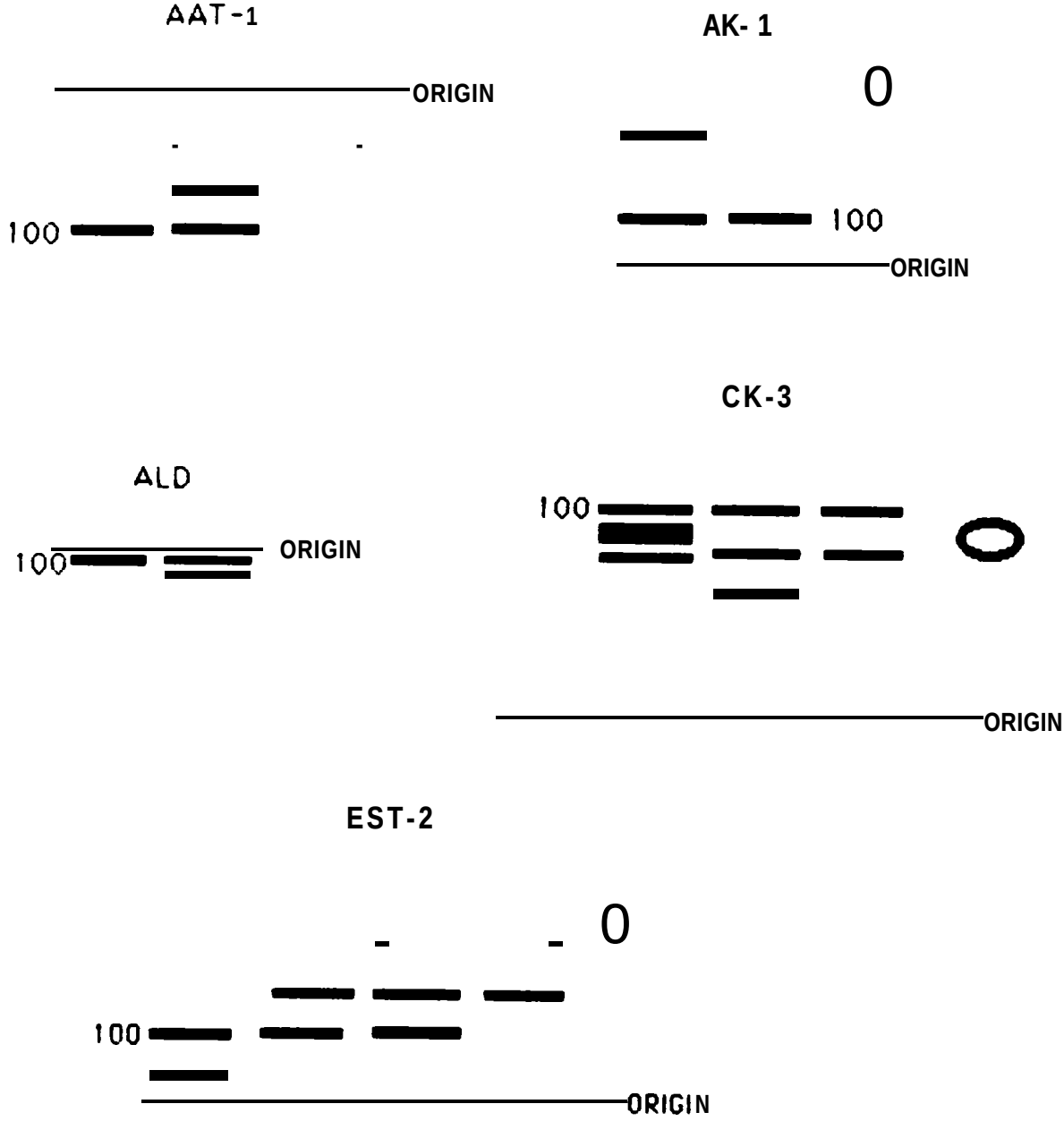
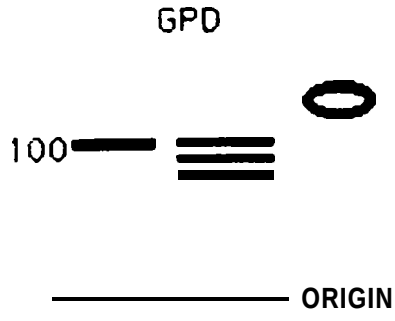
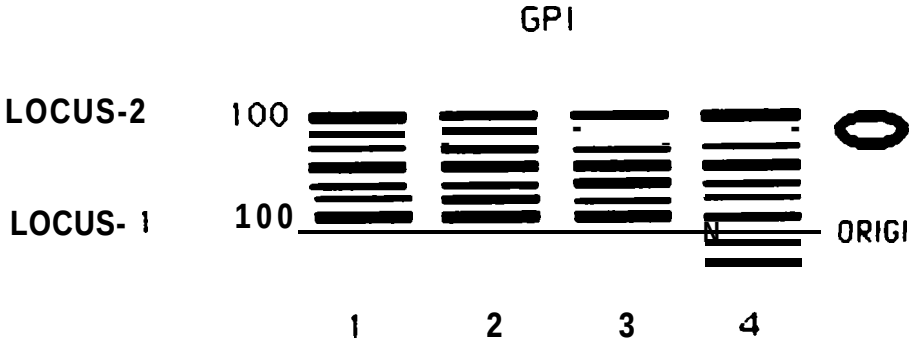
Appendix Fig. 1 Drawings of all other polymorphic loci.



Appendix Fig. 1 (cont.1 Drawings of all other polymorphic loci



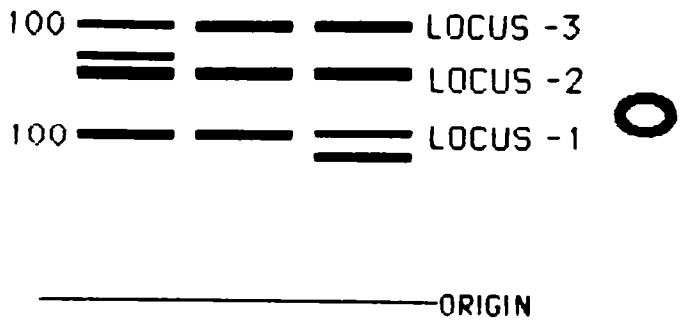


LEGEND

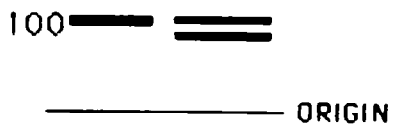
1= L-2 homozygote, L- 1 homozygote
2= L-2 al ternate. L- 1 homozygote
3= L- 2 heterozygote, L- 1 homotygote
4= L - 2 homozygote, L- 1 heterozygote

Appendix Fig. 1 (cont.) Drawings of all other polymorphic loci.

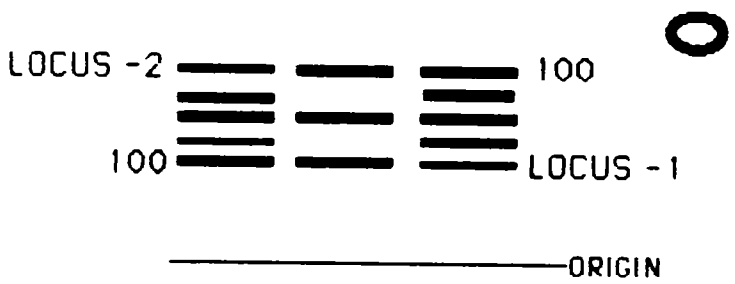
LT



ME



MDH



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

System	Allele 1	Allele 2	Allele 3
AAT-1	-100	-62	
AH-l	**100**	145	167
AK- 1	**100**	**140**	
ALD-1	-100	**-106**	
CK-3	100	**107**	96
EST-2	100	138	85
GAP-l	**-100**	-12	
GD-1	**100**	106	
GPD- 1	100	94	
GPI- 1	100	-127	
GPI-2	**100**	**76**	
LDH- 1	-100	**-271**	
LT-1	**100**	**88**	
LT-3	**100**	**92**	
MDH-1	**100**	136	
MDH-2	100	84	
ME-1	**100**	**44**	
PGM-1	**100**	**91**	107

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 frequency data by area.

LOCUS	POPULATION SNAKE	BB	I LWACO	ROOS	nro-c
AAT-1					
(N)	12	37	133	49	124
A	.917	.919	.906	.939	.911
B	.083	.081	.094	.061	.089
ADA-1					
(N)	12	39	50	51	84
A	1.000	1.000	1.000	1.000	1.000
ADA-2					
(N)	12	40		51	84
A	1.000	1.000	1.X80	1.000	1.000
AH- 1					
(N)	12	40	82	51	77
A	.792	.850	.927	.853	.864
B	.208	.150	.073	.118	.136
C	0.000	0.000	0.000	.029	0.000
AK- 1					
(N)			173	45	150
A			.983	1.000	1.000
8			.017	0.000	0.000
ALD-1					
(N)			140	51	136
A			.904	.931	.915
B			.096	.069	.085
CK- 1					
(N)			173	51	150
A			1.000	1.000	1.000
CK- 2					
(N)			173	51	150
A			1.000	1.000	1.000
CK- 3					
(N)			140	48	102
A			.868	.865	.892
B			.132	.073	.108
C			0.000	.063	0.000
EST-1					
(N)	12	40	173	51	150
A	1.000	1.000	1.000	1.000	1.000
EST-2					
(N)				32	49
A				.828	.857
B				.141	.112
C				.031	.031

LOCUS	POPULATION SNAKE	BB	I LWACO	ROOS	nro-c
GAP-1					
(N)	12	40	173	49	101
A	1.000	1.000	1.000	1.000	1.000
GD- 1					
(N)	12	40	83	42	50
A	1.000	1.000	.982	1.000	1.000
B	0.000	0.000	.018	0.000	0.000
GPD-1					
(N)	12	39	153	42	150
A	.833	.859	.977	.929	.963
B	.167	.141	.023	.071	.037
GPI-1					
(N)	12	40	173	51	134
A	1.000	1.000	.988	1.000	.993
B	0.000	0.000	.012	0.000	.007
GPI-2					
(N)	12	40	173	51	134
A	.792	.888	.896	.735	.877
B	.208	.113	.104	.265	.123
IDH-1					
(N)	12	40	143	48	150
A	1.000	1.000	1.000	1.000	1.000
LDH-1					
(N)	12	40	173	49	150
A	.625	.950	.922	.776	.837
B	.375	.050	.078	.224	.163
LT- 1					
(N)	12	40	173	51	139
A	1.000	.988	,960	1.000	.978
B	0.000	.013	.040	0.000	.022
LT- 3					
(N)	11	40	83		71
A	.955	.963	.904		.930
8	.045	.038	.096		.070
HAN- 1					
(N)			100	51	150
A			1.000	1.000	1.000
MDH-1					
(N)			173	51	150
A			.942	.892	.947
B			.058	.108	.053

Appendix Table 2 (cont.) Allele frequency data by area.

LOCUS	POPULATION				
	SNAKE	BB	I LWACO	ROOS	MID-C
MDH-2					
(N)			140	51	150
A			.986	1.000	1.000
B			.014	0.000	0.000
ME- 1					
(N)	12	40	173	51	141
A	1.000	1.000	,994	1.000	1.000
B	0.000	0.000	,006	0.000	0.000
PGD-1					
(N)			79	23	150
A			1.000	1.000	1.000
PGM-1					
(N)	12	40	165	48	134
A	.833	.925	.918	.958	.929
B	167	.075	.082	.031	.071
C	0: 000	0.000	0.000	.010	0.000
PGM-2					
(N)	12	40	173		150
A	1.000	1.000	1.000	1.04090	1.000
SOD-1					
(N)	12	40	173	51	84
A	1.000	1.000	1.000	1.000	1.000

Appendix Table 3 Chi-square statistics by area

POPULATION: BELOW 8

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	32	31.243			
	A-B	4	5.514			
	B-B	1	.243	2.788	1	.095
AH- 1						
	A-A	28	28.900			
	A-B		10.200			
	B-B			1.246		264
GPD- 1						
	A-A	28	28.776			
	A-B	11	9.449			
	B-B	0	.776	1.051	1	.305
GPI -2						
	A-A	31	31.506			
	A-B	9	7.988			
	B-B	0	.506	.643	1	.423
LDH- 1						
	A-A	36	36.100			
	A-B	4	3.800			
	B-B	0	: 100	. 111	1	.739
LT- 3						
	A-A	39	39.006			
	A-B	1	.987			
	B-B	0	.006	.006	1	.936
PGH- 1						
	A-A		34.225			
	A-B	34	5.550			
	B-B	8	.225	.263	1	.608

Appendix Table 3.(cont.) Chi-square statistics by area.

POPULATION: ILWACD

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	109	109.175			
	A-B	23	22.650			
	B-B	1	1.175	.032	1	.859
AH- 1						
	A-A	70	70.439			
	A-B	12	11.122			
	B-B	0	.439	.511	1	.475
ALD-1						
	A-A	115	114.302			
	A-B	23	24.396			
	B-B	2	1.302	.459	1	.498
CK- 3						
	A-A	106	105.445			
	A-B	31	32.111			
	B-B	3	2.445	.168	1	.682
GPD- 1						
	A-A	146	146.080			
	A-B	7	6.840			
	B-B	0	.080	.084	1	.772
GPI-2						
	A-A	139	138.873			
	A-B	32	32.254			
	B-B	2	1.873	.011	1	.917
LDH-1						
	A-A	147	147.053			
	A-B	25	24.893			
	B-B	1	1.053	.003	1	.955
LT- 3						
	A-A	160	159.283			
	A-B	12	13.434			
	B-B	1	: 283	1.970	1	.160
HDH-1						
	A-A	153	153.578			
	A-B	20	18.844			
	B-B	0	.578	.651	1	.420
PGH- 1						
	A-A	139	139.105			
	A-B	25	24.791			
	B-B	1	1.105	.012	1	.914

Appendix Table 3.(cont.) Chi-square statistics by area.

POPULATION: HID-C

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	**112**	**113.059**			
	A-B	**24**	**21.882**			
	B-B	**0**	**1.059**	1.274	1	**.259**
AH- 1						
	A-A	**64**	**64.899**			
	A-B	**24**	**22.202**			
	B-B	**1**	**1.899**	**.584**	1	**.445**
ALD-1						
	A-A	**115**	**114.965**			
	A-B	**21**	**21.069**			
	B-B	1	**.965**	**.001**	1	**.969**
CK- 3						
	A-A	**84**	**82.175**			
	A-B	**16**	**19.650**			
	B-B	**3**	**1.175**	3.555	1	**.059**
GPD-1						
	A-A	**148**	**147.347**			
	A-B	**13**	**14.306**			
	B-B	1	**.347**	1.349	1	**.245**
GPI-2						
	A-A	**112**	**110.473**			
	A-B	**30**	**33.055**			
	B-B	4	**2.473**	1.247	1	**.264**
LDH-1						
	A-A	104	**109.191**			
	A-B	**58**	**47.617**			
	B-B	**0**	**5.191**	7.702	1	**.006**
LT- 3						
	A-A	145	**145.060**			
	A-B	**6**	**5.881**			
	B-B	**0**	**.060**	**.062**	1	**.803**
HOH-1						
	A-A	135	135.424			
	A-B	16	**15.152**			
	B-B	**0**	**.424**	**.473**	1	**.492**
PGH-1						
	A-A	**124**	**123.906**			
	A-B	**21**	**21.188**			
	B-B	1	**.906**	**.012**	1	**.914**

Appendix Table 3 (cont.) Chi-square statistics by area.

POPULATION: **ROOS**

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
AAT-1						
	A-A	43	**43.184**			
	A-B		**5.633**			
	B-B	8	: 184	**.208**	1	**.648**
AH- 1						
	A-A	**36**	**37.103**			
	A-B	**12**	**10.235**			
	A-C	**3**	**2.559**			
	B-B	**0**	**.706**			
	B-C	**0**	**.353**			
	C-C	**0**	**.044**	1.516	3	**.679**
ALD- 1						
	A-A	44	**44.240**			
	A-B	**7**	**6.520**			
	B-B	**0**	**.240**	**.277**	1	**.599**
CK- 3						
	A-A	**37**	**35.880**			
	A-B	**3**	**6.052**			
	A-C	**6**	**5.188**			
	B-B	**2**	**.255**			
	B-C	**0**	**.438**			
	C-C	**0**	**.188**	14.255	3	**.003**
GPD- 1						
	A-A	**36**	**36.214**			
	A-B	**6**	**5.571**			
	B-B	0	**.214**	**.249**	1	**.618**
GPI-2						
	A-A	**28**	**27.574**			
	A-B	**19**	**19.853**			
	B-B	4	**3.574**	,094	1	**.759**
LDH-1						
	A-A	**27**	**29.469**			
	A-B	**22**	**17.061**			
	B-B	**0**	2: 469	4.106	1	**.043**
HDH- 1						
	A-A	**40**	**40.593**			
	A-B	**11**	**9.814**			
	B-B	0	.593	**.745**	1	**.388**
PGH- 1						
	A-A	**44**	**44.083**			
	A-B	**3**	**2.875**			
	A-C	**1**	**.958**			
	B-B	**0**	**.047**			
	B-C	**0**	**.031**			
	C-C	**0**	**.005**	**.091**	3	**.993**

Appendix Table 4. Chi-square contingency table statistics for entire river.

Locus	No. of alleles	Chi-square	D.F.
AAT-1	2	1.02	3
AH-l		5.69	3
AK-l	3	6.88	3
ALD-1	2	0.74	3
CK-3	3	0.81	2
EST-2	3	1.07	2
GD-1	2	5.24	3
GPD-1	2	0.99	2
GPI- 1	2	0.38	1
GPI-2	2	19.14 **	3
LDH-1	2	25.42 **	1
LT-1	2	3.30	2
LT -3	2	2.91	2
MDH-1	2	4.17	2
MDH-2	2	5.83	3
ME-l	2	2.82	3
PGM-1	3	1.81	3

Appendix Table 5. Systems where **ANOVA** indicated a significant difference between areas. In all cases except LDH and GPI-2 an allelic differentiation was apparent. In LDH and GPI-2, several areas had an excess of heterozygotes.

Enzyme system	F stat	Table value @(.05)
AH- 1	2.783	> 2.40
AK-l	3.474	> 3.02
CK-3	8.555	> 3.03
GPD- 1	7.290	> 2.39
GPI-2	4.756	> 2.39
LDH- 1	11.324	> 2.39
MDH-2	2.930	> 2.63