

Columbia River White Sturgeon

Early Life History and Genetics Study

Final Report
1984 - 1985



DOE/BP-18952-1

December 1985

This Document should be cited as follows:

Utter, F., E. Brannon, M. Miller, A. Setter, S. Brewer, W. Hershberger, "Columbia River White Sturgeon", Project No. 1983-31600, 77 electronic pages, (BPA Report DOE/BP-18952-1)

Bonneville Power Administration
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)
Early Life History
and
Genetics Study

FINAL REPORT

August 1, 1984 to December 31, 1985

By

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Funded by

Fred Holm, Project Manager
U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
Contract No. DE-AI-84BP18952
Project No. 83-316

December 1985

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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 Dr. Gary Winans and Mr. Paul Aebersold for sharing their 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 was given by Ms. Gayle Kreitman (Wash. Dept. Fish.), Mr. Steve King and Ms. Karen Moyer both from Oregon Fish & Wildlife. The Kettle Falls National Park Service rangers collected valuable samples from Lake Roosevelt. Cynthia and Charlene at the store nearest the Marcus Island campground stored samples in their freezers and made helpful inquiries among fishermen who then donated samples. Dennis Jensen and Mike Jones for their voluntary effort in the collection of samples made a helpful contribution.

Countless sport fishermen along the entire river were generous enough to allow us to sample their fish. Uncle Rob's and Jesse's in Ilwaco were extremely accomodating by allowing us to sample during their processing.

Additional thanks to Mr. Jim Lukens and Bob Griswold of Idaho Fish and Game for their donated time, equipment and effort in obtaining Snake river samples, which we had no other way to obtain. Their site knowledge was remarkable and we hit every good sturgeon hole between Lewiston and Hells Canyon dam.

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 to contribute more knowledge for the management of the sturgeon in the Columbia river.

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ABSTRACT

Research on Columbia River white sturgeon has been directed at their early life history as it may apply to production and enhancement strategies for management of the species. Spawned on the surface of substrate in high velocities, sturgeon eggs are exposed to possible losses from predation or dislodgement. After hatching and swimming up in the water column, the larvae enter a distribution phase for a period of hours determined by water velocity. Faster water appears to encourage a quicker return to the substrate where they find cover during the remaining portion of larvae incubation. Fry emerge from hiding a few days later and begin their life long activity of continually foraging for food. Activity never ceases among the juvenile stages, and their search for food encourages a general movement with the current. Salt water tolerance, however, is very poor at least during the first four to six months which limits their use of the estuary for feeding.

Behavioral characteristics and feeding strategies of sturgeon have evolved with the morphology of the species. Feeding behavior is specialized for use in dark, bottom oriented habitats where contact identity of their prey is necessary and facilitated by the evolution of highly sensitive taste receptors around their mouth. The act of capturing an individual prey item is mediated primarily by the barbels, contact with which triggers a carefully timed protrusion of the jaw that engulfs the item. Immobile benthic organisms have become the major target prey, but the rapid deployment of the protruding jaw mechanism upon contact with potential prey, allows predation on other mobile organisms when approached under cover of darkness.

The river environment in which sturgeon historically migrated, spawned, and reared has changed through development. Habitat changes are expected to precipitate genetic changes in the fish, as well as reduce the fitness in populations. Genetic analysis of samples taken from various locations over the length of the Columbia River have indicated that observed gene frequencies in all areas sampled were not in Hardy-Weinburg equilibrium, which could suggest that the general population is experiencing perturbation in the system. Analysis thus far has exposed few differences between samples from the lower, middle, and upper portions of the system. Allelic differences were identified in fish from the Roosevelt Lake, which may be evidence of unique characteristics among fish from that general area.

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.

Columbia River white sturgeon have become one of the most 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 harvest, and has been able to sustain an annual catch of from 30,000 to 56,000 fish since 1979 (King, 1983). The status of the species above Bonneville is much weaker and in some cases populations can not sustain any harvest. To manage the populations, the life history of the species and population structures must be understood. Enhancement and harvest measures can then be developed that will apply to the particular needs of the populations distributed throughout the system.

The present report is the second on 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. Phase 2 has extended the life history studies, and initiated stock identification by electrophoretic examination of allelic frequencies between populations. The objectives of the study were to:

1. Characterize the distribution behavior of Columbia River Basin white sturgeon larvae and fry.
2. Determine the influence of certain environmental conditions on the survival and quality of white sturgeon larvae and fry in the Columbia River.
3. Characterize the feeding behavior of larvae and fry, their responses to the presence of food, time of feeding initiation, and how food is captured and consumed.
4. Examine the influence of hydroelectric development on isolation of Columbia River white sturgeon populations.

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. It

would have been difficult to have achieved the objectives of the study without the generous assistance of Idaho Fish and Game, and University of California at Davis in providing samples for analysis and experimental material for laboratory testing.

DESCRIPTION OF STUDY AREA

Investigations were undertaken in both the laboratory at the School of Fisheries and the field. Observations and tests in the laboratory were used to describe juvenile responses as they developed from eggs to fry exposed to simulated environmental variables that characterize their natural range. The laboratory facilities were equipped with test arenas to present the fish with test variables under controlled conditions of flow, light, water quality, feed levels, and in a limited degree, temperature. The laboratory was equipped with incubation and rearing containers to hold experimental animals. After tests were conducted, the fish were placed in the adjacent hatchery for continued rearing.

Field data provided a qualitative environmental base from which laboratory studies could simulate environmental situations in the Columbia. Effort was expended to sample the juvenile fish population in the river to determine where young sturgeon inhabited the system, examine stomach contents, and assess environmental conditions.

Field samples were collected for electrophoretic analysis from Roosevelt Lake, the Snake River, the mid-Columbia and below Bonneville Dam as areas representing the distribution range of white sturgeon in the Columbia River. Samples were obtained from the sport and commercial harvest along the river and from fish packing facilities. A special field sampling effort was conducted on the Snake River with the assistance of Idaho Fish and Game.

METHOD AND MATERIALS

Sturgeon eggs and larvae were supplied for the study by the University of California at Davis and "The Fishery" at Elk Grove California, and Covert's Landing, Oregon, on the Columbia below Bonneville. Eggs were removed from the fish, fertilized, water hardened, incubated four days and transferred to the laboratory. In those circumstances where eggs were involved in the test situation they were spawned and adhered to substrate before incubation and transfer, or transferred as water hardened eggs and placed on substrate at the beginning of the study. Young sturgeon used in tests were taken from the holding tanks at the laboratory and placed in the test arenas.

Most of the tests were conducted in four doughnut-shaped observation arenas (Fig.1). Clear acrylic sheets 30.5 cm wide were shaped to form the walls and fitted onto a 1.9 cm thick plywood sheet cut in the shape of a doughnut with an outside diameter of 122 cm and a surface area of 81 square cm, forming a 30 x 30 x 383 cm circular trough. The doughnut arenas were set up to simulate river conditions including substrate and continuous flow of water. The shaded center observation area allowed the behavior patterns and responses shown by naive sturgeon, to be recorded with minimal disturbance of the sturgeon.

Grades of detritus, sand and stones recovered from the Columbia were placed on the floor of each doughnut providing four distinct areas of substrate type (Fig. 1). Dechlorinated city water was supplied to each doughnut arena, with the temperature reflecting that of the ambient water supply. Lighting was provided by placement of a 25 watt incandescent lamp 60 cm above each doughnut on the side housing the drain and water inlet. Photoperiod corresponding to ambient daylength was maintained using automatic timers. Additional lighting was provided by incidental light located elsewhere in the laboratory and a red 25 watt incandescent lamp placed one meter directly over the center of each doughnut. All lighting was timed so as to simulate sunrise, daytime, and sunset lighting regimes. Red lights would turn on first, then the incidental lab lights within 30 minutes to one hour later, and finally the doughnut arena lights thirty minutes later.

Each test condition and test routine used in separate tasks was different. The details involved with preparations before executing each task will be presented as "procedures" in the section describing the tests associated with each of the four objectives.

Field sampling- procedures involved several sampling techniques. A 30 m beach seine with 0.3 cm mesh was used at all of the sample areas which had suitable seining conditions. In areas with offshore pilings, three 45 cm and one 120 cm square fyke nets, with 1 mm and 5 mm mesh respectively, were tied

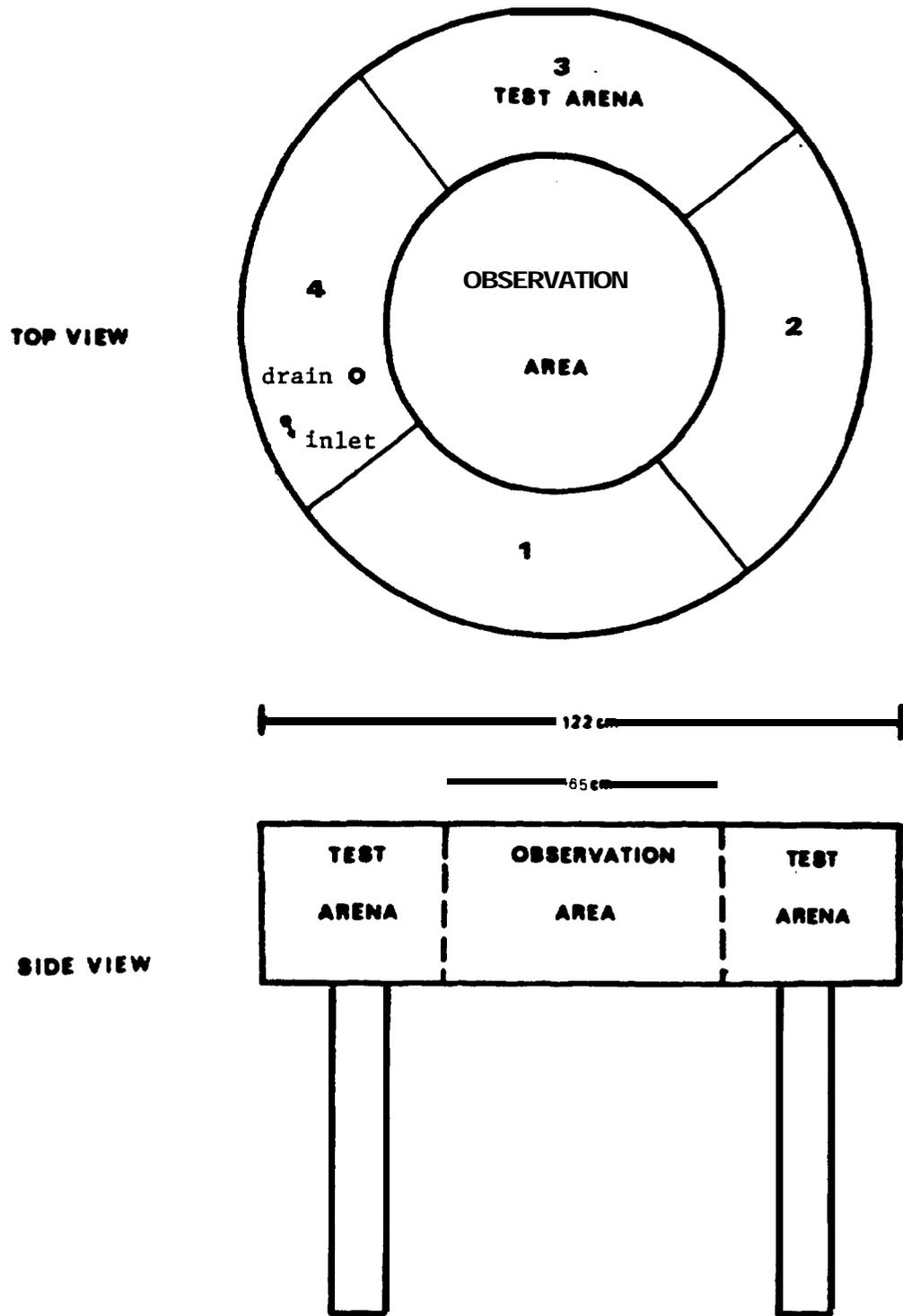


Figure 1. Doughnut shaped test arena.

to pilings and anchored about 15-20 m downriver. These nets were designed to capture any larval and juvenile sturgeon that might be moving downstream with the current at depths of 3-10 m.

An epibenthic sled with a 16x35 cm opening and very fine mesh was used to sample depths ranging from 1-8 m. The sled was used to sample various habitats for larval and juvenile sturgeon located on or very close to the bottom. The small mesh on the sled also provided qualitative information about the invertebrate community of the areas sampled.

In July and August, a 6 m otter trawl with 2.5 cm mesh side panels and .3 cm mesh bag at the cod end, was used to sample depths ranging from 3-12 m. This technique sampled the fish and macroinvertebrate communities near the bottom. In August several small crayfish traps with 5 cm openings were baited with dead fish and salmon meal and set off pilings at 7-14 m depths. Most sampling was done during the daylight hours, but the beach seine, epibenthic sled and fyke nets were sometimes used after dark in depths up to 8 m.

Tissues collected for electrophoretic analysis were taken from individual fish. When it was possible eye, heart, liver, and muscle tissue were cut from a specimen at the time fishermen were cleaning their catch. In those instances when the fish could 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 2). The tissue was then placed in a ziploc bag, set on dry ice for immediate freezing, and transferred back to the University. At the laboratory samples were stored at -85° C in a super cold freezer to prevent breakdown of tissue proteins.

Prior to analysis, without allowing a sample to completely thaw, each tissue was cut and a 1/4" by 1/8" piece was put into a test tube. The test tube contained 7 ml of a tissue prepping solution (PTP; Aebersold et al. , In Press) which helps to produce better banding patterns with some of the enzymes when stained. The test tube was put into the freezer for storage. Each tissue type was kept in a separate rack in a specific ordered sequence, which was repeated for all tissues. Tissues obtained from fifty individuals from each sample area were stored in the same test tube rack.

Starch gels were prepared the day before electrophoresis was started. Gels were poured using Sigma starch and the buffers shown in Table 1. 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 regulated high voltage power supply for 4-6 hours. Marker

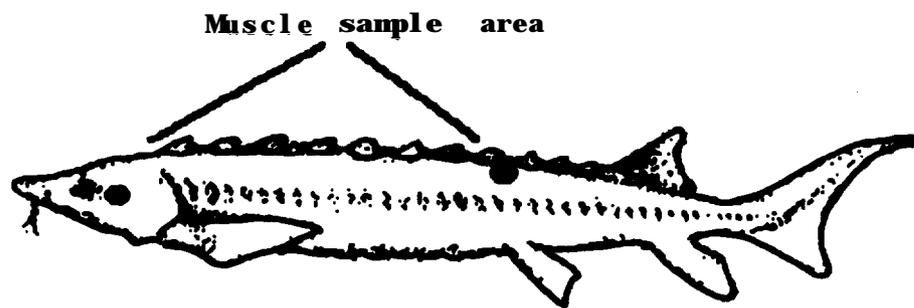


Figure 2. Side view of a sturgeon showing the area where muscle plug samples were taken.

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)
2.	Tris-borate (pH 8.7)	Tris-borate (pH 8.7)	(Aebersold et al, In Press)
3.	Citric Acid (pH 6.5)	Citric Acid (pH 6.1)	(Aebersold et al, In Press)
4.	Tris-citrate (ph 7.0)	Tris-citrate (ph 7.0)	(Shaw and Prasad, 1970)
5.	Tris-phosphate (pH 8.2)	Tris-phosphate (pH 8.2)	(Busack et al., 1979)

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 which react to produce banding patterns. Each protein has a different mobility and banding pattern which represent the genotypes of the individuals. The banding patterns are recorded and used to calculate allele frequencies. Data is collected from each individual and analyzed by area. Analysis of variance was run using minitab on the University of Washington computer.

RESULTS

Objective 1: To characterize the distribution behavior of Columbia River Basin white sturgeon larvae and fry.

Dispersal mechanisms of larval fish like sturgeon are related primarily to environmental phenomena. Distribution behavior, therefore, will place the juvenile fish in a position to take advantage of such influences. Water currents, substrate type and photoperiod are important factors affecting behavior of sturgeon larvae and fry in the Columbia River. Studies conducted in 1983 suggested that yolk sac larvae swim randomly in the water column several days before seeking cover on the stream bottom (Brannon, et. al. 1984). After yolk absorption, young fry frequently leave the bottom and swim with the current for considerable lengths of time. During both periods their behavior suggested that a major displacement occurred downstream. In the present study an attempt was made to characterize such distribution behavior. Four factors were selected as having potentially major influences on distribution, and they were identified for examination as four separate tasks; 1) the influence of current velocity, 2) the influence of substrate, 3) the influence of temperature, and 4) the influence of photoperiod on distribution behavior.

Task 1: Examine larvae and fry responses to current.

Statement of Problem

In the first phase of the life history study, larvae remained in the water column several days after hatching and were found easily displaced by water currents. Larval displacement appeared to be the primary mode of distribution, and may be a major factor in larval survival, especially where adult access upstream is limited by dams. To determine the influence of velocity on the displacement behavior of newly hatched larvae, the length of time that larvae remain in the water column at different velocities was examined.

Null Hypotheses: Sturgeon larvae remain in the water column in a free swimming pattern for a given length of time unrelated to water velocity.

Procedures

Current velocity in each of the four doughnut arenas was set at 2-3 cm/sec with a flow of 1.5 liters per minute. Eighty developing ova (Columbia River white sturgeon eggs from "The Fishery") were distributed uniformly onto the

substrate in each doughnut. Once hatch occurred, the current in doughnut 1 was increased two to three times the current in doughnut 2.

Routine observations began at hatching, designated as day 0. Each day three periods of observation were conducted in each doughnut arena, beginning from one to three hours into the photoperiod, mid-photoperiod and within the last three hours of the photoperiod. Upon entering the observation area in the center of the arena, the observer would count the number of fish observed in the water column. Next, the observer noted how many fish were on the substrate within each of the four designated sections of the arena. After the numerical count, the observer noted qualitative behavior of the fish for at least two minutes. An overall activity rating,, based on a scale of 1 to 5, was assigned the fish in each doughnut for the observation. A rating of 1 meant fish were motionless on the substrate, with occasional tailbeat and slight opercle movement. Five as an activity rating meant the fish exhibited burst activity with erratic, frantic swimming around the doughnut arena at accelerated speeds. A rating of 3 was considered normal and was given to fish that were constantly moving with paced flowing motions. After making an observation in each doughnut the temperature was recorded using a hand held thermometer. Observations were conducted every day from day 0 through day 25. From day 25 through day 69 observations were conducted every other day. From day 69 through day 82 observations were conducted every three days.

Fish were fed tubifex worms daily beginning on day 11. Mortalities were promptly removed from the arenas. Dissolved oxygen was measured once per day using a YSI Dissolved Oxygen meter model 54. Current was also measured daily using a Marsh-Birney portable current meter at a depth of 10 cm in the middle of each section. Current averaged 7.9 cm/sec throughout doughnut 1 and 2.0 cm/sec in doughnut 2. As fish grew it became necessary to remove some to prevent overcrowding from affecting behavior. Doughnuts were thinned to forty fish at day 26, and to thirty on day 76. Fish that were removed were anesthetized with MS 222, as well as ten fish periodically from the holding tanks, for representative weight and length data of the age class being tested at that period.

Observations

Major events characterizing the early life history of Columbia white sturgeon are summarized in Table 2. Temperature during egg incubation averaged 16.4 °C. hatching first occurred during the later part of the photoperiod on May 25, 1985, with three larvae in test arena 1 and one in test arena 2. By the next day almost complete hatching had occurred in both of the doughnut arenas. Observations on the time of hatching indicated that it occurred primarily during darkness. The freshly hatched fish swam up in the water column with their heads breaking the surface and continued to swim in a cycle to the substrate and back to the surface throughout the

Table 2. Major behavioral events characterizing the early life history of Columbia River white sturgeon.

<u>DAYS POST HATCH</u>	<u>EVENT</u>
HATCH	Hatching occurred during darkness in 16.4 °C water
0	Upon hatch yolk sac larvae enter water column
1	Larvae in high velocity arena begin to settle upon substrate within cover
5	All fish in both arenas upon substrate within cover
9	Yolk sac almost depleted, fry moving out onto open substrate, initiation of feeding
13	Yolk sac depleted, all fry upon open substrate actively feeding (Water temperature 16.0 °C).

observation period. Although larvae possessed a yolk sac, they were able to swim both into and with the 2.0 cm/set. current, but in a pattern that appeared random. Activity in these larvae was rated at 3. Day 0 was designated as the day on which nearly 100% of the larvae were hatched.

After the velocity was increased in arena 1 the fish in the water column were pointing into the current, constantly moving with their heads up and the bodies at a forty-five degree angle. Larvae would hold a position against the current then move with the flow while appearing to regulate the pace. As they moved downstream, larvae would drop down to the substrate, lay for three or four seconds, then move back into the water column. Larvae observed in arena 2 also moved constantly in the up and down fashion described above, but struggled less than those in the faster velocity.

Figure 3 shows the percentage of fish in the water column in both test arenas from 0 to 20 days after hatching. As day 0 progressed, less arena 1 fish were noted in the water column, with only 50% of the fish in the column by mid-day, and 13% by the end of the photoperiod. Mid-day in arena 2 showed 75% of the fish still in the water column, and at the end of the day 34% remained. Beginning on day 1, only one fish (3% of observable larvae) was observed in the water column of arena 1, whereas in arena 2, 30% of the observed fish were swimming. At mid-day one larvae still remained swimming in arena 1. In arena 2, 15% of observed larva appeared in the column, but decreased to one fish at days end. During the initial observation period of day 2, no fish were swimming in arena 1, but 33% of observable fish in arena 2 were back in the water column. By mid-day, 14% of the fish were swimming in arena 2, and at the end of the day 9%. This pattern for arena 2 was repeated again on day 3 while no fish were seem swimming in arena 1. Not until day 5 had all fish in arena 2 moved to the bottom. After completion of the experiment it was determined that hatching success approached 95% in both arenas, but the nearly 75 larvae in each arena were never observed at one time.

White sturgeon eggs are adhesive and attach to any substrate they come in contact with. Unless the eggs are spread out, high egg density coupled with even slight initial egg mortality causes fungal matting and eventual mortality to incubating larvae. It is believed that upon hatching the larvae rise up in to the column to exit the incubation area, with large yolk sacs remaining. Hatching during the hours of darkness may allow the larvae to avoid visual predators. Immediately moving out of the areas of potential fungus problems increases the larva's chances of survival.

Moreover, rising into the water column to be carried by a rapid current may decrease the probability of an encounter with foraging predators. As eggs are spawned, high concentrations of very vulnerable egg clusters and larvae will tend to be easy prey for fish capable of sustaining the high

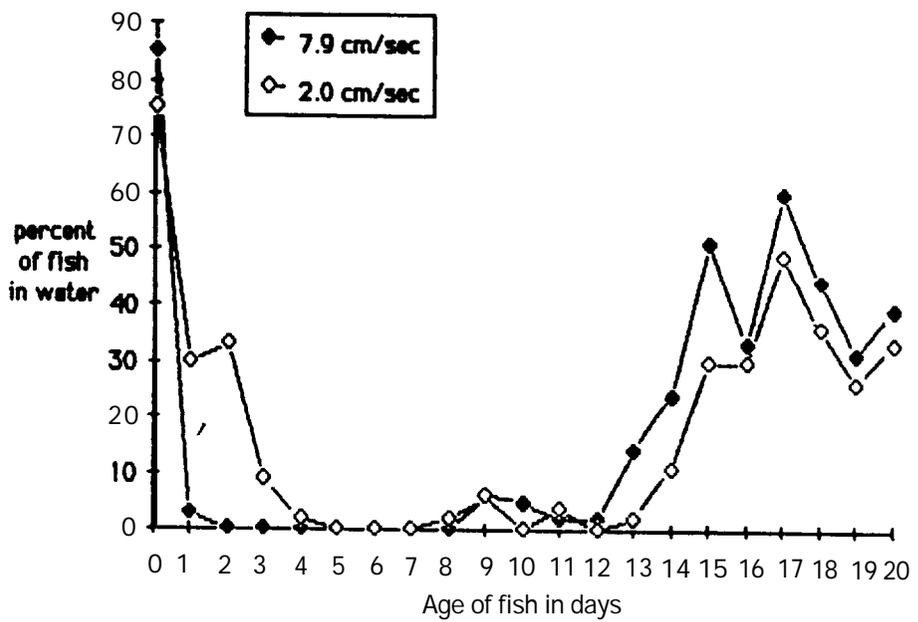


Figure 3. Distribution of white sturgeon larvae and fry in the water column during the test period from 0 to 20 days post hatch at two velocities (2.0 and 7.9 cm/sec in the doughnut shaped test arenas).

velocities characteristic of sturgeon spawning areas. Since other sturgeon may be predators on newly spawned or hatched larvae, moving away from the spawning grounds would reduce the chance of predation by sturgeon.

Being displaced from the spawning grounds and entering cover further downstream is a dispersal mechanism to lower larvae density at the initial incubation site. Cover provides a predator avoidance refuge for the remainder of the incubation period that wasn't available at the site of spawning, nor was it provided for by the placement of the eggs. While fish were in the substrate, they occupied interstices between gravel, wedged themselves under rocks or in amongst plants. The fish often had their head in the "hiding" place with their tail out, showing a constant tail beat or oscillation. It did not appear that the larvae changed location throughout the remaining incubation period. It was curious, however, that during the hiding phase larvae were often found in touch proximity with other larvae. Other similar cover areas just a few centimeters away would attract very few or no larvae.

The pattern of behavior was the same for both the fast and slow current arenas, except for the time that passed before cover was sought. Our investigations demonstrated that slower velocity delayed the movement of larvae into the substrate, which also appears to be an adaptive trait. Slower current will displace the larvae at a lower rate, and thus more time would be required to gain similar dispersal experienced at higher velocities.

By day 9 very little of the yolk sac could be seen, and the larvae began to move out onto the substrate. Food had been introduced at that time and it appeared the fish were actively foraging over the substrate. Distribution around the arenas became more uniform with each of the four substrates being represented by several fish. Prior to this point in the experiment only 1 or 2 larvae could be spotted on the sand-gravel substrate of either arena. As the fish moved out on the open substrate most faced into the current. Intensity of current did not influence emergence timing from hiding, nor fry behavior. Larvae in both arenas showed similar patterns.

Once feeding had commenced the fry were constantly active on the substrate and in the water column. By day 13, more fish were in the water column relative to those still on the substrate in arena 1 than in arena 2. On day 18 this difference between the arenas was no longer apparent. Yolk sacs on all fish were completely absorbed and no fish were found hiding. Temperatures by this time had reached 17.0°C in each doughnut.

Most of the fish were oriented either to the bottom substrate or the sides of the test apparatus. Those upon the substrate tended to stay in a localized area and usually at or near where food had been placed previously. When food was introduced, fish in the water column would drop to the

substrate and begin foraging behavior. If food was encountered, the activity intensified and the group around the food became very aggressive. After the food was consumed, the distribution of fish spread out, but they remained on the substrate. When food was absent, particularly for long periods, more fish moved into the water column.

Net movement of the fish was generally downstream, but they would often move to the substrate and forage into the current. Initially the rate of movement with the current was related to the current velocity. However, as the fish grew their rate of downstream movement became more independent of water velocity, as though they moved at will. If no food was detected, the fish would rise up into the column and continue downstream again. Behavior of the young sturgeon was determined by the presence or absence of food. Substrate type appeared insignificant as the fish would inhabit any area containing a food resource. No difference occurred in growth of the fish between arenas. Fish in either current regime were equally able to exploit the available food source.

Results indicate that the null hypothesis was disqualified. Current velocity influenced the length of time that larvae remained in the water column before entering the hiding phase. Slow velocities lengthened the free swimming period of larvae. After feeding commenced, the fry were not influenced differently by the velocities tested.

Task 2: Larvae and fry responses to substrate.

Statement of Problem

Substrate composition in a river may influence both the emergence and settling response of sturgeon larvae and could affect whether they remain in an area once they become bottom oriented. Substrate type could be integral to the behavioral adaptations white sturgeon have developed for survival. Should a substrate type be limited in a river impacted by hydroelectric development and operation, survival for larvae and fry could also be limited. Present studies test the influence of substrate type on distribution behavior of white sturgeon larvae and fry.

Null Hypothesis: Larvae and fry do not show a preference for a particular substrate.

Procedures

Task 2 utilized the same procedures as task 1. The number of fish in each of the four designated sections (Fig.1) was recorded and the analysis

was based on a quantification of the distribution of the fish throughout the arena. Substrate types in each of the four designated sections of the test arenas are described as follows:

1. Section 1 substrate consisted of small gravel placed on top of a sand-gravel mixture. Patches of sand were scattered randomly. One medium size rock with sand piled around was placed in the middle of the section. In the area just downstream of the water inlet, channelized areas occurred and fines were washed from the gravel continuously .
2. Section 2 consisted of a sand-gravel mixture with random patches of sand and other patches of only gravel. One large rock providing cover underneath was placed in the middle of this section.
3. Section 3 substrates were a sand gravel mixture with small rocks and a patch of 3-4 aquatic plants placed in the middle of the section, toward the outer perimeter.
4. Section 4 was an open section consisting of a sand-gravel mixture. The sand-gravel mixture was level throughout the tank.

Observations

Upon hatching larvae enter the water column and are subject to the influence of current. Larvae then seek the substrate for places that provide cover. Larvae remain in the substrate until yolk is absorbed and feeding initiated. Larvae were noted to enter just about every conceivable space where they could hide their head. Beneath rocks, gravel interstices, amongst plants, and under detrital material were the places harboring the larvae during the "hiding" phase. A few fish were noted with their head in an expended egg case and remained there until initiation of feeding.

This general behavior would suggest that the larvae at this time were photophobic. The vast majority of the fish, however, preferred the area underneath the large rock placed in section 2. Sixty percent of the larvae observed hiding in arena 1 and 52 % in arena 2 were found in section 2. The difference between the two arenas showed more arena 2 larvae hiding in section 3 than larvae in arena 1. It isn't known why fish in a slower current regime preferred both the plants of section 3 and the rock of section 2, while those in arena 1 seemed to prefer the rock of section 2 solely. Fish in arena 2 did spend more time in the water column thus having an increased chance of encountering the plants. Larvae in both arenas tended to clump together in places resulting in high density situations. Substrates offering cover nearby were neglected suggesting some intraspecific attraction among the fish.

Once feeding commenced no particular substrate type seemed to be preferred. Fish were more uniformly distributed throughout the tanks. Sections of the arenas where food was introduced were the places the fish congregated until the food was gone. Fish were constantly on the move at this period of their life. It was noted that when fish were upon sand they would linger, tending to slow movement some. When upon the gravel or rocky areas the fish would skim and move along the substrate rarely stopping or spending much time until reaching sand again. However, the fish would not stop every time sand was encountered.

Results indicate that the null hypothesis was disqualified. Larvae preferred areas where cover was maximized, such as under large rocks and in plant material. After feeding was initiated, however, no substrate appeared preferred, although the young fish touched the substrate and moved slower when they were over sand.

Task 3: Larvae and fry responses to changing temperature.

Statement of Problem

Operation of hydroelectric facilities can result in rapid temperature changes immediately downstream from the dam when temperature stratification has occurred in the reservoir. Daily fluctuations of two degrees centigrade were recorded on certain days in 1982 at both Ice Harbor and McNary Dams (U.S. Army Corp of Engineers, 1982). While gradual fluctuations of two degrees from one day to the next would be within the range of normal temperature variation, a sudden change in temperature may have an influence on distribution behavior of white sturgeon larvae and fry. The present task investigated the behavioral response of larvae and fry when exposed to a rapid temperature change.

Null Hypothesis: Sturgeon larvae and fry will show no response to temperature changes of 5°C above or below ambient temperature.

Procedures

Tests were conducted in the doughnut-shaped observation arenas described in Task 1. Substrate type, substrate placement, food, lighting, and water supply were uniform for all tests. Dechlorinated city water was supplied to each doughnut arena, with the temperature reflecting that of the ambient water supply. Lighting was provided by placement of a 25 watt incandescent lamp 60 cm above each doughnut on the side housing the drain and water inlet. Photoperiod corresponding to ambient daylength was maintained using automatic timers, and the current velocity in each arena was set at 2-3 cm/sec with a flow of 1.5 liters per minute. During the first month after hatching, only three of the test arenas (2 [control], 3, and 4) were

utilized. For the second month of testing a second velocity was added to the test and all four arenas were used. Arena 1 (control) and 3 were operated at 7.9 cm/sec, and arena 2 (control) and 4 continued at 2-3 cm/sec.

Fertilized eggs on substrate were placed in the test arenas. Tests began at two days post-hatch, were repeated at 12 days post-hatch, and once a week thereafter for two months. Observations were conducted at the beginning of the testing period noting the number of fish on the substrate, location of fish, activity, and behavior. After the initial observation the test temperatures were added to doughnuts 3 and 4. Observations were made each thirty minutes in the two hour test period.

Temperature changes were imposed by the supply of warmer or cooler water being delivered through hoses directly into the doughnuts. Heated Lake Washington water was used to provide the warmer temperature regime and chilled dechlorinated city water provided the cooler temperature regime. Flows of the incoming test waters were regulated to give an approximate increase or decrease of five degrees centigrade over a two hour period. When temperatures were changed the water exchange rate increased, but current velocity remained unchanged. At the conclusion of the experimental period, temperature sources were shut off and ambient conditions returned. Fish were not removed, as the same group continued to be tested week after week. The warm and cool tests were alternated between the two test arenas.

Observations

White sturgeon larvae and fry appear to be most sensitive to fluctuations in the temperature regime during the first few days after hatch. Two day old larvae exhibit increased activity when the temperature rose from 16.5°C to 21.9°C over a two hour period. Sixty seven percent of the fish observed after two hours were moving in the water column compared to 41% at the initial observation in the beginning of the test (Fig 4). In contrast, when the temperature lowered from 16.4°C to 11.7°C the two day old fish became sluggish and less active. At the beginning of the test, 24% of the fish observed were in the water column. After one hour only 7% remained. In the control arena, where the temperature remained unchanged, about 25% of observable larvae were in the column throughout the test period. Larvae at this stage are entering the time when they hide under rocks, plant material, and within the gravel interstices.

By day 12 post-hatch very little difference existed between the test and control arenas (Fig. 5). Larvae by this time are completing yolk absorption and beginning to feed. Behaviorally, larvae are starting to move out from cover and onto the substrate in search of food. Once fish were actively feeding they would exhibit an insignificant response to the temperature fluctuations. The percentage of fish in the water column relative to those on the substrate did not change during the test.

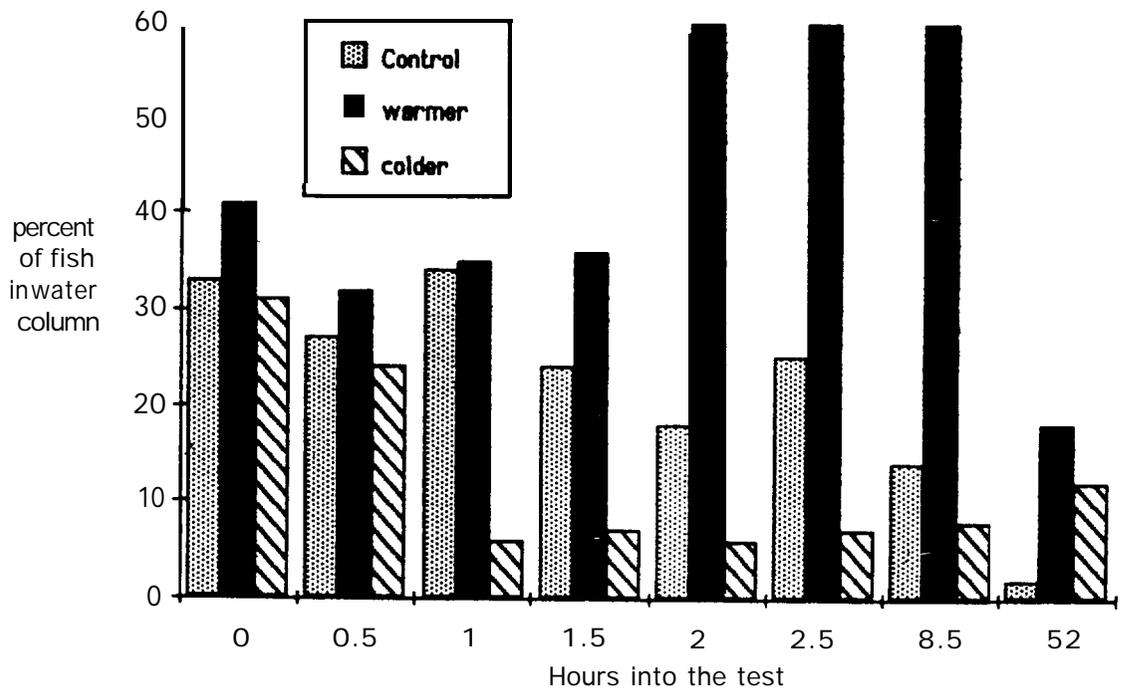


Figure 4. Distribution of 2 day post hatch larvae exposed to increasing or decreasing temperatures in the doughnut arena.

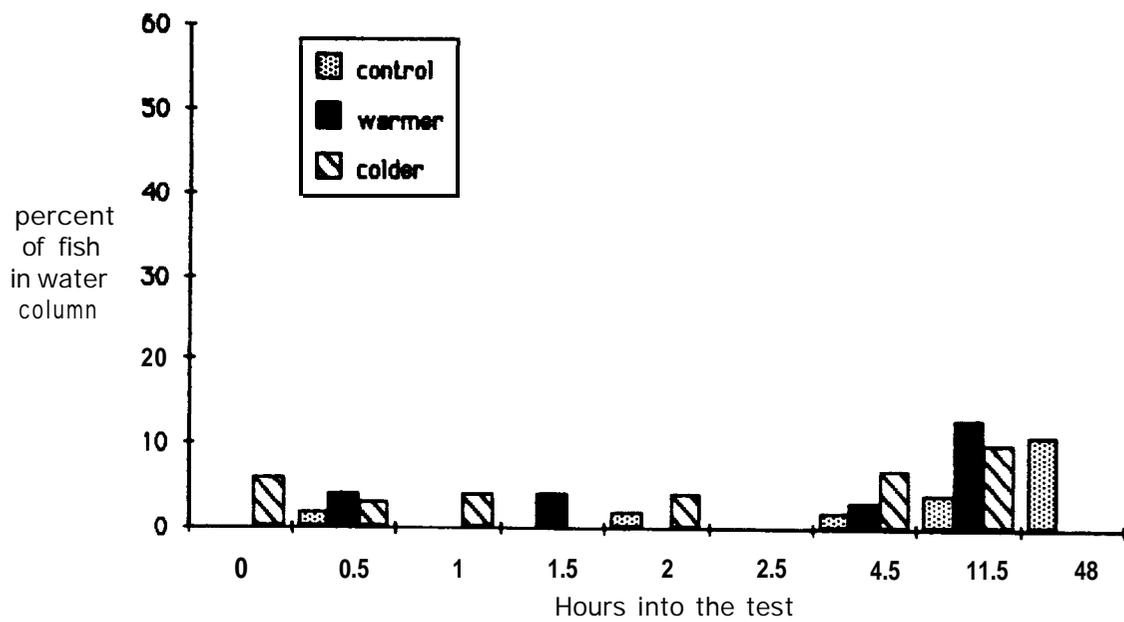


Figure 5. Distribution of 12 day post hatch fry exposed to increasing or decreasing temperatures in the doughnut arena.

As fish became older, differences in activity resulting from changes in temperature were not apparent (Fig. 6). Differences in current did not result in different behavior. Feeding fry were oriented towards seeking food, unaffected by temperature changes in the short term. With older fish the use of lake water generated a response similar to that when food was introduced to the tanks by increasing their substrate foraging activity.

Results indicated that the Null hypothesis was disqualified. A marked increase in temperature induced larval emergence from hiding, and increased their activity. In contrast, a reduction in temperature resulted in a significant reduction in activity and induced a greater number of the larva to enter the substrate. As the fry get older rapid temperature changes do not result in behavioral changes.

Task 4: Larvae and fry response to photoperiod.

Statement of Problem

White sturgeon larvae and fry exhibit a sensitivity to light which may influence their distribution behavior. Displacement by current during darkness could extend the downstream drift of larvae if darkness causes their disengagement from the bottom after settlement. Activity and behavior may be directly related to photoperiod. The 1983 studies suggested that young white sturgeon were more active at night than during the day. (Brannon et al., 1984). In 1985 focus was on the influence of photoperiod on larval and fry swimming patterns and orientation.

Null Hypothesis: There is no influence of photoperiod on larvae and fry activity, swimming patterns, and current orientation.

Procedures

Task 4 was integrated with Task 1 procedures. Lighting was provided by placement of a 25 watt incandescent lamp 60 cm above each doughnut on the side housing the drain and water inlet. Photoperiod corresponding to ambient daylength was maintained using automatic timers. Additional lighting was provided by incidental light located elsewhere in the laboratory and a red 25 watt incandescent lamp placed one meter directly over the center of each doughnut. All lighting was timed so as to simulate sunrise, daytime, and sunset lighting regimes. Red lights would turn on first, then the incidental lab lights within 30 minutes to one hour later, and finally the doughnut arena lights thirty minutes later. Analysis of the influence of photoperiod consisted of correlating time of day with number of fish in the water column. Light intensity was kept at low levels of illumination.

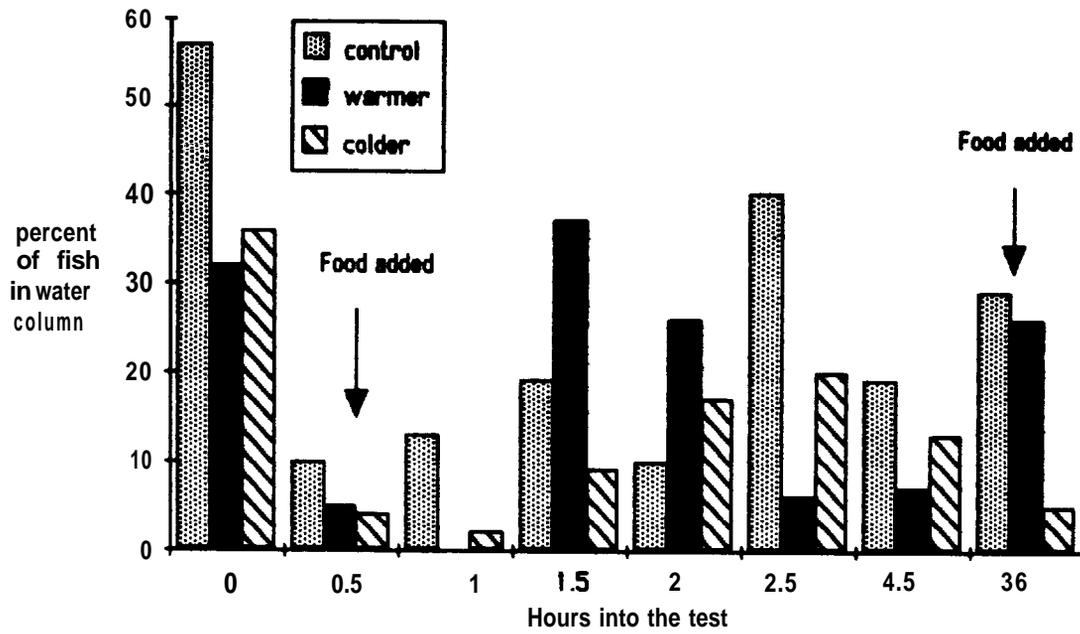


Figure 6. Distribution of 27 day post hatch fry exposed to increasing and decreasing temperatures in the doughnut arena.

Observations

Photoperiod was most influential during hatching and the first few days after hatching. Hatching and the immediate emergence into the water column occurred during darkness. The number of fish in the water column relative to those on the substrate varied with photoperiod, but also with time (Table 3). In arena 2, on day 0 more fish were noted swimming during the first few hours of the photoperiod. As the day progressed the number appearing in the water column decreased. On day 1 post-hatch the number of arena 2 larvae in the water column was found slightly elevated again during the early hours of the day. The pattern of many fish in the water column in the morning and decreasing throughout the day continued in arena 2 throughout day 3. By day 5 all fish in arena 2 were within the substrate.

Observations on numbers of fish in the column at night with the aid of red lights revealed that fish were present, but difficult to enumerate. Fish were noted swimming in the water column at first light and dropped off as the day progressed which indicated that larval movements may increase at night, during the swim-up mode. The general pattern, however, was a continued reduction of number of fish in the water column as time progressed. A slight increase in swim-up occurred during darkness. Larvae at this time appear to be photophobic. They sought cover in any space that could shelter their head, including discarded egg casings out in the open substrate. During the settling phase after initial swim up, the majority of fish were under a rock in section 2 which also was the area of the tanks furthest from the light source.

Once feeding was initiated photoperiod was not as influential on the behavior and movements of the fish. Soon after feeding commenced a slight pattern of more fish in the column during the morning and decreasing throughout the day was evident. Intensity of activity, however, did not change during the photoperiod at this time. Once fish were fully into the feeding mode by day 19, their location in the arenas was independent of photoperiod.

Results indicate that the null hypothesis was disqualified. Photoperiod has a major influence on larvae behavior. Larvae are photonegative during the hiding mode and they become much less photonegative with increased age.

Objective 2: To determine the influence of certain environmental conditions on the survival and quality of white sturgeon larvae and fry.

Certain environmental factors in the Columbia River system that may

Table 3. The number of fish in the water column/number of fish on substrate expressed as 40/13 from 0 to 20 post hatch during three daily observation periods.

AGE OF FISH (DAYS)	OBSERVATION PERIOD		
	1 <u>HRS. 1-3</u>	2 <u>HRS. 7-9</u>	3 <u>HRS. 14-16</u>
0	40/13	40/13	12/37
1	16/37	7/40	1/44
2	17/35	6/38	4/41
3	4/43	0/48	1/45
5	0/46	0/49	0/42
7	0/39	0/42	0/32
9*	2/31	0/34	3/35
12**	0/57	0/43	2/54
15	20/46	20/47	8/62***
20	20/40	11/53***	12/57

Photolength = 18 hours

Current velocity= 1.5-3.0 cm/sec; Avg. 2.25 cm/sec.

Temperature = 15.7-17.7 C; Avg. 16.6 C.

* - Beginning to move out upon substrate exhibiting feeding behavior, see Task 7 observations.

** - Feeding commenced on day 11 at observation period 3.

*** - Fish fed before this observation period.

have an impact on juvenile sturgeon are related to conditions created by hydroelectric projects. Where spawner upstream movement is limited by Bonneville Dam, spawning is forced to occur closer to salt water and juvenile sturgeon do not have as many river miles over which to distribute before they approach the estuary or marine conditions. Preliminary tests in 1983 (Brannon et al, 1984) indicated that juvenile sturgeon were intolerant of high salinities, which suggested that arriving too early in the estuary could be a source of juvenile mortality. From another perspective, however, dams may severely limit the production potential of a river by denying access to the estuary when sturgeon are of the size to enter and take advantage of the rich food resources there.

Another hydro related environmental condition is the high level of dissolved oxygen prompted by the plunging water over dams, or low oxygen from water standing in side channels. Although water depth frequented by sturgeon will generally protect them from supersaturation, juvenile sturgeon may concentrate in areas where they can be exposed to supersaturated water, or possibly low oxygen water unless they are able to detect and avoid undesirable conditions. This part of the study examines the influence of salinity and oxygen level on the behavior and survival of young sturgeon.

Task 5: The influence of salinity on survival and behavior.

Statement of Problem

White sturgeon are characterized as an anadromous species, capable of utilizing the ocean (Hart, 1973). White sturgeon are caught incidently in trawl fisheries off the coast of the Pacific Northwest (Paltie and Tagart, 1984) and tagging studies show movement into saltwater (Chadwick, 1955). Anadromous fish spend most of the adult life in the ocean and migrate into fresh water to spawn. Ocean environments, particularly an estuary, are rich in food resources allowing for the tremendous growth inherent in the species. River systems, like the Columbia River Basin, affected by hydroelectric facilities, contain populations whose movements to and from the ocean are inhibited by such impoundments. Length of time young white sturgeon spend in fresh water before they are able to exploit the ocean is unknown. Although responses to salinity are of interest with regard to the maximum level they can withstand, the main issue is how soon can they utilize the estuary which represents the most productive environment available, and what influence would denial of access to the estuary have on the young fish.

Studies conducted in 1983 showed that 29 day old, 0.4 g Columbia River white sturgeon could-not survive 15 ppt saline water (Brannon et al 1984). McEnroe and Cech (1985) reported low survival for Sacramento River White sturgeon (several weeks old and weighing 0.4-0.6 g) when exposed to 10 ppt, but did not investigate larvae and fry. Upon hatching in rivers where

spawning populations are prevented from moving upstream, larvae may be subject to currents which displace them to the estuary or the open ocean. Present studies sought to examine through bioassays and behavioral observations the ability of young white sturgeon to tolerate abrupt transfer to various salinities.

Null Hypothesis : Sturgeon larvae and fry show no tolerance or preference for salinities greater than freshwater.

Procedures

Larvae and fry were held in five foot circular tanks supplied with dechlorinated city water. Upon initiation of feeding young white sturgeon were fed artificial diets for the duration of the experiment. Bioassay tests were conducted at days 1, 8, 15, 25, 31, 38, 45, 52, 59, 63, 70, and 83 after hatching. Sixty fish were sampled from the experimental stock at each testing period, and at the conclusion, ten fish were randomly selected from the experimental stock, weighed, and measured. Replicate four liter test aquaria were supplied with three liters of the appropriate test water representing salinities of 0, 8, 11, 16, 23, and 31 ppt. Aeration was provided to each aquaria to ensure adequate dissolved oxygen supplies and constant mixing of the saline solutions. Each aquaria was placed in a continuous flow water bath to maintain temperatures consistent with rearing conditions. Salinities were achieved by combining fresh dechlorinated city water with appropriate amounts of Marine Environment, a commercial saltwater mixture. Salinity was measured using a YSI S-C-T Meter model 33. Test water was renewed after two test periods. Temperature and dissolved oxygen content were measured prior to testing. Dissolved oxygen was monitored using a YSI Dissolved Oxygen Meter model 54.

Fish were randomly selected and placed one at a time into each test aquaria until five fish per test aquaria was achieved. Observations of mortality and fish behavior were conducted at 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours into the test. Mortalities were not removed until conclusion of the twenty-four hour period or once all fish had died in an aquaria.

Behavior observations were undertaken by placing substrate consisting of a sand-gravel mixture into two preference arenas (ten-gallon glass aquaria) providing a slope running diagonally over their length (Fig.7). One arena served as a control and was filled with 20 liters of fresh water. To the other arena was first added 10 liters of fresh water (0 parts per thousand), then 6.5 liters of 15 ppt saline water, and finally 3.5 liters of 30 ppt saline water. Test waters were added to the arena through a 3/16 " I.D. Tygon tube, with one end placed in a head tank positioned above the arena and the other end in the lower corner of the arena. Water moved from

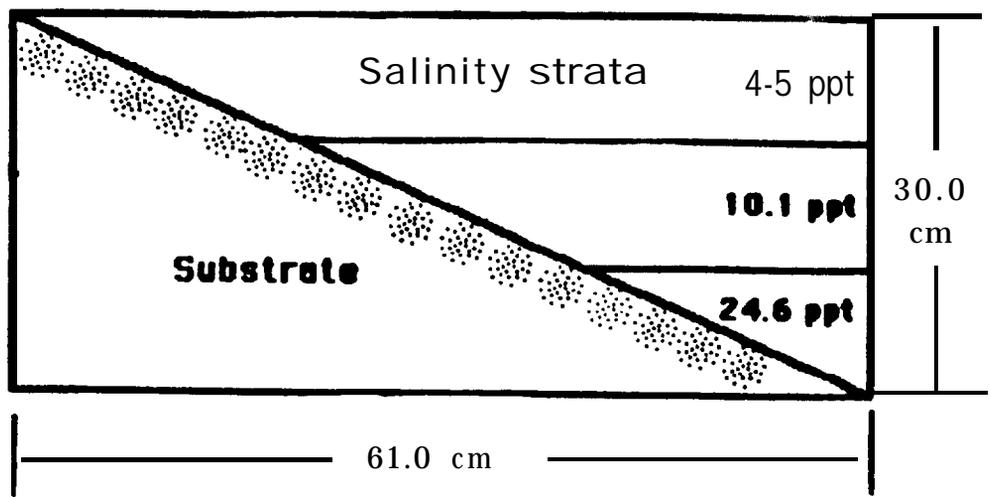


Figure 7. Salinity preference arena.

the head tank to the arena by siphon action, regulated by attachment of a Hoffman Clamp to the tubing to prevent mixing of salinity strata. Due to the different densities of the three solutions a vertical salinity gradient was achieved. The arenas were marked to show approximate boundaries of the three layers. Both arenas were allowed to stand until room temperature was reached. Twenty fish were randomly selected from the experimental stock, placed in a static, aerated, fresh water aquarium and allowed to stand 12 hours or until temperature acclimated. After this acclimation period ten fish were placed into each arena. Observations, two minutes after placement and every five minutes for thirty minutes, noted location of fish and behavioral responses. Tests were conducted with fish aged 33 (.16 g), 38 (.35 g), 45 (.55 g), 52 (.96 g), 60 (2.29 g), and 66 (2.15 g) days post hatch. Fish over 150 days old (10 g) were exposed to small increases in salinity in an attempt to also improve their tolerance by acclimation. Mixing of the different salinity strata occurred as fish swam through the three layers. Layer 3, the highest saline water, became more dilute while layer 1, fresh water, became more saline. Sections 1, 2, and 3, after thirty minutes, averaged salinities of 4.5, 10.1, and 24.6 ppt throughout all tests. As fish grew larger mixing of the three test salinities was more complete and occurred at a faster rate.

Observations

Results of the investigations on salinity tolerance to salinity during the early life history stages show that sturgeon larvae and fry would be unable to survive a transfer to salinities greater than 11 ppt (Fig. 8). The fish were considered tolerant if at least 50% survived twenty-four hours in the test salinity, but at 16 ppt and higher none of the test fish survived. Even at 11 ppt, those that did survive were sluggish and sometimes unable to exhibit a fright response.

Tolerance did not markedly change with age or size. Survival was relatively high for one day old fish (21.1 mg) in 11 ppt. Fish at this stage of life are typically up in the water column and susceptible to current before becoming substrate oriented to begin the hiding phase. Their tolerance declined slightly thereafter until fish were 45 days old (0.55 g), and then returned to the 11 ppt level through day 86 (3.5 g). Even at this moderate salinity, the surviving fish were sluggish in response, and showed lower levels of activity after only three hours exposure. They swam erratically and of ten become sedentary on the bottom of the test aquarium without responding to delicate probing. The 86 day old fish showed increased survival through twelve hours in 16 ppt, but were unable to survive the full twenty-four hour test period. Larger fish (10 g) acclimated by small increments of increased salinity over a period of four weeks, showed a tolerance to 15 ppt. After exposure to each elevated salinity, however, their color darkened and activity was reduced.

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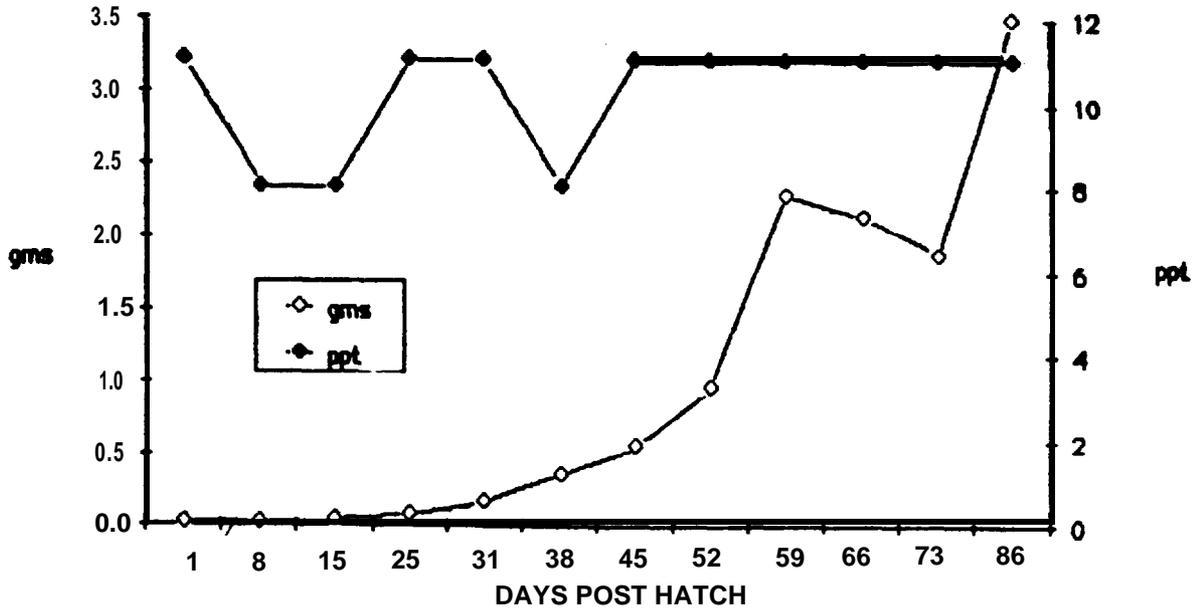


Figure 8. Salinity tolerance of young white sturgeon from 0 to 86 days post hatch based on at least 50% survival for 24 hours.

Observations of the response to salinity in the preference arena showed that when larvae and fry are given the opportunity to venture into saline areas they tend to congregate in the lowest salinity of the test tank (Table 4). Throughout all test periods fish in the fresh water control moved actively throughout the tank. Fish presented with salinities of 5, 10, and 25 ppt after thirty minutes of testing tended toward the low salinity section. Comparing the control response in the preference arena with freshwater to the response in the salinity gradient, showed a highly significant preference for the lowest salinity stratum ($P[X^2] < .001$). The fish would swim into the deeper section containing the more saline water, but with a burst of swimming they would return to the upper, low salinity section. Fish that entered the high salinity sections did not repeat the action regularly. Fish in the fresh water control were found throughout the water column constantly, and occasionally lingered in the lower level. Rarely were fish in the preference arena observed to settle on the substrate or within the lower, more saline section.

One potential problem with the analysis of salinity preference was the design of the apparatus used to determine preference. To allow equal substrate contact opportunity within each salinity and still permit salinity stratification, the floor of the apparatus was sloped which resulted in unequal volumes of water representing each stratum. Less volume in the higher salinities could bias the results in favor of fresh water. To correct for that possibility, the counts of fish in the fresh water strata (0 - 4.5 ppt) was compared with the sum of the medium and high salinities (10 - 30 ppt). The analysis was between counts from nearly equal volumes of water representing similar space through which to swim. The result was the same, however, with the distribution in the upper stratum significantly greater than the lower combined strata, $P(X^2) < .005$.

Results indicated that the null hypothesis was only partly disqualified. Sturgeon larvae and fry were intolerant of salinities at or above 16 ppt, but tolerated salinities of 11 ppt with some mortality. Larvae and fry showed no preference for the salinities they were exposed to.

Task 6: Response of sturgeon fry to low and high dissolved oxygen and their ability to discriminate between major differences in concentration.

Statement of Problem

Oxygen supply is an environmental condition integral to the survival and quality of white sturgeon larvae and fry. Inadequate supplies for developing fish could result in slowed growth and decreased survival (Hamer and Garside, 1976). Fish tend to inhabit areas where oxygen concentration is adequate (Bishai, 1962; Shelford and Allee, 1913). These fish, particularly

Table 4. Salinity preference of white sturgeon, 33 days old, when exposed to low (0-4.5 ppt), medium (10-15 ppt) and high (24-30 ppt) salinities.

PREFERENCE ARENA(freshwater)

	MINUTES INTO THE TEST					
	5	<u>10</u>	<u>15</u>	<u>20</u>	<u>25</u>	<u>30</u>
Low	2	5	7	3	4	7
Medium	6	4	2	4	3	2
High	2	1	1	3	3	1

PREFERENCE ARENA(salinity gradient)

	MINUTES INTO THE TEST					
	5	<u>10</u>	<u>15</u>	<u>20</u>	<u>25</u>	<u>30</u>
Low	7	8	7	8	7	8
Medium	2	1	2	1	1	1
High	1	1	1	1	2	1

salmonids, have the ability to detect and avoid water containing abnormal concentrations of dissolved gas. Although it is unlikely that large areas of the Columbia would exhibit significant oxygen deficits, larvae and fry may encounter low and high levels of dissolved oxygen in effluent water or from hydro operations. The task was designed to study the behavioral response of white sturgeon larvae and fry when exposed to either high or low concentrations of oxygen, and their ability to detect such concentrations.

Null Hypothesis : Sturgeon larvae and fry will not behave differently when in the presence of low or high dissolved oxygen levels, nor will they be able to detect them.

Procedures

Three of the doughnut shaped arenas described in Task 1 were utilized. Substrate, fish placement, food, water supply, and lighting were the same as described in Task 1 in all arenas. Current regimes for the three doughnuts were set at 2-3 cm/sec and doughnut 2 was used as a control for comparison with the other two arenas. When fish were 5 days post hatch the normoxic water supply was allowed to increase from 10.0 ppm oxygen to 10.6 ppm oxygen. In the other arena dissolved oxygen was allowed to drop to 5.7 ppm. High oxygen levels were achieved by running water into the top of a fifteen foot, three inch diameter PVC pipe while compressed air was bled in at the bottom of the column. Air passed up through the water filled column and escaped from the top. Supersaturated water was drawn off at the bottom of the column and supplied to the doughnut arena. Low oxygen levels were created by running the water supply in to the top of a four foot, two inch diameter PVC pipe while nitrogen gas was bled in at the bottom of the column. Deoxygenated water was drawn off at the bottom and supplied to the other doughnut arena.

Modified water was supplied to the arenas in continuous flow over a two hour period. Observations of behavioral response were conducted initially when the test water was supplied and every thirty minutes thereafter. The two experimental arenas were compared with the control supplied with normoxic water. Temperature and dissolved oxygen were monitored at each observation period. Ten fish were randomly selected from the experimental stock described in Task 1, weighed and measured to represent the size of fish used in the doughnut arenas. Larvae weighed 30.5 mg with a length of 16.6 mm.

Investigations conducted on the ability to detect differences in oxygen concentration utilized a variation on a Y-maze. Tests were conducted in a rectangular plywood box 122 x 20 cm. Ramps were positioned at a forty-five degree angle, sloping up from the center towards the ends at a height of 7 cm and terminated 5 cm from the ends, leaving a catch basin to collect the fish. A drain was fixed at the center, dividing the box into two halves. Water was

supplied by hoses into each end section of the box. One end received normoxic water while the other end received the modified water. Both water supplies met and drained from the middle compartment providing a continuous flow to the drain. Water depth was maintained at 10 cm. Illumination was achieved by placement a 60 watt bulb four feet above the center of the box.

Ten fish were randomly selected and placed in the center section of the test box while normoxic water was supplied to both ends of the apparatus. Fish were allowed to adapt to the surroundings for two minutes before test water was turned on at one end. Fish were observed and the number appearing in each section was noted every two minutes for the duration of the ten minute test. Replicate tests were conducted at each test period for the water supply combinations of low/normal and high/normal. Test periods in the test box coincided with fish aged 39 (0.35 g), 53 (0.96 g), 62 (2.29 g), 67 (2.15 g) and 76 (1.88 g) days post hatch. Tests conducted at day 39, 53, and 67 post hatch compared only low oxygen water with normoxic water.

Observations

The alteration of oxygen content of water in the doughnut arenas at the 5 day post-hatch stage showed that as the modified water supplies were introduced the larvae became more active (Fig. 9). Those in the control arena were in the hiding phase and did not move out onto the substrate for the duration of the test. As the test period progressed, fish in both the low and high oxygen supplied arenas increasingly moved out of the hiding spaces onto the open substrate surface. No differences in behavior was observed between the fish that were confronted with either the low and high oxygen conditions. Both low and high oxygen levels induced movement from the substrate, increased activity, and resulted in swimming patterns characteristic of fish in the same velocity in Task 1. Observations of larvae and fry in the test box with a choice between water sources with low and normal oxygen levels appear to indicate that the juvenile fish are able to recognize modified water. Tables 5 and 6 present results of tests conducted with fish 39, 62 and 76 days old. After six minutes of exposure to low oxygen test conditions, fish of the three ages tested avoided the section containing the low oxygen source. Fish ventured into the section of low oxygen early in the test. Within four minutes movement was confined to the center sections. As the test proceeded fish began to move out of the center regions and towards the section supplied with saturated water. Such results suggest that young white sturgeon could detect the lower oxygen and would avoid that area, moving instead toward water richer in oxygen. on two occasions a couple of fish remained in the section containing low oxygen water, exhibiting strong opercle movement, but were not successful finding their way out of the chamber and eventually become moribund. When unmodified water was restored after testing the fish revived. Results were analyzed

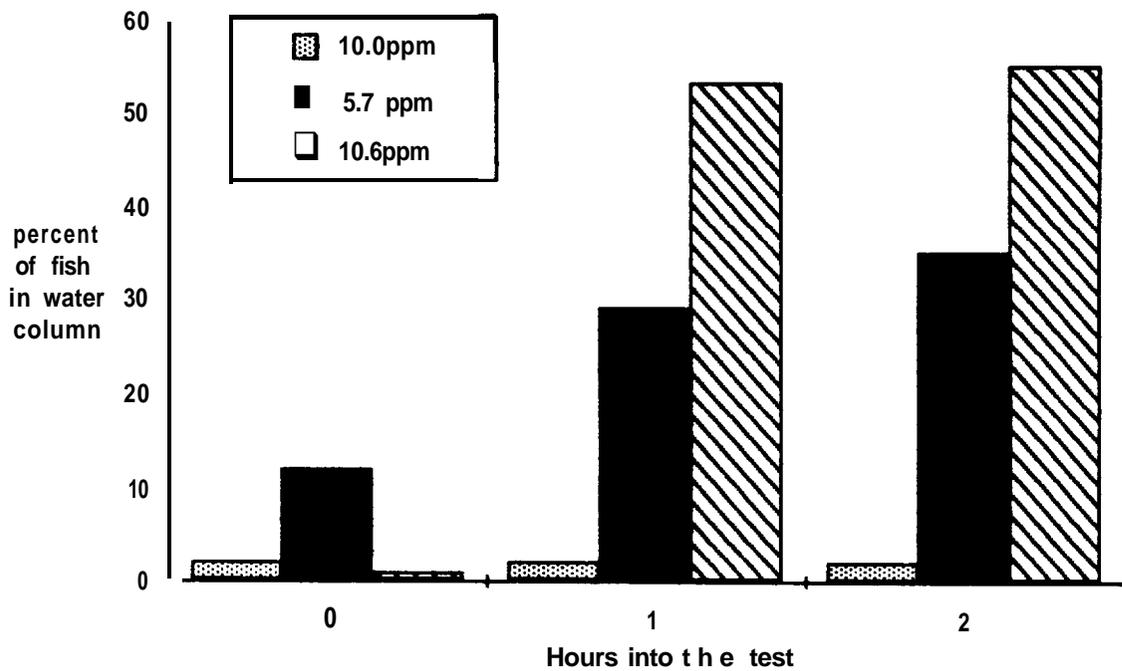


Figure 9 Distribution of 5 day post hatch larvae exposed to decreased (5.7 < 10.0 ppm) and increased (10.0 > 10.6 ppm) dissolved oxygen concentrations in the doughnut arena.

Table 5. Distribution of white sturgeon, age 39 and 62 days, during increasing and decreasing dissolved oxygen concentrations.

<u>DAY 39</u>							
NORMOXIC vs. HYPOXIC							
MINUTES INTO THE TEST							
SECTION	<u>0</u>	<u>5</u>	<u>10</u>	<u>15</u>	<u>20</u>	<u>25</u>	<u>D.O. (ppm)</u>
1	0	0	0	4	1	4	9.3
2	7	6	9	5	8	5	7.0
3	2	3	0	0	0	0	1.0

<u>DAY 62</u>							
NORMOXIC vs SUPERSATURATED							
SECTION	<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>10</u>	<u>12</u>	<u>D.O.(ppm)</u>
1	2	2	2	2	1	0	8.0
2	3	4	2	3	3	5	8.2
3	2	1	4	2	3	3	9.1
4	1	1	0	1	1	0	9.5

NORMOXIC vs HYPOXIC							
SECTION	<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>10</u>	<u>12</u>	<u>D.O.(ppm)</u>
1	3	2	0	0	0	0	1.0
2	2	2	2	1	1	2	2.6
3	3	4	4	7	5	5	5.1
4	0	0	2	0	2	1	7.8

Table 6. Distribution of white sturgeon, 76 days old, during increasing and decreasing dissolved oxygen concentrations.

NORMOXIC vs NORMOXIC								
MINUTES INTO THE TEST								
SECTION	<u>0</u>	2	-	6	-	-	-	<u>D.O.</u> (ppd)
1	1	0	0	0	0	0	1	8.7
2	3	2	4	3	2	6	5	8.7
3	3	6	3	3	3	2	2	8.7
4	1	0	1	2	3	0	0	8.7

NORMOXIC vs HYPOXIC								
SECTION	<u>0</u>	2	4	6	8	-	<u>12</u>	<u>D.O.</u> (ppm)
1	1	1	0	0	2	2	3	8.5
2	3	3	4	4	3	5	4	6.8
3	3	4	4	4	3	1	1	4.4
4	1	0	0	0	0	0	0	1.5

NORMOXIC vs SUPERSATURATED								
SECTION	<u>0</u>	2	4	6	<u>8</u>	<u>10</u>	<u>12</u>	<u>D.O.</u> (ppm)
1	2	1	1	1	1	1	1	8.6
2	3	2	4	2	2	3	4	8.7
3	2	4	3	4	5	3	2	9.3
4	1	1	0	1	0	1	1	9.7

based on the sum of fish in sections 1 and 2 compared with the sum in sections 3 and 4 at the 6, 8, 10, and 12 minute intervals. There were significant preferences for the saturated water compared to hypoxic levels in all ages tested (39 day $P(X^2) < .001$, 62 and 76 day $P(X^2) < .025$).

Tests conducted on preference between normal water with high oxygen water showed evidence that sturgeon could detect the presence of supersaturated water, but did not show a preference for it over saturated water. When presented with supersaturated and saturated water the fish moved into the modified water source without hesitation and appeared unaffected. However, when the responses of 5 day old larvae in the doughnut arena are taken into consideration which revealed the ability to identify increases in oxygen level, it appears that juveniles have the ability to detect supersaturated water, but choose not to avoid it.

Results indicated that the null hypothesis was partly disqualified. Sturgeon fry show responses to water with lower and higher dissolved oxygen levels by changes in activity patterns. Lower oxygen levels are avoided, but supersaturated oxygen levels evoke neither attraction or avoidance.

Objective 3: To characterize the feeding behavior of Columbia River white sturgeon larvae and fry.

Feeding behavior of larvae and fry has not been examined in any detail in Columbia River white sturgeon, and yet such basic need may be a key to the success of this species in the Columbia. When a species has evolved such a unique life history as sturgeon, with the adaptive behavior patterns associated with large river systems, changes in the environment through hydro development and other uses of water will alter the selective forces that created and sustain this species. If the changes are severe enough, the species may disappear from the system, or those parts of the system most affected. If the adaptive characteristics of the species can accommodate the changes, no deleterious effects may occur. In the case of highly fecund substrate spawners, eggs are very small and the resulting larva go through morphological changes as well as behavioral changes that make the species highly vulnerable to mortality factors. In an altered environment vulnerability to mortality factors could be greatly accentuated. White sturgeon are an example of such a species, and the Columbia River is an example of an altered river system. Since mortality of larva and fry is naturally very high in sturgeon, any information on life history needs that may show how mortality could be reduced would be most worthwhile. Feeding behavior was selected as one potentially limiting life history phenomenon that needed investigation under the present conditions of the river. The study was designed to determine when feeding was initiated, behavioral responses to the presence of food, and mechanisms used to obtain food.

Task 7: Feeding initiation, feeding responses, and feeding mechanisms in juvenile white sturgeon.

Statement of Problem

During early larval stages the white sturgeon is completely dependent on yolk supplies and can remain under cover in the substrate. When yolk reserves are depleted, the young fish must begin exogenous feeding. Initiation of feeding behavior may be required prior to emergence for successful entry into the foraging phase of their life history. Feeding behavior and the mechanisms used in foraging can affect the success of the young fish in the altered river environment, and may benefit from measures designed to provide access to natural feed, or allow the exercise of natural feeding patterns.

Null Hypothesis: Initiation of feeding will not be demonstrated by changes in larval behavior, nor will unique feeding responses or feeding mechanisms be demonstrated by young fish.

Procedures

Initiation of feeding was determined by observing larvae that developed in the doughnut arenas, flow-through plexiglass arenas and static aquaria. The doughnut arenas used were described in Task 1. The arenas had a temperature range of 16.0-16.7°C during the period of observation on initiation of feeding . The rectangular plexiglass tank used in the study was divided into four separate 25x30 cm chambers, each with identical sandy substrate and several flat stones on the surface. Each chamber was supplied with a constant flow of dechlorinated city water with a temperature range of 15.3-15.8°C. The aquaria used in the study were of 15 gallon capacity, with undergravel filtration and small gravel substrate. The temperature range in the aquaria during the study was 20.1-21.3°C.

Eighty eggs hatched in each of the doughnuts, 50 two day post-hatch larvae were put in each plexiglass chamber and 30 two day larvae were put in the aquarium. Tubifex worms and daphnia were added occasionally to the doughnuts and aquaria, before the sturgeon had begun to emerge from hiding. In two of the four plexiglass chambers, tubifex worms and occasional daphnia were fed each day throughout the experiment. One of the remaining two chambers didn't receive food until the larvae were beginning to emerge and the other chamber received no food during the experiment. This feeding arrangement was designed to allow behavioral and morphological comparison between fish that had food available and those that did not. In all of the experimental arenas observations were made before and after each feeding to

determine if any fish were responding to or eating the food. Daily observations of distribution and activity patterns were also performed to monitor the overall behavioral development of the larvae.

Behavioral observations of sturgeon fry of several ages were carried out to gather information about their feeding mechanism. Details of sturgeon foraging behavior were assembled by observing sturgeon fry before and after the addition of live food to the doughnuts and a variety of aquaria settings. Feeding events with tubifex worms, daphnia, mysid shrimp and carp larvae were observed with sturgeon fry (30-80 mm). Larger sturgeon (80-200 mm) were observed feeding on tubifex worms, mysid shrimp, salmon eggs, salmon alevins and salmonid fry. Video recording of various feeding events was performed and analyzed. The video camera was used to record group foraging patterns and for close-up observation of the dynamics of prey capture. Individual feeding events with tubifex worms and salmon eggs were filmed from the side and below the fish. As an aid to understanding the behavioral phenomena associated with feeding, basic morphological observations of the external features of the head and jaw were made using a dissecting scope.

Observations

Developing larvae were observed to emerge from hiding and initiate feeding in all three of the experimental arenas. No larvae were observed to be feeding or growing when exposed to live food before emergence. This behavioral change from a strictly hiding mode to a strictly mobile or foraging mode appears to be developmentally controlled and temperature dependent, since the sturgeon in warmer water emerged much sooner than identical fish in colder water.

Emergence of the larvae appears to coincide with yolk absorption and occurs after the head region has developed all of the basic sensory and jaw structures of older sturgeon (Fig. 10). In the doughnuts at 16°C sturgeon began to emerge from hiding at about 10 days post-hatch and by 13 days all fish were out in the foraging mode and most were feeding. The fish in the plexiglass tank at temperatures of 15-18°C, had clearly begun feeding by 14 days post-hatch. In aquaria, fish in temperatures of 18-20°C started to emerge at 7 days post-hatch and by 10 days all were out and were feeding.

After emergence juvenile sturgeon behavior seems to be dominated by random searching or foraging movement. If food is not present, sturgeon appear to enter the water column and move to another area to check the bottom for food. When benthic food or food odor is present, the sturgeon increase their foraging activity and spend most of their time frantically searching the bottom. After encountering suitable prey items sturgeon fry are able to respond to the presence of free-swimming prey by modifying their foraging patterns to facilitate their chances of prey capture.

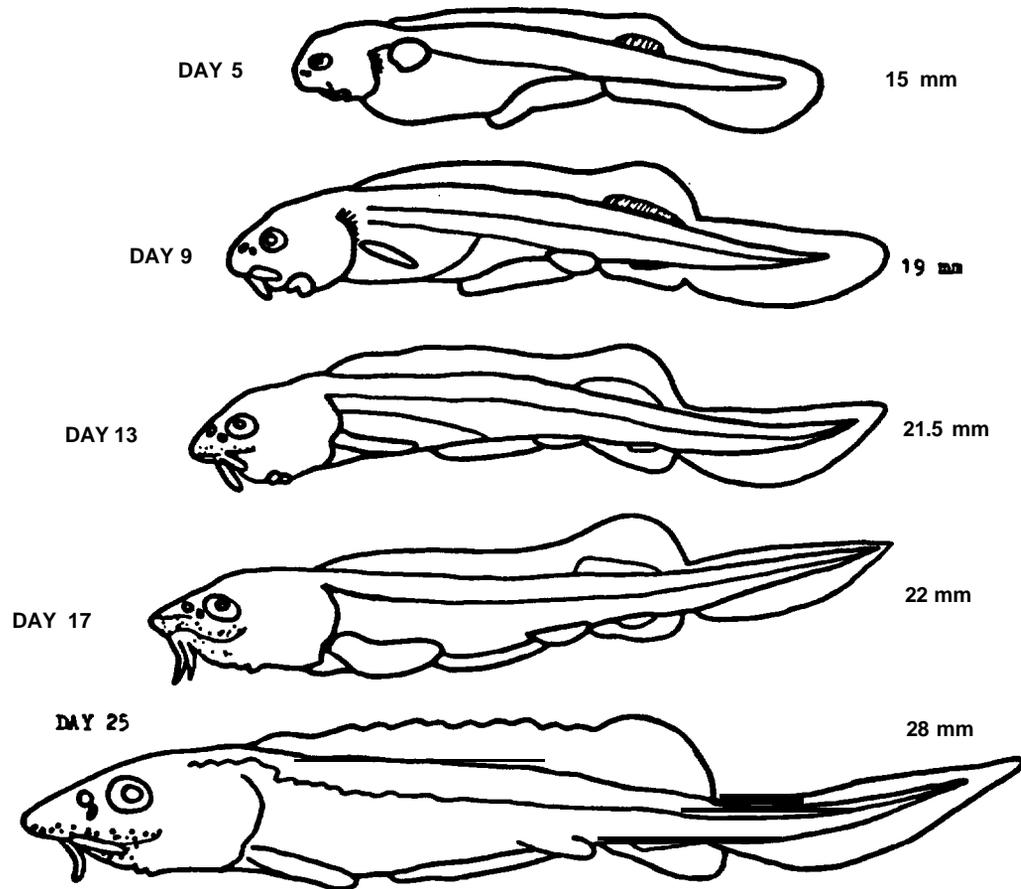


Figure 10. Side views of various stages of white sturgeon larvae and fry fixed in 10% formalin. All fish developed in water at 16-17°C and are drawn to the same scale. Day 5 and day 9 larvae have unabsorbed yolk material and the day 13 fish has reached yolk absorption. The day 17 fish has been eating the artificial diet and by day 25 considerable growth has occurred.

In aquaria, early stage fry were observed to be able to capture carp larvae, Cyprinus carpio, daphnia, *Daphnia* spp., and benthic tubifex worms. While able to occasionally encounter and capture mobile individuals the sturgeon were most successful at capturing carp larvae that were attached to plants or sides of the aquaria by skimming these surfaces with their barbels. Sturgeon are also able to capture mobile daphnia, but unless present in high densities the chances of random encounter are low. When dead daphnia collected at the surface of one tank, some sturgeon soon began to skim the surface with their barbels and consume the daphnia while swimming upside-down. Juvenile sturgeon were observed to be able to capture mysids, Neomysis mercedis, but their success seemed very dependant on the ability of the mysids to rapidly avoid being captured by an approaching sturgeon. When large enough (250 mm), sturgeon are able to capture mobile salmonid fry by employing a free-swimming foraging strategy. All ages of juvenile sturgeon appear to exhibit similar random searching patterns that are very adaptable once a particular food type has been identified.

White sturgeon have six separate sets of sensory receptors that could provide information used in feeding. Sturgeon have dorso-laterally oriented eyes and two anteriorly oriented olfactory rosettes (Fig. 11). On the underside of head or snout are four very sensitive barbels, two lateral canals, and numerous pores that probably contain electroreceptors (Jorgenson 1972; Teeter et al. 1980). Several lobes of the lips appear to have numerous taste buds that are positioned for contact with food items when the jaw is in the resting position or protruded.

Without more complex experimentation it is impossible to determine exactly which sensory systems, are used in feeding, but certain basic patterns of sensory utilization were very apparent from the numerous observations of feeding events. In all of the various feeding situations that were observed there was no indication that vision in prey location and capture. Under circumstances where food items did not emit much odor and were not contacted, the sturgeon would not show any response to their presence regardless of their proximity. However, sturgeon were very responsive to olfactory stimulus and were observed to rely on olfactory cues to locate odorous food. Addition of small quantities of odor of chopped tubifex worms to tanks normally fed tubifex worms always stimulated feeding activity such as increased foraging intensity and substrate orientation.

General observation and slow-motion video analysis of feeding events indicate that the barbels contain the primary receptors that trigger prey capture by the jaw apparatus. However, the jaw apparatus will sometimes respond to a strong food odor in the absence of an actual food item. If the barbels are removed the jaw appears to respond to prey items contacted by the

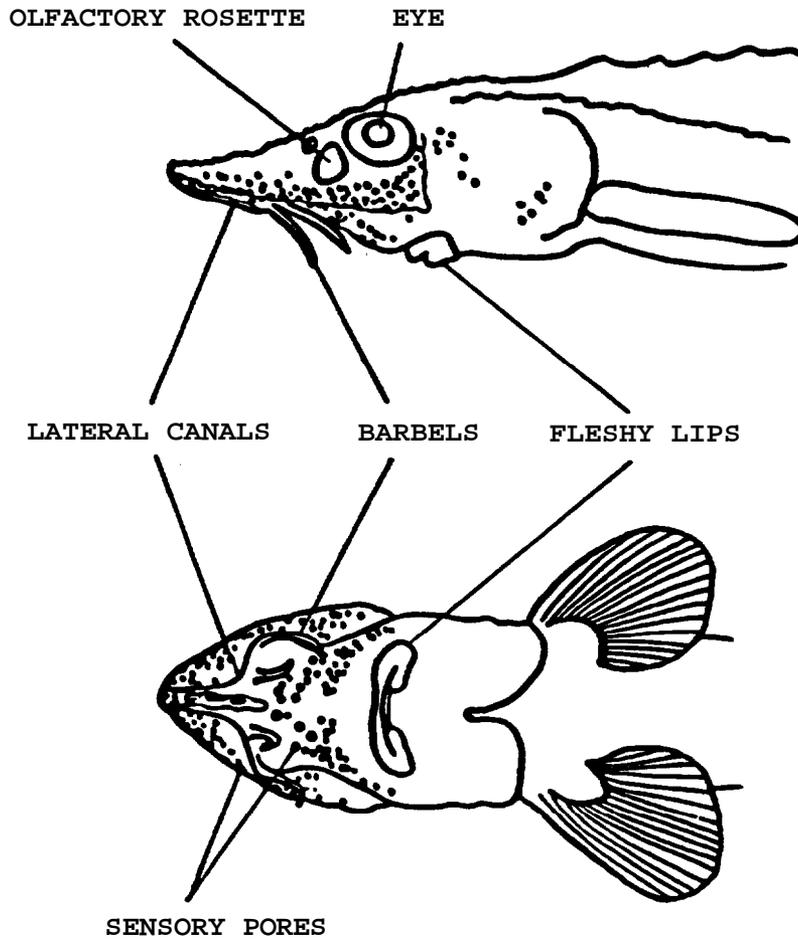


Figure 11. A side and ventral view of the anterior position of a 55 mm white sturgeon fixed in 10% formalin. The diagrams show the precise location of the six different sets of sensory receptors that are present in juvenile sturgeon.

lips.

Sturgeon capture their prey using a rapidly protrusible ventral jaw mechanism that is designed for immediate reaction to certain stimuli . Video analysis indicates that the jaw can extend down a considerable distance to capture prey items (Fig. 12). This protrusion appears to create a suction force by expansion of the buccal cavity that rapidly pulls prey items into the mouth. After the prey item is inside the mouth the retraction of the jaw appears to jam the food through the esophagus. When attempting to swallow larger items sturgeon exhibit a complex array of rapid jaw protrusions and internal processing functions, the success of which determines whether or not the prey can be successfully ingested. The jaw apparatus is relatively feeble and is essentially unable to bite off parts of a food item so the ability of sturgeon to utilize various prey items when captured is determined by their ability to swallow it.

Results indicate that the null hypothesis is disqualified. Sturgeon larvae-fry transition is marked by the initiation of feeding, a change from hiding to foraging behavior, and a demonstration of olfactory and taste sensory mechanisms that are the primary mode of food recognition. Feeding behavior is adapted to food sources, but the mechanics of feeding limit the behavior to prey types and conditions that are accommodated by the sturgeon's physical feeding structure.

Objective 4 : To make a field examination of the distribution behavior of larvae and fry, and to assess the influence of isolation of Columbia River white sturgeon populations from hydroelectric development.

Distribution of sturgeon in the Columbia River is generally known, but little evidence is available on what habitats they use, what their main source of food is in the different reservoir or river areas, and what influence has isolation from hydro development had on the status of the different populations. The present study was an attempt to gather field information on these subjects that will assist in management and enhancement of Columbia River white sturgeon in the future.

Tasks 8 & 9: Larvae and fry distribution and food source.

Statement of Problem

Laboratory studies have been conducted on distribution behavior and feeding of juvenile sturgeon. To make the laboratory studies applicable to the Columbia River, field observations were necessary to confirm laboratory conclusions.

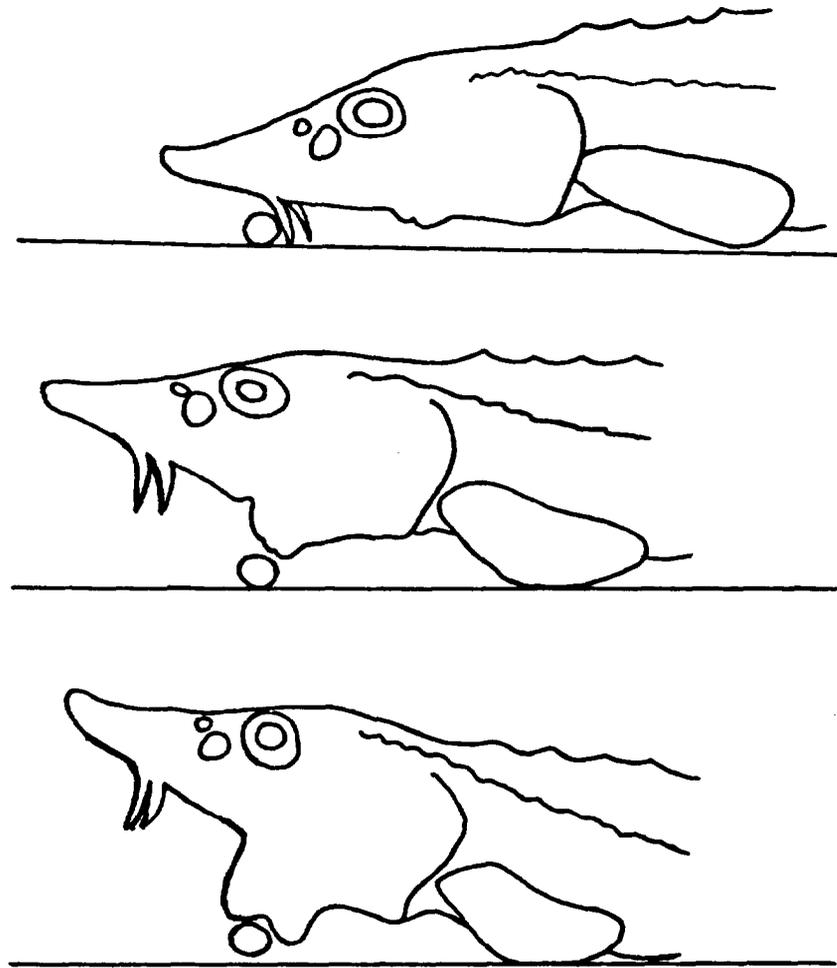


Figure 12. A diagrammatic representation of a juvenile sturgeon eating a salmon egg, illustrating detection of the egg by the barbels, the upward thrust of the snout and the downward protrusion of the jaw.

Null Hypothesis: Distribution and diet content of young sturgeon is uniform.

Procedures

A limited field sampling program on the Columbia River at sites from the Bonneville Dam to the estuary was performed once a month during the period of April to September 1985. The eight general areas and the dates on which they were sampled are as follows:

- 1) Downriver from Beacon Rock near Skamania, WA. (May 25)
- 2) Upriver from the I-205 bridge near Vancouver, WA. (May 24)
- 3) Caterpillar Island, a few miles downriver from Vancouver, WA. (April 27, July 23, August 21)
- 4) Bachelor Island, near the mouth of the Lewis River. (July 23)
- 5) Several sites from Kalama to Longview, WA. (April 27, August 21)
- 6) Areas around Puget Island near Cathlamet, WA. (June 24, July 23)
- 7) Areas between Skamokawa, WA and Three Tree Point. (June 22)
- 8) Baker Bay near Ilwaco, WA. (June 23)

Sampling techniques in Methods and Materials were employed in shallower water at the beginning of the season, and included more effort at depths greater than 3 m as the season progressed.

Observations

A wide variety of fish species were captured in the beach seine, otter trawl, and epibenthic sled. Salmonids, cyprinids, sticklebacks, sculpins, sandrollers, and starry flounders were captured in the beach seine at the freshwater sites. In Baker Bay, the fish community was dominated by more marine species. The beach seine captured numerous individuals of several species, and the sled caught a few fish up to 10 cm in length. In freshwater the sled occasionally captured larval cyprinids, small sticklebacks and sculpins. The crustacean, Neomysis mercedis, was clearly the dominant invertebrate in terms of biomass in the sled and trawl samples. The areas that were trawled yielded sculpins, cyprinids, and sturgeon; Two sturgeon of approximately equal size (40-50 cm) were captured at 9-10 m depths, close to the main channel. One was captured in July near Austin Point downriver from the mouth of the Lewis River and the other was caught a few miles downriver from Kalama, Washington. Despite extensive use of fyke nets in the spring and summer months, no sturgeon and only one salmonid was captured. The limited use of the crayfish traps yielded a few sculpins and crayfish.

Our field sampling efforts were by necessity focused on the more shallow habitats, and were designed to determine if large numbers of sturgeon larvae remain in the water column and are displaced downstream. The lack of

sturgeon in the beach seine, fyke nets, or sled, is interpreted as an indication that relatively few larval sturgeon are displaced by the current into the shallower habitats that were sampled. Large mysid populations were found in many of the shallow riverine areas, but there was no indication that young sturgeon use these areas during the day. However, there were shallow areas that have too much large debris on the bottom to permit sampling with active gear, and juveniles may have sought cover during their hiding phase in these areas without detection.

The National Marine Fisheries Service has captured juvenile sturgeon at depths around 11 meters using a larger bottom trawl and they have made stomach analysis on these fish (Bob McConnell, personal communication). The general indication from their sampling is that there seems to be a tendency to find more juvenile sturgeon in areas of debris accumulation, especially around 10 or 11 meters. It was also found that the gammarid amphipod, Corophium, is the primary food item of young of the year sturgeon, and becomes less important in the diet of older fish.

Sampling efforts to obtain tissue samples provided information on some of the distribution and feeding patterns of larger sturgeon. When working with Mr. Percy Brigham of the Umatilla Indian Tribe it was apparent that his experience on the river has resulted in considerable knowledge on the the behavior of sturgeon. He finds that fish of the same size often group together in the same pool. Some pools consistently yielded larger fish, while sub-adult fish could be found with high regularity in other pools. In general sturgeon are found in areas where the bottom was at a depth of 11 meters. Many of the fish caught by Mr. Brigham have stomachs stuffed full of thumbnail size china clams, Corbicula manilensis. It is interesting that a fish with its stomach filled with clams will still take bait. In Lake Roosevelt, clams were not found in sturgeon stomachs, but crayfish claws and river vegetation generally filled the gut. Below John Day Dam fishermen mentioned having found sturgeon stomachs full of salmon smolts that were apparently killed during by-pass difficulties at the dam. The evidence points to the fact that sturgeon are opportunistic feeders and take advantage of whatever is available.

Sampling sturgeon in the Snake River below Hell's Canyon Dam also indicated that adult fish appeared to reside in home pools. While movement from that area may occur to some extent, residence in a certain location was common enough to identify a particular fish with a certain site. Certain areas were frequented by young sub-adults and their residence in those areas was consistent enough that catching that size of fish could be anticipated with a degree of certainty. Of the twelve fish sampled below Hell's Canyon Dam, four had been intercepted and marked previously. Growth rate of the sturgeon in this section of the river, based on the marked fish, appeared very slow (Lukens, personal communication).

Task 10: Genetic assessment of Columbia River white sturgeon.

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 hydro 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 (Hayes 1978), and also interrupt the normal maturation cycle and 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 pre-dam years. The present field sampling program is a survey of the genetic similarity of fish distributed over the Columbia River system.

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

Procedures

Four areas of the Columbia River were selected for sampling white sturgeon in 1985 to begin an investigation of the genetic population structure within the drainage. Sturgeon are found from the estuary at the mouth of the river, up the Snake River, and well into Canada in the Kootenai drainage. The pools behind Chief Joe and Grand Coulee dam are inaccessible for upstream migration because fish passage facilities are lacking. For this reason, Lake Roosevelt was chosen as one sampling area.

Lake Roosevelt would only have sturgeon that existed or have descended from fish residing above Grand Coulee at the time of dam completion. The Snake river drainage in Idaho presents a similar scenario. Most likely sturgeon which are between dams below Hell's Canyon, represent fish that have been there for many years even though fish passage facilities are present downstream. Individual adult sturgeon have been found in the same area year after year during tagging studies in the Snake (Coon et al., 1977). The stretch of the river below McNary dam down to Bonneville dam was, the third region sampled and is referred to as the mid-Columbia in this report. This

area has three large lakes and supports a substantial commercial and sport fishery. Below Bonneville dam out to the estuary was the fourth area chosen. This region alone provides a harvest of over 50,000 fish annually (King, 1983). Sampling was undertaken at these four areas of the river from April through September. The area below Bonneville dam was divided in two with the area immediately below the dam analyzed separate from the rest of the lower river.

Observations

Gels were run initially using ten individuals and tissue from muscle, liver, eye, and heart as a preliminary survey to determine where activity occurred for the various enzymes. Different buffers were employed to obtain the best resolution of the enzymes tested. Once the analysis of enzyme systems began, photos were taken of the gels for later reference. Enzyme recipes were tried at least once using buffer systems 1, 2 and 3 shown in Table 1. When no activity was found the other buffers were tried.

The estimate of sturgeon population genetic structure in the Columbia depends on the ability 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 (1985) found 7 polymorphic loci in white sturgeon from four different river system in the Pacific Northwest, but were limited by sample size to distinguish major differences. In the overall electrophoretic analysis of the present study, a total of nineteen loci showed banding patterns which could be scored (Table 7). Twelve of the loci scored from the four areas were polymorphic.

Aspartate aminotransferase (AAT) showed a cathodal locus which was storable in both sturgeon muscle and heart. The variant out of this locus was slower migrating than the common allele.

Adenylate kinase (AK) had one anodal (AK-2) and one cathodal locus (AK-1). There was a fast variant out of each loci.

Aldolase (ALD) showed one locus in muscle which migrated anodally. There was a fast variant from this locus.

Esterase (EST-) was monomorphic in all areas but Roosevelt Lake where a single slow variant was found.

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

Table 7. Allele frequencies from variable loci at five areas in the Columbia River.

Locus	Alleles	Roos.	Lk.	Ilwaco	Mid-Col.	Snake	Below Bonn.
AAT-	100	.934		.831	.857	.916	.918
	59	.066		.168	.142	.083	.081
AK-1	-100	.978		.87	.91	**	**
	-175	.021		.13	.09	**	**
AK-2	100	1.0		.971	.987	1.0	.962
	287	0		.029	.012	0	.037
ALD	100	.87		.928	.892	.958	.925
	114	.13		.072	.108	.041	.075
EST(-)	100	.978	1.0	1.0	1.0	1.0	1.0
	76	.021	0	0	0	0	0
GPD	100	.956		.967	.933	.833	.859
	93	.043		.033	.067	.167	.141
GPI-1	100	1.0		.994	.982	1.0	1.0
	-120	0		.006	.018	0	0

Table 7. (continued) Allele frequencies from variable loci at five areas in the Columbia River.

Locus	Alleles	Roos.	Lk.	Ilwaco	Mid-Col.	Snake	Below Bonn.
GPI-2	100	.761		.869	.879	.791	.89
	74	.239		.131	.121	.209	.11
LDH	100	.783		.917	.826	.625	.95
	-150	.217		.083	.174	.375	.05
ME-1	-100	.95		.981	.938	**	.912
	-60	.05		.019	.062	**	.087
MDH	100	.869		.905	.958	.958	.913
	152	.131		.095	.042	.041	.075
	56						.012
PEP-3	100	.935		.886	.898	1.0	.95
	92	.065		.114	.10	0	.05

** Means the system was not scored for that area.

Glucosephosphate isomerase (GPI) was scored as being coded for by two loci. The first loci was on the origin and had a variant that migrated cathodally. The second loci was anodal and also had a slow variant. GPI was scored assuming a simple two-loci model although more loci may exist. This should be resolved by treating the samples with a thiol reagent and removing the shadow bands.

Lactic dehydrogenase (LDH) had one loci which was storable in sturgeon muscle. The common allele was on the origin and its variant migrated cathodally. A large percentage of heterozygotes were seen in the Snake River samples from this area. The structure of LDH and MDH in Russian sturgeon has been described by Slynko (1976).

Malic dehydrogenase (MDH) showed two loci anodally, with only MDH-1 being polymorphic. MDH-1 had a fast variant allele which fell on the heteropolymeric band between the two loci. There was also a super slow allele that showed itself once.

Malic enzyme (ME) had two loci, but only ME-1 was polymorphic. ME-1 migrated cathodally and had a slow variant.

Peptidase (PEP) showed three loci of which one was polymorphic. PEP was scored from the peptide leucyl alanine (LA) which showed a banding pattern identical to leucylglycylglycine (LGG) . A slow variant was scored out of the third locus.

Adenosine deaminase (ADA), Glyceraldehyde-3-phosphate dehydrogenase (GAP), A-Mannose (A-MAN), Malic enzyme (upper locus: ME-2), Malic dehydrogenase (MDH-2), Peptidases 1 + 2 (PEP), and Glucosidase (GLU) were all monomorphic.

In general, so called "shadow" bands were seen when staining for PGM, GPI and AH, and this confused the interpretation of the gels. Often samples which have not been frozen rapidly enough or for some reason are of poor quality exhibit such a pattern. Because of the procedure used to obtain samples, tissue may not have been taken immediately after the fish was caught. Fish were often kept tied on a rope in the water for several hours or days before a tissue sample was allowed to be taken from the fish. Some samples were collected by volunteers and frozen in a standard freezer for a couple weeks prior to being put in the super cold freezer. There was no difference in the amount of shadow banding between samples frozen immediately on dry ice and those stored in a freezer, there were differences in the strength of the banding.

Variation exists in AH and PGM, but the model for the number of loci was unclear. This will be clarified by the use of fresh samples . The average

heterozygosity by area was calculated as an index of the amount of variation that existed within each area sampled (Selander and Johnson, 1973). There was little variation in the individual heterozygosity between areas (Table 8). There was a number of enzymes for which no activity or poor activity was found (Table 9).

While sample sizes were small in both the Roosevelt Lake and Snake River areas, they were large enough to yield a heterozygosity estimate which would probably fall within 1% of the estimate that would have been obtained from a large sample if a larger number of loci had been sampled (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 stain. For instance, in LDH there is another locus in heart, and probably another in eye which could be scored if the samples were attainable (Bartley et al, 1985). Genetic distance estimates were made from gene frequencies using the method of Nei (1972) and showed the areas to be very similar (Table 10). Also, one way analysis of variance was used to analyze the raw data (obtained from scoring the gels) both between and within each area (Zar, 1974). No significant difference at $p < .05$ was found in either case.

The observed and expected gene frequency values were tested using the log-likelihood ratio which utilizes the G statistic and chi-square p values. Each enzyme system was tested between and within each of the five areas. The G statistic was significantly different in every case at $p < .001$. This means that the observed frequencies did not fit the model of expected values which would be obtained from a population in Hardy-Weinburg equilibrium. Because of the high number of heterozygotes and the lack of alternate alleles found in several of the systems scored, further refinement of the substrate will be undertaken to verify that the population character is in fact out of Hardy-Weinburg equilibrium.

Table 8. Number of polymorphic loci and average heterozygosity by area.

Areas	Number of Polymorphic Loci	Average Heterozygosity	# Fish Sampled
Ilwaco	11	.049	84
Mid-Columbia	11	.044	83
Roosevelt Lake	10	.047	24
Snake	6	.051	12
Below Bonneville	9	.038	40

Table 9. Enzyme resolution results from initial screening.

(Enzyme systems that produced no banding patterns during screening)

Abbreviation	Enzyme
ACP	Acid Phosphatase
ADH	Alcohol dehydrogenase
AGPDH	Alpha-glycerophosphate dehydrogenase
DIA	Diaphorase
ENO	Enolase
EST	Esterase +
FUM	Fumarate hydratase
GAM-1	Glyoxalase I
GAM-2	B-Galactosidase
GD	Glucose-6-phosphate dehydrogenase
GDA	Guanine deaminase
GL	Glycylleucine
GPT	Glutamate-pyruvate transaminase
GR	Glutathione reductase
GUS	B-glucuronidase
GUK	Guanylate kinase
HAGH	Glyoxalase II
LAP	Leucine aminopeptidase
NP	Nucleoside phosphorylase
NTP	Nucleoside triphosphate pyrophosphatase
PK	Pyruvate kinase
SDH	Sorbitol dehydrogenase
XDH	Xanthine dehydrogenase

Table 9.(continued) Enzyme resolution results from initial screening.

(The systems below have shown activity but poor resolution)

Abbreviation	Enzyme
AAT-2	Aspartate aminotransferase (upper locus)
AH	Aconitase hydratase
FDP	Fructose 1,6-diphosphatase
GPI	Glucosephosphate isomerase
GLD	Glutamate dehydrogenase
GR	Glutathione reductase
HK	Hexokinase
MPI	Mannose phosphate isomerase
PGD	Phosphogluconate dehydrogenase
PGK	Phosphoglycerate kinase
PGM	Phosphoglucomutase
TAT	Tyrosine amino transferase
TK	Thymidine kinase
TPI	Triosephosphate isomerase
XO	Xanthine oxidase

Table 10. Genetic distance estimates between white sturgeon of different areas on the Columbia River.

	Ilwaco	Mid-Columbia	Snake	Below Bonneville
Roosevelt Lake	.003	.002	.003	.003
Ilwaco		.001	.002	.001
Mid-Columbia			.004	.002
Snake				.007

DISCUSSION

Management of Columbia River white sturgeon, including whatever enhancement measures are necessary, depends on understanding the life history of the species, and what stages are most vulnerable to mortality. The present study has shown that environmental factors have a major influence on the distribution behavior of sturgeon larvae and fry. Egg, larvae, and fry mortalities are extremely high in a species that has such a large fecundity and deposits the eggs on the substrate surface without covering them. After hatching and rising into the water column, sturgeon larvae leave the displacement phase and enter the hiding mode which reduces their vulnerability to predation. Larvae have the ability to determine something about their rate of displacement, and enter the hiding phase earlier when in the presence of faster currents. Such adaptive behavior allow the larvae to distribute away from the spawning grounds and potential predators that feed on incubating sturgeon without being swept too far. Seeking cover during yolk absorption prevents prolonged exposure at their most vulnerable stage. Current is used as the major distribution mechanism, and the larvae appear equipped to alter their response to it. Although spawning penetration upstream is limited by Bonneville Dam, previous concerns that larvae would be displaced to the marine environment by river currents, are not completely justified based on the larvae's ability to escape from high velocity to start their hiding phase sooner.

Photonegative behavior characterizes the larval stage, which may help them make a transition from the distribution mode to the hiding mode. The importance of low illumination during these early stages is emphasized with the fact that hatching occurs primarily during darkness, which provides immediate cover to the new larva. More fry were obvious in the water column in the morning hours prior to going into hiding. Absence of fry in the shallow areas of the river suggests that photonegative behavior also characterizes the more advanced stages. Juvenile white sturgeon are rarely observed in nature until they reach two or three years of age. As discussed in some detail later, the feeding and predator avoidance strategy of sturgeon favors low illumination.

Substrate composition is important to larvae in the hiding mode. Material providing recesses to get out of sight, such as large rocks and plant material was found most frequently used. When the foraging phase, has begun, fry and fingerling sturgeon do not appear to prefer a substrate type, with the exception of perhaps sand. They settle on sand, but it may be that sand is the expected- habitat of many prey items. Other substrate was no less attractive to the fry if food could be found there, although settling on other substrate without food seldom occurred in the laboratory. Once foraging has started, from that time on the juvenile fish are moving

continuously, and with the exception of food the main environmental factor influencing their area of preference is light.

Sudden temperature fluctuations tend to alter responses to current. Rapidly increasing water temperatures induced larval activity and moved them out from their cover. Decreases in temperature at this time did not perceptively change activity patterns but fewer larvae were noted swimming in the water column after temperatures dropped. Once fish began to feed temperature fluctuations had less affect on their behavior, and no affect as they advanced well into their foraging phase. Therefore, it is concluded that in the Columbia River temperature flucuations would have the greatest impact on young white sturgeon during the first few days after hatching. Any influence that tends to bring larvae out from their hiding mode prematurely would increase their vulnerability to predation and downstream dispersion by the current.

Low oxygen also brought larvae out of their hiding phase. This response indicates that larvae are able to detect low oxygen levels and seek more oxygen rich areas. The response could also increase the vulnerability of these fish to predation and displacement by current. Researchers studying adult white sturgeon showed that under hypoxic conditions these fish would lower their activity, thus consuming less oxygen (Burggren and Randall, 1978). Adaptations such as these would allow white sturgeon to wait out a temporary oxygen debt in a river. Typically an oxygen deficit in a river like the Columbia would be short-lived as continuous large flows replenish water supplies. White sturgeon are either able to escape, or wait out the temporary low oxygen situations.

Supersaturated conditions are likely to be encountered in the Columbia and fish could avoid such situations through adjustment of their swimming depth. No evidence was provided, however, that would indicate sturgeon avoid supersaturated oxygen levels, and increased water depths were not available to the fish to retreat to if such a response would be typical under such situations. Our investigations merely suggest that larvae and fry do not exhibit an abnormal response when confronted with oxygen supersaturated water. Potentially, such a response could make larvae vulnerable to gas bubble disease if they are in shallow water during the hiding phase.

Throughout the early life stages investigated, any larvae and fry displaced by the current into the estuary or open ocean would experience very high mortality. Present studies showed 80% survival at 11 ppt for fish in the 20 mg size range after twenty-four hours, but zero survival for fish tested in 16 ppt. Even after only three hours exposure to 11 ppt, activity levels had decreased which would suggest that if larvae and fry were displaced in to areas greater than or equal to 11 ppt salinity they would

perish or be susceptible to predation and harsh environmental conditions (currents, tides). Based on preference behavior, in the early life stages white sturgeon avoid salinities over 10 ppt. Since all life stages are bottom oriented, if dispersed into the estuary the fish would avoid areas of salt water penetration. Even if the larvae and fry had the opportunity to acclimate to higher salinities, it is unlikely that they would develop salinity tolerance. McEnroe and Cech (1985) in their investigations of Sacramento River white sturgeon tested the juveniles' ability to tolerate saltwater after gradual increase of the salinity level. Acclimation of fish (4.9-9.5 g) to 15 ppt for one week did slightly increase survival at 25 ppt, but not 35 ppt. Survival, however, was below fifty percent in 25 ppt. Our studies on acclimation of 10 g fish over a four week period increased their ability to tolerate 25 ppt, but their sluggish behavior and low activity accompanying the acclimation period, would increase the vulnerability of the fish to predators.

Larvae and fry could move into a food rich area of an estuary for a brief period before returning to fresh water. At all sizes tested fish survived for at least one hour and sometimes through three hours at 16 ppt while appearing not to suffer ill-effects. Fish could move between saline and fresh water to take advantage of feeding in the nutrient rich estuarine areas before returning to fresh water as salinity becomes physiologically intolerable. Based on the results of the tests, however, it is unlikely that white sturgeon will utilize the ocean environments during the first few months of life.

Insight on the characteristics of juvenile feeding behavior were provided from the studies. The behavioral change associated with the onset of exogenous feeding is a very important transition point in the life of juvenile sturgeon. It represents a switch from a hiding behavioral mode to a foraging phase in which no attempt to hide is ever made again. If sufficient cover is available during the hiding phase, the developing sturgeon larvae can probably avoid most predators. However, once their yolk is absorbed and the morphological development of feeding structures is complete, the sturgeon must leave this safety and emerge from hiding to search for food. At this point the young fry must find quality feeding habitat that will allow them to grow rapidly to a larger size and become less susceptible to predation. The behavioral analysis of the timing of initiation of feeding in this study confirms the results of Buddington and Doroshov (1984), which showed that under hatchery conditions at 16-17°C, 50% of the sturgeon had initiated feeding on tubifex worms at 12 days post-hatch. Since yolk is stored in and absorbed through the stomach (Detlaf et al. 1981), it appears that waiting until yolk absorption before feeding will prevent any interference with yolk metabolism from the intake of food. However, the larger the larvae become before having to begin foraging provides greater selection for feeding opportunity.

The seemingly random foraging patterns used by young sturgeon are probably a result of their poor ability to use visual cues to locate and capture food. Juveniles of other species of sturgeon have been shown to be non-visual feeders (Sbikin 1973), and it is generally assumed that most sturgeon use other senses than vision when feeding (Buddington and Christofferson 1985). This means that the success sturgeon have with mobile prey could be dependant on the amount of light available for prey to detect their approach. A non-visual predatory strategy would be an advantage to sturgeon when feeding on large populations of visually oriented prey species in habitats that are often turbid (Miller 1978). A dependence on sensory systems other than vision would also be advantageous when foraging at night or in areas too deep for light penetration. A random searching pattern is characteristic of all ages of juvenile sturgeon that were observed in laboratory and hatchery settings. Some of the larger hatchery sturgeon rest on the bottom at times, but the early stage fry in a variety of habitat simulations were observed to be constantly moving. This constant motion allows the fish to continually monitor areas for taste or olfactory stimuli that will lead them to food.

Olfactory cues are clearly very important for sturgeon when feeding on odorous food types. Sturgeon have large olfactory rosettes with both ciliated and microvillus receptors (Hara 1972), and we have observed that the behavior of sturgeon is instantaneously affected by contact with food odors. Sturgeon will often stop after detecting an odor and begin circling the general area in an attempt to contact the food item. The intensity of their reaction to an odor appears to be related to the strength of the stimulus, which is presumably proportional to the distance from a food item. Buddington and Christofferson (1985) have described preliminary work on the responsiveness of white sturgeon to food odors. Other studies have shown that sturgeon fry are able to use weak food odors to make precisely directed movements in an attempt to find the source of the odor (Miller in preparation). In the river environment, odor could be very important in locating food items. When larger prey are captured by sturgeon, the odor that is released could serve to signal other fish that a particular area could be good feeding habitat.

While odor is very important in locating distant food, the act of capturing an individual prey item is usually mediated by the barbels. Barbel contact with a food item triggers a carefully timed protrusion of the jaw that usually engulfs the item. Unless contacted by the barbels, non-odorous foods remain undetected. However, it is possible that highly mobile and relatively odorless prey such as small fish can be detected at a short distance using motion detectors in the canal system. Benthic and mobile prey could also create weak electric fields that might be detected by the electroreceptors in the snout of the sturgeon.

The rapid jaw mechanism of sturgeon is very well adapted for engulfing small benthic prey. The downward protrusion of the jaw creates a powerful suction that is capable of pulling in prey that are too large to be ingested. The jaw mechanism reacts so fast that its basic function can only be observed in slow motion films. This rapid mechanism even enables sturgeon to capture mobile salmonid fry.

Our observations indicate that white sturgeon are opportunistic feeders that will consume any aquatic animal that they are able to capture and ingest. This is confirmed by the few food habit studies which indicate that white sturgeon eat a relatively wide variety of prey types in numbers often reflecting their temporal and numerical abundance (Radtke 1966; Semakula and Larkin 1968; McKechnie and Fenner 1971). Food habit studies of other species of sturgeon have suggested opportunistic feeding patterns in which the most common, easily captured food types are utilized most by sturgeon (Zakora 1978; Dadswell 1979; Dadswell et al. 1984). The opportunistic, non-visual predatory mechanism of white sturgeon is clearly best adapted for relatively immobile benthic prey or high density groups of more mobile prey. Individuals in low density populations of mobile prey would probably be encountered too infrequently to compensate for the foraging effort required to locate and capture them. The density of mobile water column prey such as zooplankton populations is probably the most important factor in determining whether or not sturgeon can feed on them.

Field sampling did not result in enough sturgeon for direct evidence to describe their residence locations. However, good indirect evidence was obtained about where juveniles preferred not to be. The Columbia River from Bonneville Dam to the mouth of its estuary is an enormous river with numerous islands and sloughs. Although the main channel of the Columbia often exceeds 20 meters in depth, much of the river is probably less than 13 meters. Our field sampling efforts were by necessity focused on the more shallow habitats, and were designed to determine if large numbers of sturgeon larvae remain in the water column and are displaced downstream. The lack of sturgeon in the beach seine, fyke nets, or sled, could be an indication that few larvae are displaced by the current into the shallower habitats sampled. Large mysid populations were found in many of the shallow riverine areas, but there was no indication that young sturgeon use these areas during the day. However, there are shallow and deep microhabitats that have too much large debris on the bottom to permit sampling with active gear.

Laboratory analysis of feeding strategy has indicated that areas with low levels of illumination should be favored foraging habitats for sturgeon, and that their feeding behavior would select immobile prey as preferred food. These conclusions have been supported from observations made in the field. Information from the National Marine Fisheries Service and Percy Brigham have

indicated that both juvenile and adult sturgeon are found at depths around 11 meters. Gammarid amphipods, Corophium is the primary food item of young of the year sturgeon, and china clams, Corbicula manilensis was found to be more important in the diet of older sturgeon in some areas. Changes in the Columbia River that alter light penetration or food sources would result in severe habitat perturbations that would limit the success of sturgeon in that area of the river.

Habitat changes may be implicated in the results of the genetic analysis also. Analysis was performed on fish throughout the Columbia, but there seems to be very weak evidence thus far of any genetic differences between the upper and lower reaches of the river. In 1985 emphasis was placed on screening for enzyme systems that show activity. Nineteen loci have been isolated and the conclusion that little genetic difference exists is based on data from those loci. Such a conclusion could change as more loci are scored from each individual which would provide a better estimate of population characteristics. When a locus shows different allelic patterns between areas its a good indicator that subjective selectivity may have occurred. This situation presented itself in only one system (EST-) which was scored this year, but another enzyme system (AH) will probably give further evidence of different allele patterns among the areas sampled. This would be an indication that differences exist, and as more data is examined there will be a greater opportunity to support or dispute the allele differences associated with any population segregation.

Perhaps of most concern is the fact that the observed gene frequencies were out of Hardy-Weinburg equilibrium. This situation could mean that the populations are undergoing selective mortality in response to the changes in the river. Alternatively, the reproductive success of various age groups could be influencing the allele frequencies. These migratory fish have been trapped by dam construction very recently in the evolutionary time scale of such a long lived species. With drastic changes having occurred in their environment, selective genetic changes would be expected to occur.

The implications of this study are severalfold. Behavioral characteristics and feeding strategies of sturgeon have evolved with the morphology of the species. The larva are distributed by water current and must find cover which provides protection during the incubation period. Fry also distribute by river currents as they continually forage for food. Feeding behavior is specialized for use in dark, bottom oriented habitats where contact identity of their prey is necessary and facilitated by the evolution of highly sensitive taste receptors around their mouth. The act of capturing an individual prey item is mediated primarily by the barbels, contact with which triggers a carefully timed protrusion of the jaw that engulfs the item. Immobile benthic organisms have become the major target prey, but the rapid deployment of the protruding jaw mechanism upon contact

with potential prey, allows predation on other mobile organisms when approached under cover of darkness. The river environment in which sturgeon historically migrated, spawned, and reared, however, has changed and those changes are expected to have precipitated genetic changes in the fish, as well as being reflected as reduced fitness in populations.

The observed gene frequencies being out of Hardy-Weinburg equilibrium may be an indication of selective changes occurring within Columbia River white sturgeon populations. 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. Similarly, hatchery fry may be of little value in the Snake below Hell's Canyon. If sturgeon growth rate is as slow as it appears, it would suggest that productivity of that habitat would not sustain large numbers of fish, and efforts to enhance the stock may best be placed in other measures. 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.

When hatchery fish introduction is judged appropriate in an area, the genetic question surfaces. If genetic differences are found among 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.

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