Bull. Environ. Contam. Toxicol. 30, 522–529 (1983)

Effects of Grays Harbor Estuary Sediment on the Osmoregulatory Ability of Coho Salmon Smolts (*Oncorhynchus kisutch*)

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A major problem faced in salmonid enhancement programs is the low adult return rates of artifically propagated fish (SAUNDERS and ALLEN 1976). This often occurs despite the generally higher survival rate of hatchery fish during the hatching and early developmental stages.

One suggested explanation of this problem addresses the ability of the hatchery released fish to undergo a parr/smolt transformation and function as true smolts upon arrival at the seawater/freshwater interface. A number of environmental factors affect the physiological and behavioral processes commonly referred to as smolting. Among these factors is the effect of trace heavy metal exposure during the development of smolts in freshwater. Usually the source of these contaminants is mineral deposit drainages or nonpoint source industrial pollution (LORZ and MCPHERSON 1976). Partial or complete inactivation for the gill-ATPase system occurs if fish are exposed to 20-30 microgram per liter (ug/l) of copper during the parr/smolt transformation (LORZ and MCPHERSON 1976). The biological damage is not apparent unless the fish are placed directly into seawater, where hypo-osmoregulatory failures and severe mortalities occur. Synergistic effects with other heavy metals have also been demonstrated (LORZ et al. 1978). However, if a 5-day freshwater recovery period is allowed before exposure to seawater, survival returns to normal.

In estuaries which have experienced large amounts of heavy metal pollution, the bottom sediments tend to bind the heavy metals from the water column and act as a sink for storage of the contaminants. Dredging activities may resuspend these toxicants in the water column and contact with this material by migrating juveniles may have a deleterious effect on smoltification and early marine survival (WEDEMEYER et al. 1980).

This study examines the effect of sediments from Grays Harbor, Washington, on the hypo-osmoregulatory ability of smolting coho salmon (Oncorhynchus kisutch) through the measurement of blood sodium levels following seawater challenge. The presence of high blood sodium levels is indicatory of osmoregulatory impairment, which has been shown to occur in salmonids exposed to heavy metals during smoltification. These fish were subjected to conditions which were designed to simulate, as closely as possible,

the effects on migrating juvenile coho salmon of the periodic high suspended sediment levels created by hopper dredges while dredging in the inner harbor. These bioassays, performed on four groups of coho smolts, attempted to identify the effect of resuspended contaminated sediments on the hypo-osmoregulatory ability of the smolts under a variety of conditions.

METHODOLOGY

Sediments were collected from five upper estuary sites in Grays Harbor in April 1982. Collection was performed using a teflon coated 1 meter^2 Van Veen sampler. A total of 25 gallons of material was collected and placed in five 5-gallon sealed plastic containers and stored at 0° C within 4 hours after collection.

The fish used in this study were obtained from the Washington State Department of Fisheries' Simpson Hatchery on the Satsop River in Grays Harbor County, Washington. The fish were acclimatized for 2 weeks and fed 1/8-inch Oregon Moist Pellets two times per day to satiation. The experiment did not commence until the true smolt status of the fish was verified. This was done by periodically subjecting a 10-fish subsample to a 24-hour seawater challenge test as described by CLARKE and BLACKBURN 1979. It was considered that no affect on smoltification had occurred when blood sodium levels under 170 millequivilents per liter (meq/l) were found in all fish which had undergone this test (WEDEMEYER 1982, personal comm.). This condition was met on 16 May, when 10 fish were given the 24-hour seawater challenge test and blood sodium levels were measured and compared to the blood sodium levels of 10 control fish.

On 18 May, the sediment exposure period was started. Two-hundred and forty fish of approximately equal length and weight were divided into groups of 20 and transferred to 12 experimental tanks, each holding approximately 80 liters of water. Waterflow rates into these tanks were initially set at 0.5 gallons per minute (gpm) or greater and were lowered to 0.25 gpm on the third day of contaminant exposure in an attempt to raise the turbidity levels in the tanks and prolong the usage of the available sediment.

Each one of the sealed 5-gallon buckets containing the frozen sediments was thawed as needed at room temperature for 36-48 hours prior to use. A measured amount of sediment was initially combined with 5 gallons of water in plastic buckets to form a concentrated sediment-water slurry. This mixture was poured through a 950 micron mesh size Nitex screen to reduce organic debris and then further diluted with water to a final sediment: water volumetric ratio of 1:166 in two 44-gallon plastic drums (slurry tanks) which were set up at a level above the experimental tanks. The plastic drums were connected by two 2-inch-diameter PVC pipes near their bases. This procedure was repeated as necessary (once every 4-6 hours) to keep the slurry

tanks full throughout the 9-day exposure period. On day three of the contaminant exposure period, this ratio was decreased by half to 1:83 to increase the turbidity levels in the experimental tanks. The sediment in these slurry tanks was kept suspended through the use of a 5-gpm stainless steel centrifugal pump which drew the slurry out of a hole in the bottom of each slurry tank and discharged it back into the tank at the top via 1-1/2-inch PVC piping.

Mounted above the slurry tanks, nine diaphragm-type chemical metering pumps each withdrew an average of 97 milliliters per minute (ml/min) of slurry from the tanks. Each pump injected the slurry to an experimental tank via vinyl tubing and introduced the mixture into the tank next to the water inflow nozzle. Because the slurry tanks were above the level of the experimental tanks, back-pressure valves were installed at the sediment injection point at each tank to prevent siphoning of the slurry into the experimental tanks. No injection tubing was present in the control tanks. The chemical metering pump flow rates into each tank were adjusted to create turbidity levels between 20-200 nephelometric turbidity units (NTU) in the tanks on each day of the experiment.

Turbidity levels, dissolved oxygen (DO) levels, temperature, and waterflow rates were checked daily. Photoperiod was adjusted weekly. The amount of light reaching the control tanks was reduced to the approximate level of the experimental tanks by placing brown paper over the clear plastic tank lids. Metering pump flow rates and waterflow rates were reset throughout the experiment to maintain the turbidity levels which occur during hopper dredging activities.

On 21 May 1982, the fourth day of the experiment, a random water sample was taken from one of the experimental tanks, which had a turbidity level of 180 NTU, for an elutriate analysis of heavy metals in the water of experimental tanks. This sample was filtered through a 0.45-micron membrane filter; then the filtrate was analyzed by atomic absorption. (Reference: Methods for Chemical Analysis of Water and Wastes, U.S. ENVIRONMENTAL PROTECTION AGENCY, Office of Research and Development, Cincinnati, Ohio, March 1979.)

Four experimental groupings were set up, each consisting of three replicate tanks containing 20 fish per tank. The control group was held in noncontaminated freshwater for 9 days, then immediately subjected to a 24-hour seawater challenge test as described by CLARKE and BLACKBURN 1977. The second, or 0-hour recovery group, was exposed to Grays Harbor sediment for 9 days, then immediately subjected to the same 24-hour seawater challenge test in clean seawater as the control group. The third, or 5-day recovery group, was placed in freshwater which was contaminated with Grays Harbor sediment for 9 days, and were then placed into clean freshwater for a 5-day recovery period. At the end of this time, they were subjected to the 24-hour seawater challenge

test as described above. The fourth group, or 10-day recovery group, was placed in freshwater which was contaminated with Grays Harbor sediment for 9 days and was then placed into clear freshwater for a 10-day recovery period. After this, they were subjected to the same 24-hour seawater challenge test as described above.

Feeding of the fish was conducted until the day before the experiment commenced. None of the 240 experimental fish were fed during the experiment since this species is a sight feeder and the high turbidity levels in the experimental tanks could affect the results by allowing differential feeding rates between the clear control tanks and the turbid experimental tanks.

After each group had undergone the seawater challenge test, the fish were anesthesized, five at a time, in 200 parts per million (p.p.m.) of MS-222. Each fish was weighed and measured and then its tail was removed at the caudal peduncle. Blood from the dorsal aorta was collected in 280-micron Natelson capillary tubes which had been treated with ammonium heparin. The tubes were sealed at one end and centrifuged in groups of six for 5 to 10 minutes until distinct separation of plasma and hematocrytes had occurred. The capillary tubes were then broken at the plasma-hematocryte interface and a portion of the tube containing the plasma was sealed at one end and immediately placed in a nonfrost-free freezer. The samples were kept frozen until analysis of blood sodium levels was performed on an atomic absorption flame spectrophotometer. Two standards were used for quality controls, and readings were checked every five samples to account for drift.

RESULTS

The mean blood sodium level for the preliminary seawater challenge test was 168 meg/l with a standard error of 3.9 meg/l on 16 May 1982. Since a level of 170 meg/l was considered to be the upper limit of smolt classification for this experiment, the experiment commenced on 18 May 1982.

The mean turbidity level in the three control tanks was 0.89 NTU. The turbidity readings in the other tanks fluctuated randomly from 6 to 220 NTU's throughout the experiment. The mean for the nine tanks containing sediment throughout the entire experiment was 75 NTU. No attempt was made to correlate turbidity levels with suspended solids concentrations.

Fish length and weight measurements can be summarized as follows: The shortest mean length per tank was 123.1 millimeter (mm) in tank 48, and the longest mean length per tank was 129 mm in tank 54. The greatest standard error from a mean was 1.9 mm in tank 54. The mean fish weight varied from 15.9 grams in tank No. 47 to 20.2 grams in tank 51. The largest standard error was 0.8 grams in both tanks 53 and 54. Because the fish were not

fed throughout the experiment, a comparison of length to weight was made for each tank to determine length or weight changes during the experiment. In table 1, a greater length to weight ratio can be seen in the 10-day recovery group compared to the other three groups. The fish in the 10-day recovery group were noted to be visibly emaciated in comparison to the three other groups. No fish mortalities occurred in any of the experimental tanks during the experiment.

TABLE 1

LENGTH TO WEIGHT OF RATIO OF EXPERIMENTAL FISH AFTER THE SEAWATER CHALLENGE TEST (mm:grams)

Group	Tank Number	L:W Ratio
Control	50	6.76
	51	6.31
	55	6.79
0-Hour Recovery	56	6.92
	57	6.62
	58	6.64
5-Day Recovery	52	6.70
	53	6.93
	54	6.45
10-Day Recovery	47	7.75
	48	7.69
	49	7.06

The results of the elutriate chemical anaysis performed on 21 May are contained in table 2.

Water temperatures and pH levels in the freshwater and saltwater intake systems were monitored throughout the experiment. Freshwater temperatures ranged from 11.0° C to 12.8° C, and pH ranged from 7.72 to 7.90. Saltwater temperatures ranged from 10.0° C to 11.0° C and pH ranged from 7.98 to 8.02. The DO level was not below 7.0 milligrams per liter in any tank measured during the experiment.

Blood sodium levels for the four groups in the experiment can be seen in table 3. The three control tanks had a mean blood Na level of 157 meg/l and a standard error of 2.3 meg/l. The three zero-hour recovery tanks had a mean of 145 meg/l and a standard error of 2.9 meg/l. The three 5-day recovery tanks had a mean of 168 meg/l and a standard error of 4.0 meg/l. The 10-day recovery tanks had a mean of 181 meg/l and a standard error of 2.2 meg/l. Statistically invalid data were removed and not used in the above calculations (WEDEMEYER 1982, personal communication).

TABLE 2

ELUTRIATE ANALYSIS OF GRAYS HARBOR SEDIMENTS

Metal Type	Quantity Measured (mg/l)	Current EPA Criteria (mg/l)
Copper	0.002	0.023
Zinc	0.012	0.058
Lead	0.001	0.025
Nickel	0.001	0.007
Cadmium	0.0001	0.005
Chromium	0.0010	0.018
Mercury	0.0002	0.0001

Quality Control: Analysis performed on atomic absorption spectrophotometer using three standards, a blank, and an EPA reference standard. Standards were checked at least once in every 10 samples.

TABLE 3

BLOOD SODIUM LEVELS
BY EXPERIMENTAL GROUP
(meq/l)

Group	Tank Nos.	Fish No.	Mean (meq/l)	S.D.	S.E.
Control	50, 51, 55	60	157.3	18.2	2.3
0-Hour Recovery	56, 57, 58	60	145.2	23.3	2.9
5-Day Recovery	52, 53, 54	60	168.0	31.0	4.0
10-Day Recovery	47, 48, 49	60	181.0	16.6	2.2

DISCUSSION

A great deal of variation in turbidity levels occurred in the experimental tanks throughout the contaminant exposure period. This variability correlates closely with naturally occurring variations in turbidity during hopper dredging operations. This variation was due to several factors. Sediment clogging of the chemical feed pumps and back-pressure valves, variations in particle sizes within the samples, the movement of the fish in the tanks, and siphoning of the sediment slurry from the slurry tanks to the experimental tanks all contributed to the fluctuations observed in the turbidity readings.

Although turbidity readings of about 200 NTU's were occasionally reached, the waterflow rates in the experimental tanks were not sufficient to keep the heavier material in the slurry suspended. This was evidenced by the effect of flow rate and slurry input rate changes on turbidity levels during the experiment. Despite doubling the slurry input rate and halving the water inflow rate on day three of the contaminant exposure period,

significant increases in subsequent turbidity readings were not observed. Deposition of sediment up to 5 centimeters deep occurred in the experimental tanks, indicating that the water movement in the tanks was not great enough to keep all of the material suspended.

Because turbidity readings are widely used in conjunction with dredging operations, turbidity measurements were used for this experiment rather than suspended solids concentrations measurements.

Chemical analysis of the elutriate from the Grays Harbor samples showed the presence of a variety of heavy metals. The concentrations found in the elutriate analysis were all below the levels which are considered to have an effect on juvenile salmon during parr-smolt transformation (WEDEMEYER et al. 1980). The question of the availability of these metals must also be considered; however, tests to examine this would not be justified unless an effect from heavy metal contamination was apparent. The effect of hydrogen ion concentration (pH) on the availability of heavy metals should also be noted. Analysis of data for the Chehalis River near Grays Harbor shows an average pH of 7.2 for the last several decades. The pH at the freshwater inflow pipe was approximately 7.8-7.9 during the experiment. This increase in pH would tend to make less heavy metals available in their free ion state, and so this experiment would, therefore, tend to slightly mute any heavy metal effects occurring during dredging operations in Grays Harbor.

The intent of this experiment was to perform effect/no effect analysis of typically dredged Grays harbor sediments on the osmoregulatory ability of smolting juvenile salmon migrating through the estuary. A blood sodium level of 170 meg/1 or lower in exposed fish subjected to a 24-hour seawater challenge test was established as a range which demonstrated no effect of heavy metals on the osmoregulatory capacity f the smolts. A blood sodium level of 171 meg/1 or greater was designated as an indication that osmoregulatory impairment was occurring (WEDEMEYER 1982, personal communication).

Previous work (LORZ et al., 1978) has shown that if 5-day recovery periods before saltwater challenge are allowed, the effects of heavy metal exposure on seawater tolerance are reversed. However, in this experiment, the 0-hour recovery group and the 5-day recovery group were below the "effect" threshold and are, therefore, not considered to have suffered any osmoregulatory impairment due to the exposure to the sediments. The smolts allowed to recover for 10 days showed signs of regulatory impairment, but these results are the reverse of what would be expected, based on previous work, and may be an artifact.

The comparison of length to weight ratios provides a hypothesis to explain te cause of the higher blood sodium levels in the 10-day recovery group. This form of stress may have affected

the osmoregulatory capability of the fish. The question of whether this aspect of the experimental design affected the final blood sodium levels obtained cannot be answered without further investigation.

ACKNOWLEDGEMENTS

Research was supported by the U.S. Army, Corps of Engineers. Many thanks to the U.S. Fish and Wildlife Service and especially to the National Fisheries Research Center at Marrowstone Island, Washington. Special thanks to Dr. Gary Wedemeyer.

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Accepted February 16, 1983