

EVALUATION OF FISHWAY DESIGNS FOR DOWNSTREAM PASSAGE OF
SPRING CHINOOK SALMON AND STEELHEAD TROUT SMOLTS, 1987

Final Report

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P.O. Box 3621
Portland, Oregon 97208
Project No. 86-47
Contract No. DE-AI79-86BP64234

March 1988

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ACKNOWLEDGEMENTS

We thank T. Barila, R. Emmert, J. Ferguson, D. Johnson, M. Lindgren, R. Ringe, and S. Willis for assistance in planning and carrying out this project.

ABSTRACT

The stress response of chinook salmon and steelhead trout smolts to passage through three different flumes was tested by assaying plasma cortisol concentrations before and after flume passage. In addition, descaling of fish was recorded before and after flume passage, and the ability of the flumes to pass adult chinook salmon and debris was determined. The three flumes were a corrugated metal flume (CMF), a 4-foot wide baffled flume (BF4), and a Z-foot wide baffled flume (BF2). Each flume was tested under three conditions: 1) at night, 2) during the day with a perforated metal cover, which reduced the amount of light entering the flume by about half (partially darkened), and 3) during the day with the perforated cover and an additional double layer of black plastic (completely darkened).

Plasma cortisol concentrations were not significantly elevated in chinook salmon smolts after passage through any of the flumes ($P > 0.2$, ANOVA). In daytime tests of partially and completely darkened flumes cortisol concentrations were consistently decreased following flume passage. We attribute this to pre-test stress (holding of fish in small tanks) and to the absence of a strong stress response to flume passage. Flume design did not have a significant effect on cortisol concentrations ($P = 0.9$). Total darkening of the flumes during daytime was beneficial: cortisol concentrations were lower ($P = 0.03$) in chinook salmon smolts passing through completely darkened flumes than in smolts passing through partially darkened flumes.

In steelhead trout smolts, plasma cortisol concentrations were significantly elevated after passage through the flumes, and flume design did have a significant effect ($P < 0.0001$, ANOVA). Cortisol concentrations were most increased in fish that passed through the BF2, followed by fish that passed through the BF4. The smallest increase occurred in fish that passed through the CMF. Complete darkening of the flumes during daytime tests did not have a significant effect on cortisol concentrations ($P = 0.4$).

Plasma cortisol concentrations were significantly higher in daytime than in nighttime samples of chinook salmon smolts held in darkened and undarkened tanks and raceways. This diel cortisol cycle was unaffected by light intensity. The cortisol response to passage through darkened flumes was greater in nighttime than in daytime tests with both species.

None of the flumes tested caused descaling of fish. Descaling was measured in two ways: as mean percent of body area descaled, and as the percent of fish in a sample with greater than 5% descaling in any of 10 body zones. Neither of these descaling measures was significantly increased after flume passage, and flume design did not have a significant effect.

When woody debris was introduced into the flumes, a number of pieces lodged in the two baffled flumes. Most of the pieces that lodged in the BF4 were 1.3 to 2.4 meters in length. All debris passed freely through the CHF. Adult chinook salmon (N=3) passed through each of the flumes in 5 min or less.

INTRODUCTION

The collection facility at Little Goose Dam for downstream migrating chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) smolts will be rebuilt in the near future and will include a new bypass system to carry fish from the dam to the facility. Alternative designs to the pressurized-pipe bypass now in use at Little Goose Dam were evaluated by Congleton and Ringe (1985). They tested a corrugated metal (CMF; tested at 3.4% slope) and a baffled flume (BF; tested at slopes of 3.4 and 8.3%) and concluded that:

1) In daytime tests, plasma cortisol concentrations in both chinook salmon and steelhead were significantly higher (indicating a stronger stress response) after passage through the CMF than after passage through the baffled flumes. In nighttime tests, cortisol concentrations did not differ significantly between groups after passage through the CMF and baffled flumes. These results suggested that light intensity affected plasma cortisol concentrations and contributed to the elevation of plasma cortisol in fish passing through the CMF during daylight hours (the interior of the unpainted CMF was much brighter than the interior of the BF).

2) Descaling of test fish was relatively low in both test flumes, but somewhat greater in the CMF than in the BF (day tests only). A hydraulic analysis by Army Corps of Engineers personnel suggested that hydraulic conditions over the perforated dewatering plate at the terminus of the CMF could have been responsible for the descaling observed.

3) Floating debris passed readily through the CMF, but several water-logged sticks lodged in the baffled flume.

After review of the 1985 studies, the Fish Passage Committee of the Columbia Basin Fish and Wildlife Council asked several questions:

1) Could the increase in plasma cortisol concentrations in fish passing through the CMF during the daytime be reduced by darkening the interior of the flume?

2) Could the dewatering screens at the terminus of the CMF be modified to eliminate descaling of fish?

3) How well would a wider baffled flume pass debris?

4) Would adult salmonids, particularly upstream-migrating chinook salmon, remain in a baffled flume for extended periods, possibly deterring the passage of juvenile fish?

In 1986 the Bonneville Power Administration made funds available for further flume testing (see Appendix A for description of flume modifications). These funds were administered by the Ualla Walla District of the U.S. Army Corps of Engineers, who modified the flume testing

facility at Lower Granite Dam and contracted with the Idaho Cooperative Fish and Wildlife Research Unit for testing of the flumes.

For the 1987 flume comparison tests, the interior of the CMF was painted a dark gray-brown color to reduce the bright reflective glare suspected to contribute to increased cortisol levels in smolts in 1985. Hydraulic conditions at the terminus of the CMF were modified to increase the depth of water over the dewatering plate and a new, enlarged concrete sampling tank was constructed to eliminate leakage problems and reduce turbulence. The 2-foot wide (0.61 m) baffled flume (BF2) with a slope of 3.4% remained essentially the same as tested in 1985, except that the interior was lined with fiberglass to reduce leakage and painted the same color as the CMF. The BF2 with an 8.3% slope was removed and a new 4-foot wide (1.22 m) baffled flume (BF4) with a slope of 3.4% was constructed of concrete.

The performance of the flumes was compared on the basis of the following criteria:

- 1) Changes in plasma cortisol concentrations (indicative of a physiological stress response) in fish that passed through each flume under three light conditions: nighttime, partially darkened in daytime, and completely darkened in daytime.
- 2) Descaling or other physical **damage** to fish that passed through each **flume**.
- 3) Time required for passage of smolts through flumes.
- 4) Ability of flumes to pass debris.
- 5) Behavior of adult chinook salmon in flumes.

Several additional questions regarding the effects of light intensity and periodicity on plasma cortisol concentrations were addressed. This information was needed to aid in interpretation of data on changes in plasma cortisol in smolts after passage through covered and uncovered flumes (1 above).

- 1) Do plasma cortisol concentrations in chinook salmon and steelhead trout smolts fluctuate on a day-night (diel) basis?
- 2) If so, does the diel cortisol cycle continue in smolts held in constant darkness?
- 3) If the diel cortisol cycle is suppressed by constant darkness, to what extent must light intensities be reduced to attain this effect?
- 4) If a diel cortisol cycle has a daytime peak, is this a consequence of intraspecific aggressive behavior that is dependent upon visual contact?

MATERIALS AND METHODS

All tests were conducted at the fish passage facility of Lower Granite Dam, Garfield Co., Washington, between April 12 and May 21, 1987, in cooperation with personnel of the Army Corps of Engineers.

Design of Test Flumes

Three flume designs were evaluated by this study (Figure 1 and Appendix A). The first design was a corrugated metal flume (CMF) constructed of 12-gauge aluminum with 1.3-cm high corrugations on 6.7 cm centers. This flume was U-shaped in cross section with a width of 86 cm (2.9 feet). The other two flumes were square in cross section with baffles of molded fiberglass that created a serpentine water flow. One of these flumes (BF4) was 1.22 m wide (4 feet) with baffles every 1.22 m on alternate sides of the flume, and was constructed of concrete. The other baffled flume (BF2) was 0.61 m wide (2 feet) with baffles every 0.61 m and was constructed of plywood lined with fiberglass. Flows in the CMF, BF4, and BF2 were 0.85, 0.71, and 0.14 m³/s (30, 25, and 5 cubic feet/s) respectively. All flumes had a 3.4% slope. Perforated plate covered each of the flume channels, reducing the amount of incident light. The interiors of all flumes were painted a dark gray-brown.

At the head of each flume were six 168-L aluminum holding tanks (91 cm x 33 cm wide and 48 to 64 cm in depth, with bottom sloping toward door), four of which had a release mechanism to open the downstream-facing door. The tanks had opaque fiberglass lids and the overflow screens were covered with duct tape to exclude light. Water from the Snake River was supplied to each tank at 0.55-0.60 L/s, maintaining temperatures at 11-12°C. Below the holding tanks at the head of the BF2 and BF4 were sliding crowder screens used to force the smolts to swim downstream after release. Relatively high velocities at the head of the CMF precluded the need for a crowder screen.

At the downstream end of each flume was a receiving tank which the fish entered after passing through the flume. These tanks were designed to provide a quiet resting area for the fish and were supplied with fresh water to maintain adequate oxygen concentrations and temperatures. The horizontal dimensions were based on hydraulic considerations and differed for each flume (Fig. 1). Water depth varied from 1.0-1.4 m during flume operation to 47 cm after shutoff. The tanks were covered with perforated aluminum plate and a double layer of black polyethylene, which reduced the normal light intensity (up to 3800 lux) to 1-4 lux.

Procedures for Flume Tests

Chinook salmon and steelhead trout smolts were obtained from the gatewells of Lower Granite Dam between 1900-0100 hours by National Marine Fisheries Service personnel using a crane-operated sampling basket.

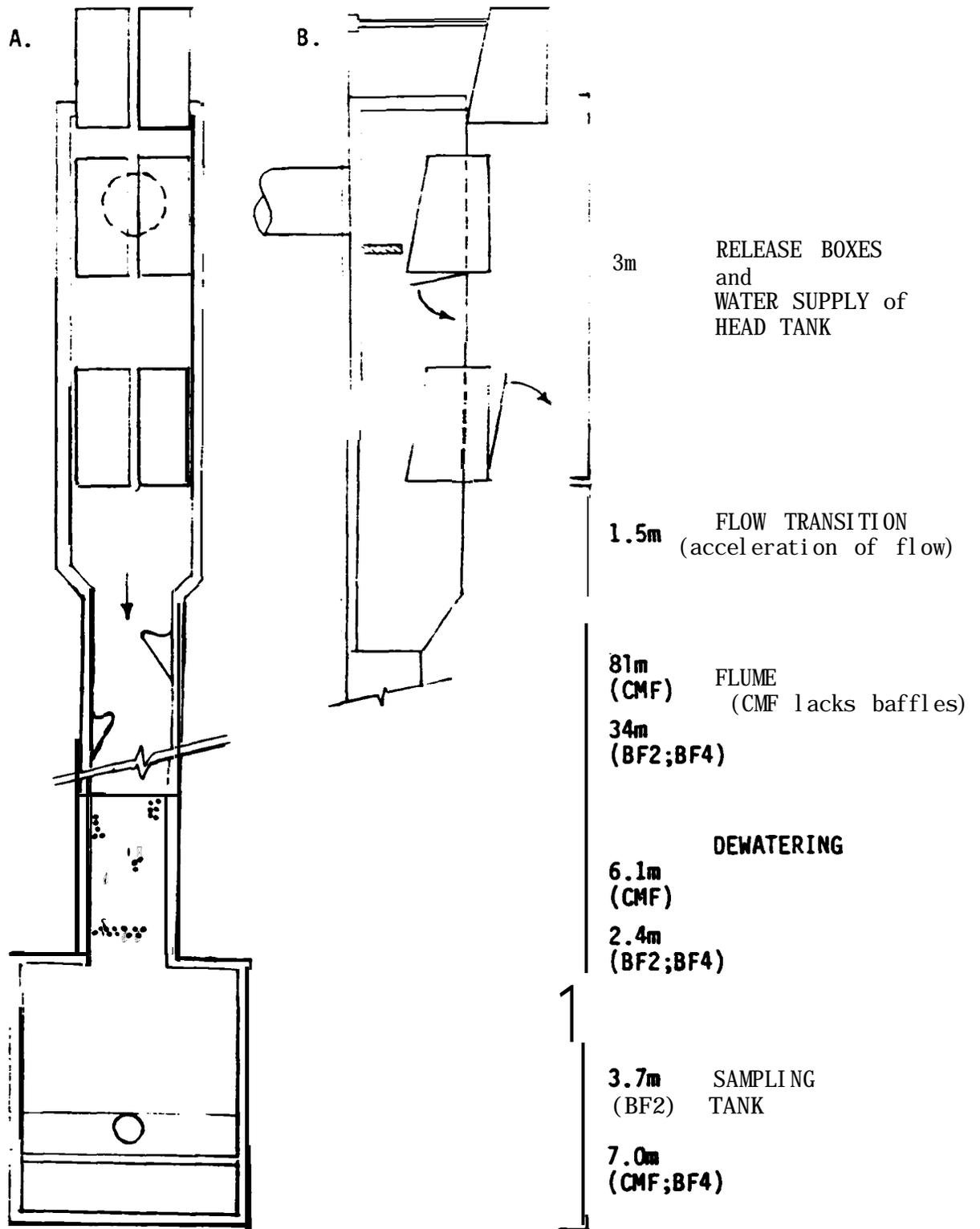


Fig. 1 Schematic (A, top view; B, side view) of the three test flumes: corrugated metal flume (CMF), baffled flumes 1.22m wide (BF4) and 0.61m wide (BF2).

Smolts that had entered the gatewells during the day were first removed by repeating dipping, so that only smolts that had recently entered were used for our tests. Fish were anesthetized prior to handling.

We loaded 20-22 smolts into each of 18 aerated 19 L buckets. Lots of five smolts were added to the buckets in rotating sequence to avoid bias in selection or treatment of fish. The smolts were transported by pickup truck to the fish passage facility and loaded into holding tanks at the head of each flume.

Nighttime tests were performed about 24 hours after loading of fish into the holding tanks. Daytime tests were performed the following day, about 38-40 hours after loading. For daytime tests the perforated metal covers over the flumes were either covered with opaque black plastic (completely darkened) or were uncovered (partially darkened). The testing sequence of the two light conditions alternated, with the partially darkened condition first in trials 1, 3, 5, and 7. The order in which flumes were tested was selected at random. Daytime tests were initiated between 1000 and 1700 h and nighttime tests between 2130 and 0100 h.

Tests were initiated by netting a baseline sample of fish (N=20) from a holding tank. After the fish were anesthetized in 75 mg/L of Tricaine Methane Sulfonate (MS-222) and examined for descaling, the caudal peduncle was severed and blood was collected in heparinized capillary tubes. Plasma was separated by centrifugation and frozen on dry ice in microcentrifuge tubes.

A second tank was randomly selected and the fish released into the flume. After the fish were released, the crowder screen below the holding tanks was pulled downstream to force them to swim downstream. Flumes were dewatered 20 min after release of fish into the BF2 and BF4 and 10 min after release into the CMF. Any fish remaining in the flumes after this time were counted and released into the Snake River. Fish that had passed through the flume were sampled from the tank at the base of each flume one hour after release in the manner described for baseline samples.

Determination of Plasma Cortisol and Lactate Concentrations

Plasma specimens were thawed, vortexed and pooled by combining 100 uL of plasma from each of four different tubes to obtain 5 subsamples for each 20-fish sample. If samples consisted of less than 15 plasma specimens, each specimen was analyzed individually. Cortisol concentrations in plasma were determined by a radioimmunoassay modified from Foster and Dunn (1974) by Redding et al. (1984). Inter-assay and intra-assay coefficients of variation were 24% and 12%.

Plasma lactate concentrations were determined by a spectrophotometric method based on the conversion of lactate to pyruvate and NAOH in the presence of excess NAO and lactate dehydrogenase (Sigma diagnostic kit 826-UV). A standard curve was prepared for each run.

Criteria for Descaling

To estimate the extent of descaling, we visually estimated the percent of descaled area within each of 10 body zones as recommended by Basham et al. (1982). The left and right sides were divided into five zones: 1) caudal fin to anterior end of adipose fin, 2) anterior end of adipose fin to posterior end of dorsal fin, 3) posterior end of dorsal fin to anterior end of dorsal fin, 4) anterior end of dorsal fin to tip of the pectoral fin when it is folded against the body, and 5) tip of pectoral fin to anterior insertion of pectoral fin. The same person generally recorded descaling data for each test, so that comparisons of baseline and postpassage samples as well as comparisons between flumes were unbiased.

The percent of body surface descaled per fish was calculated by the formula:

$$D = ((L1*0.5)+L2+L3+L4+L5+(R1*0.5)+R2+R3+R4+R5)/9$$

in which L2 represents the percent of descaled area in zone 2 of the left side. Note that zone 1 (caudal peduncle area) of each side was weighted by a factor of 0.5 due to the smaller size of the zone relative to the others. The mean descaling rate was calculated for each sample of 20 fish.

A second criterion for descaling was the percent of fish in a sample with more than 5% descaling in any one or more of the 10 body zones.

Analysis of Cortisol and Descaling Data

Baseline cortisol data were tested by analysis of variance (ANOVA, randomized block factorial design) at the 5% probability level for effects of tank location (i.e., at head of one of three test flumes) and light condition. The effect of light condition was significant, so mean baseline cortisol concentrations were calculated separately for nighttime and daytime tests and subtracted from postpassage cortisol concentrations to estimate net changes in plasma cortisol resulting from flume passage. These data were analyzed by ANOVA for effects of flume design and light condition, followed by pair-wise comparison of individual means (Tukey's HSO test) if treatment effects were significant ($P < 0.05$). ANOVA results were almost identical with log-transformed and untransformed cortisol data, so results obtained with untransformed data are reported.

Descaling data were analyzed by ANOVA, following the procedures described for analysis of cortisol data. Descaling percentages were transformed to arctangents before analysis.

Behavior of Adult Chinook Salmon in Flumes

Adult chinook salmon (685 mm to 850 mm length range) were removed from the adult trap at the dam, anesthetized, and transported to a holding tank at the head of each flume. A small balloon was attached by a hook and monofilament line to the dorsal fin to facilitate observation after releasing the fish into the flume. In addition, 20 chinook smolts were loaded into a holding tank of each flume in trials 1 and 2; 20 steelhead smolts were used for trial 3.

Trials 1 and 2 were conducted on April 29, 1987, and trial 3 on May 20. For each trial, adult salmon were released into the flumes five min before the smolts were released. After 20 min the flume was dewatered and the number of smolts remaining in the flume counted. In trial 3, the flume was shut down after 10 min, since prior experience showed that all the smolts had passed through the flume after 20 min. After flume passage, the adults were netted out of the sample tank into a plastic garbage can containing a 50-75 mg/L solution of MS222 anesthetic, loaded into the fish transport truck (containing a 25 mg/L MS222 solution), and released at the boat launch above the dam. The adults recovered in a matter of minutes, and continued their upstream migration. All trials were conducted during the day with partially darkened flumes.

Passage of Debris Through Flumes

Water-soaked debris was cast into each flume piece by piece. The water was left on until all debris had either passed or lodged. Once the flume was dewatered, the number and size of the debris that had lodged in the flumes were recorded. The same debris was used in tests with all flumes.

Debris used for the first test (May 20) had passed through the orifices at Little Goose Dam and was kept waterlogged until testing. Below normal water flow greatly reduced the amount of debris reaching Little Goose Dam, so the reserved debris was not representative of a typical year.

To rectify the problem, the test was repeated on May 27, 1987, using the debris collected at Little Goose plus an additional 15-20 pieces of woody debris chosen from the Lower Granite Dam debris pile by Sarah Willis, fishery biologist at Lower Granite, who had worked at the Little Goose facility in previous years. The debris filled three 50-gallon garbage cans.

Diel Variation in Baseline Cortisol Concentrations and Effects of

Light Intensity

Baseline Cortisol Concentrations in Chinook Salmon

The objectives of this test were to determine if plasma cortisol concentrations in chinook salmon smolts varied significantly between day and night, and to determine the effects of constant darkness upon any day to night variation.

Twelve aluminum tanks identical to the holding tanks at the head of each flume were stocked with 20 smolts each. Six of the tanks were covered with opaque lids which reduced the interior light intensity to <0.1 lux; the remaining six tanks were covered with a layer of translucent plastic which reduced the intensity of incident light only slightly (midday maximum=3800 lux). The overflow screens of all tanks were covered with duct tape. The fish were allowed to acclimate to the tanks for 24 hours before the first sample was taken. The fish were not fed. Subsequently, one uncovered and one covered tank were sampled each day (1100 h) and each night (2300 h) for three days and nights. Three trials of the test were conducted (April 13, 17, and 29).

The results were statistically analyzed by ANOVA (randomized block factorial design) comparing cortisol concentrations between fish held in covered and uncovered tanks. Day versus night differences were similarly tested (ANOVA) by comparing the overall mean of all six night samples (covered and uncovered) with the mean of the six day samples.

Cortisol Concentrations in Chinook Salmon and Steelhead Trout Held in Raceways

The objectives of this test were to determine if plasma cortisol concentrations in chinook salmon and steelhead trout held in raceways varied significantly between day and night, and to determine the effects of raceway darkening upon any day to night variation.

A barrier of plastic netting with a wood frame was placed into each of two raceways, 9.1 m from the inflow. One raceway (no. 7 for trial 1, no. 8 for trial 2) was covered for its entire length (27 m) with a double layer of black polyethylene, weighted down on the outside walls of the raceway with wood planks. Light intensities below the cover were 1.0-4.0 lux on a sunny day at 1030 h.

The 9.1 m sections of the raceways enclosed by the mesh barriers were loaded with fish that had entered the smolt collection system and passed through the bypass pipe and debris separator. Trials with chinook salmon were conducted when the percent of chinook salmon smolts was high (93-95%) and trials with steelhead were conducted when the percentage of steelhead was high (82-92%).

After a 4 to 5 h loading period (0900-1400 h), each raceway contained approximately 5000 smolts of both species. Population estimates were based on a sample of fish diverted through electromagnetic fish counters for 3-5 min of **every hour (9-12% subsample)**. The resulting densities were 27-29 **smolts/m³** in tests with chinook salmon and 24-35 smolts/m³ in tests with steelhead. Flows were maintained at 3400-3800 L/min. The biomass of chinook smolts per raceway varied between 142-176 kg (8.3-10.3 g/L) and steelhead varied between 343-372 kg (20.1-21.8 g/L).

Beginning the day that fish were loaded, blood samples were taken from 20 smolts from each raceway over the course of two days and nights at 1600, 2300, 0400, and 1100 h. The last sample was collected at 0800 rather than 1100 hours because the smolts had to be loaded into barges by 0900 h.

Two trials of the test were conducted. Fish were captured by "lift" nets (61 cm or 47 cm square) that were hauled up quickly from the bottom by ropes pulled by two persons on either side of the raceway. The net was left lying on the bottom of the raceway between samples. Since fish in the uncovered raceway could see us, we always sampled it first to avoid possible elevation of cortisol concentrations due to fright.

Blood samples were taken as described for the flume comparison tests. For statistical analysis, day versus night differences were tested (ANOVA) by comparing the overall mean of the 8 night samples (covered and uncovered) with the mean of the 8 day samples. A paired T-test was used to compare corresponding covered and uncovered samples.

Effect of Reduced Light Intensity on Cortisol Concentrations in Chinook Salmon and Steelhead Trout

The objective of this test was to determine the relationship between light intensity and plasma cortisol concentrations in chinook salmon and steelhead trout smolts held in tanks.

The 12 168-L aluminum tanks previously described were loaded at night with 20 smolts each. Each of the twelve tanks was randomly assigned one of four levels of light intensity, which were attained by covering the tanks with a single layer of translucent white plastic, with one or two layers of semi-opaque black plastic (1.2 mil), or with an opaque fiberglass lid. The overflow screens of all tanks were covered with duct tape, leaving an overlap at the bottom for the water overflow. Midday light intensities at the surface of the water in the tanks were 3800, 470, 4, and **<0.1** lux.

Fish were sampled from all tanks at 1000-1630 h, 32-36 hours after loading. Tanks were sampled in random order. Two trials were conducted using chinook and two using steelhead smolts. For statistical analysis an ANOVA, randomized block factorial design, was used.

Cortisol Concentrations in Isolated Chinook Salmon

The objective was to determine whether diel changes in agonistic behavior might be responsible for diel cycles in cortisol concentrations in chinook salmon smolts. The approach was to evaluate differences in cortisol concentrations between chinook salmon smolts that were physically but not visually isolated from other fish and smolts that were both physically and visually isolated. We assumed that any agonistic interactions would be visually mediated.

The 12 tanks previously described were modified by subdividing each tank into four compartments of equal volume. In the first two trials the dividers were plastic netting supported by a wood frame, permitting visual contact between fish in uncovered tanks. In the third and fourth trials, dividers were covered with opaque black polyethelene, thus physically and visually isolating each smolt. Six tanks were covered with opaque covers and six with translucent covers as previously described.

Smolts were sampled from three covered and three uncovered tanks at night (2230-0130 h), 24 hours after loading one fish per compartment (4 per tank). Smolts in the remaining six tanks were sampled the following day (1100-1300 h). Plasma specimens were taken as previously described, but each specimen was individually analyzed and not pooled.

For statistical analysis, a randomized block design first evaluated differences in cortisol concentrations between the four compartments within a tank. The differences were insignificant in both covered and uncovered tanks, so an average of the four samples within a tank was used for subsequent ANOVA. A randomized block design was used to test differences between covered and uncovered tanks, night versus day, and between cortisol concentrations in tanks using the two different types of dividers. Data from covered and uncovered tanks were analyzed separately and together to test the two types of isolation.

RESULTS

Plasma Cortisol Concentrations Before and After Flume Passage

Chinook Salmon

Holding tank location did not have a significant effect on baseline cortisol concentrations in chinook salmon smolts, but concentrations in nighttime samples were significantly lower than in daytime samples ($P < 0.0001$). Therefore, an average nighttime baseline cortisol concentration (109.0 ng/mL) was subtracted from nighttime postpassage concentrations and an average daytime baseline average (148.0 ng/mL) was subtracted from daytime (completely darkened and partially darkened) postpassage concentrations to determine net changes in cortisol following flume passage (Table 1).

Table 1. Mean concentrations (\pm SE, N=7 trials) of plasma cortisol (ng/mL) in chinook salmon smolts before (BL) and after (PP) passage through three flumes under three light conditions.

Flume		Light Condition		
		Night	Partially Darkened	Completely Darkened
BF2	BL	136.8 +16.4	139.7 +12.9	135.8 + 9.2
	PP	144.6 +11.9	141.0 +12.7	112.3 \pm14.9
	PP-XBL ^a	35.6	-7.0	-35.7
BF4	BL	92.0 \pm16.8	162.2 \pm10.2	161.0 \pm16.3
	PP	139.6 \pm15.0	117.4 \pm16.4	124.2 \pm15.0
	PP-XBL	30.6	-30.6	-23.8
CMF	PP	98.2 \pm15.5	143.7 \pm15.6	145.5 \pm 8.7
	PP-XBL	145.5 36.5 \pm19.5	141.5 -6.5 \pm10.7	101.0 -47.0 \pm10.4

^a Postpassage cortisol concentration minus an average nighttime (109.0 ng/mL) or daytime (148.0 ng/mL) baseline concentration.

After the smolts had experienced release from the holding tank, a tumultuous ride through the flume, and an hour in the sampling tank, one would expect cortisol concentrations to rise above those of the baseline samples. Nevertheless, for chinook salmon there was no significant difference between baseline and postpassage concentrations when data for all light conditions were tested. When data for the three light conditions were tested separately, postpassage cortisol concentrations were significantly lower than baseline concentrations in fish passing through flumes in daytime tests (partially and completely darkened). We

attribute this decline in cortisol concentrations to the combined effects of somewhat stressful conditions in the holding tanks, relatively unstressful conditions in the receiving tanks, and the absence of a strong stress response to flume passage. Differences between baseline and postpassage cortisol concentrations were insignificant in nighttime tests.

Flume design did not have a significant effect on net changes in cortisol concentration (P=0.9) in tests with chinook salmon. Analysis of data for daytime tests alone indicated that cortisol concentrations for fish passing through covered flumes were significantly lower (P=0.03) than for fish passing through partially covered flumes.

Steelhead Trout

Holding tank location did not have a significant effect on baseline cortisol concentrations, but concentrations in nighttime samples were significantly lower than in daytime samples (P=0.002). Therefore, an average nighttime baseline cortisol concentration (174 ng/mL) was subtracted from nighttime postpassage concentrations and an average daytime baseline concentration (212 ng/mL) was subtracted from daytime (darkened and partially darkened) postpassage concentrations to determine net changes in cortisol following flume passage (Table 2).

Table 2. Mean concentrations (**±SE**, N=7 trials) of plasma cortisol (ng/mL) in steelhead trout smolts before (BL) and after (PP) passage through three flumes under three light conditions.

Flume		Light Condition		
		Night	Partially Darkened	Completely Darkened
BF2	BL	159.5 ±12.2	227.9 ±16.8	210.3 ±22.5
	PP-XBL ^a	288.8 ±26.2	287.0 ±16.1	303.4 +20.6
BF4	BL	188.3 ±16.1	196.4 ±21.0	184.6 ±16.7
	PP	254.0 ±23.1	294.5 ±24.8	270.6 ±14.7
	PP-XBL	8.0	82.5	58.6
CMF	PP	174.8 ±12.9	259.0 ±13.0	215.8 ±19.3
	PP-XBL	248.2 74.2 ±24.6	231.6 47.0 ±13.0	260.7 48.7 ±13.8

^a Postpassage cortisol concentration minus an average nighttime (174 ng/mL) or daytime (212 ng/mL) baseline concentration.

Postpassage concentrations were significantly higher than baseline concentrations (**P<0.0001**), with net increases ranging up to 115 ng/mL. The three flumes differed (P<0.0001) when all three light conditions were analyzed together. Each of the three flumes differed significantly (**P<0.05**; Tukey multiple comparison procedure) from the other two. The BF2 had the highest average cortisol increase (101.4 ng/mL), followed by the BF4 (73.7 ng/mL) and the CMF (47.5 ng/mL).

Nighttime cortisol increases in cortisol concentrations were higher (P=0.02) than daytime increases in smolts passing through partially and completely darkened flumes, but absolute postpassage concentrations did not differ significantly (P=0.13) for nighttime and daytime conditions. When data for partially and completely darkened daytime tests were analyzed apart from the data for nighttime tests, no differences were apparent.

Rate of Passage of Smolts through Flumes

Some smolts often remained in the flumes when they were dewatered 20 min (BF2, BF4) or 10 min (CMF) after smolts were released (Table 3). These fish did not reach the sample tank or reached it and swam back upstream. Lower velocities in the baffled flumes allowed more smolts to stay in these flumes; fish could not swim upstream against the higher velocities in the CMF.

Table 3. Average number (N=7 trials) of chinook salmon and steelhead trout smolts remaining in the three flumes under three light conditions (22 fish were released in each trial).

Flume	Night	Covered	Partially Covered
chinook			
BF2	3	1	4
BF4	0	0	2
CMF	0	1	0
steelhead			
BF2	4	4	6
BF4	7	7	9
CMF	0	1	1

The number of chinook smolts remaining in the flumes varied from 0 to 11 (0-50% of fish released). The number of steelhead varied from 0 to 18 (0-82% of fish released). More steelhead than chinook salmon were found in all the flumes after dewatering, probably because the larger

steelhead smolts (average length=207 mm vs. 144 mm for chinook salmon) were able to fight the current long enough to find sheltering eddies behind the window ports of the CMF or the baffles of the other two flumes.

As expected, ANOVA and subsequent comparison of means showed that significantly fewer chinook (**P<0.025**) and steelhead (P<0.003) remained in the CMF compared to the baffled flumes. Light condition did not have a significant effect.

Plasm Lactate Concentrations Before and After Flume Passaae

Mean baseline plasma lactate concentrations (N=20) in chinook salmon ranged from 1 to 2 mMol/L and postpassage concentrations from 2.1 to 3.8 mMol/L (Table 4). Baseline concentrations in steelhead trout ranged from 2.1 to 6.5 mMol/L and postpassage concentrations from 2.6 to 5.8 mMol/L. No differences were attributable to flume design or light condition. Postpassage lactate concentrations in both species were generally equal to or lower than lactate concentrations measured in chinook salmon sampled from gatewells at Lower Granite Dam in 1982 (Congleton et al. 1983).

Table 4. Plasma lactate concentrations (**mMol/L**) in chinook salmon (April 6-9 tests) and steelhead trout (May 2-3 tests) smolts before (baseline, BL) and one hour after passage (postpassage, PP) through three flumes. Daytime tests were performed with flumes partially (PD) or completely (CD) darkened.

	<u>BF2</u>			<u>BF4</u>			<u>CMF</u>		
	<u>BL</u>	<u>PP</u>	<u>PP- BL</u>	<u>BL</u>	<u>PP</u>	<u>PP- BL</u>	<u>BL</u>	<u>PP</u>	<u>PP- BL</u>
<u>Chi nook Salmon</u>									
Ni ght	1.52	2.32	0.80	1.98	2.84	0.86	1.02	2.07	1.05
Daytime PD	1.42	3.78	2.36	1.84	2.25	0.41	2.70	2.34	-0.36
Daytime CD	1.92	3.59	1.67	1.07	3.16	2.09	1.54	2.97	1.43
	mean		1.61			1.12			0.70
<u>Steel head Trout</u>									
Ni ght	2.52	4.22	1.70	3.36	3.88	0.52	6.47	5.85	-0.62
Daytime PD	2.51	3.84	1.33	3.32	2.62	-0.71	2.10	3.51	1.41
Daytime CD	2.68	3.08	0.40	2.80	3.29	0.49	3.08	3.36	0.28
	mean		1.14			0.10			0.36

Descaling of Smolts in Flumes

Chinook Salmon

There was no significant difference between baseline and postpassage samples in the percent of chinook salmon with descaling or in descaling of fish that passed through the three flumes (Table 5).

Table 5. Average (\pm SE, N=7 trials) percent of chinook smolts with more than 5% descaling in any one of ten body zones before (BL) and after (PP) passage through three flume designs under three light conditions.

Flume		Night	Partially Covered	Covered
BF-2	BL	8.6 \pm 1.9	23.0 \pm 5.8	23.6 \pm 2.3
	PP	25.0 \pm 4.9	31.4 \pm 4.3	17.8 \pm 2.6
BF-4	BL	22.3 \pm 4.8	23.1 \pm 3.4	28.0 \pm 4.6
	PP	16.1 \pm 2.7	30.0 \pm 4.6	18.6 \pm 2.5
CMF	BL	21.1 \pm 2.9	19.3 \pm 3.6	28.1 \pm 4.0
	PP	20.4 \pm 3.2	24.3 \pm 3.0	24.1 \pm 5.4

The average (N=20) percent of body surface descaled ranged from 0 to 14 percent in individual samples (Table 6). There were no significant differences between flumes, between the three light conditions, or between baseline and postpassage samples.

Table 6. Average (\pm SE, N=7) percent of body surface descaled per chinook salmon smolt before (BL) and after (PP) passage through three different flume designs under three light conditions.

Flume		Night	Partially Covered	Covered
BF2	BL	6.1 \pm 1.9	2.7 \pm 0.7	4.3 \pm 1.4
	PP	5.6 \pm 2.3	5.1 \pm 1.3	4.9 \pm 1.0
BF4	BL	3.2 \pm 0.8	3.6 \pm 1.3	4.5 \pm 0.8
	PP	5.9 \pm 1.3	5.7 \pm 1.0	7.3 \pm 2.2
CMF	BL	3.9 \pm 1.0	2.6 \pm 0.7	4.3 \pm 1.0
	PP	3.7 \pm 0.9	3.5 \pm 0.7	2.7 \pm 0.4

Steelhead Trout

There was no significant difference between baseline and postpassage samples in the percent of steelhead trout with descaling or in descaling of fish that passed through the three flumes (Table 7).

Table 7. Average percent (\pm SE, N-7) of steelhead trout smolts with more than 5% descaling in any one of ten body zones before (BL) and after (PP) passage through three different flume designs under three light conditions.

Flume		Night	Partially Covered	Covered
BF2	BL	46 \pm 12	57 \pm 8	60 \pm 10
	PP	50 \pm 10	52 \pm 10	60 \pm 9
BF4	BL	47 \pm 11	53 \pm 12	55 \pm 6
	PP	39 \pm 11	62 \pm 8	65 \pm 11
CMF	BL	53 \pm 11	45 \pm 12	48 \pm 11
	PP	47 \pm 10	58 \pm 8	61 \pm 8

The average (N=20) percent of body surface descaled ranged from 0 to 12 percent in individual samples (data not shown). Values were similar to those for the chinook samples. There were no significant differences due to flume design or light condition, or between baseline and postpassage samples.

Behavior of Adult Chinook Salmon in Flumes

In the first trial in the BF4, the adult chinook salmon travelled downstream from behind one baffle to behind the next until it reached the screen at the lower end 107 s after release. Somewhere between the screen and the sample tank at the base of the flume the line to the attached balloon broke, so that the location of the fish could no longer be determined. When the flume was dewatered, the adult was above the screen, although all the smolts had passed. Apparently, the adult salmon reached the sample tank and swam back upstream. This was the only case in which an adult remained in the flume; all others passed in a few minutes (Table 8).

Table 8. Passage time (s) of adult chinook salmon through three flumes.

Trial	Flume		CMF
	BF-2	BF-4	
1	110	107	a
2	140	300-1200^b	37
3	91	97	33

a No time recorded due to escape of adult fish from holding tank.

b The balloon line broke after 5 min while the fish was still in the flume, but the adult had passed after 20 min.

All chinook salmon smolts passed through the flumes within twenty min, but 17 and 15 steelhead smolts were still in the BF4 and BF2 after 10 min (trial 3). Since the adults had passed through the flumes before the steelhead smolts were even released, smolt passage was not affected by the presence of an adult.

Passage of Debris Through Flumes

All debris passed through the CMF without lodging in both trials. In the first trial with the BF4, a log jam formed at the third baffle in which there were 5 sticks ranging from 1 m long and 3 cm in diameter to 2.4 m long and 10 cm in diameter. In the lower end of the flume, a stick 1.4 m x 13 cm and another 2.0 m x 2 cm lodged in two different places. In the second trial with the BF4, a log jam formed at the same site as the first: it contained four sticks between 1.8 m and 2.4 m, four more between 1.2 and 1.8 m, and 24 that were less than 1.2 m in length. Four sticks lodged in the lower section of the flume: one was between 1.8 m-2.4 m, two more were between 1.2 and 1.8 m, and one was less than 1.2 m in length.

Nearly every stick lodged in the BF2. Once one stick lodged behind a baffle, a debris jam quickly formed at that location.

The manner in which the sticks lodged behind baffles was consistent. The downstream end would be pointed into the upstream corner of the baffle where it attached to the wall and floor. The back end of the stick was carried by the current to the opposite side of the flume, where the current kept it pushed against the upstream baffle. Sticks that were longer than the width of the flume lodged first, creating a site for smaller debris to collect.

Diel Variation in Baseline Cortisol Concentrations and Effects of Light

Intensity

Cortisol Concentrations in Chinook Salmon Held in 168-L Tanks

Cortisol concentrations were lower in nighttime samples (99.4 ng/mL) than in daytime samples (119.5 ng/mL) and this difference was significant ($P=0.01$). Cortisol concentrations in fish held in darkened or undarkened tanks did not differ significantly. These results confirm a diel cycle in plasma cortisol concentrations in fish held in both darkened and undarkened tanks (Fig. 2).

Cortisol Concentrations in Chinook Salmon and Steelhead Trout Held in Raceways

During the first trial with chinook salmon the barrier in the covered raceway broke free sometime before the fourth sampling, allowing smolts access to the rest of the raceway and lowering the density to a third of that for the uncovered raceway. The results of this trial were used in the analysis because values were similar to those of the second trial and a third trial was not possible.

Plasma cortisol concentrations of chinook salmon were significantly lower at night than during the day, indicating a diel cycle (Fig. 3). Cortisol concentrations were similar in fish from covered and uncovered raceways, except during the first few hours after loading (first sample, 1600 h).

Plasma cortisol concentrations in steelhead trout did not differ significantly between daytime and nighttime samples, nor between samples from covered and uncovered raceways. Plotted over time, the data do not demonstrate a diel cortisol cycle (Fig. 4).

Effect of Reduced Light Intensity on Cortisol Concentrations in Chinook Salmon and Steelhead Trout

Midday light intensities ranging from 3800 to <0.1 lux did not significantly affect cortisol concentrations in chinook salmon or steelhead smolts ($P=0.8$; Table 9).

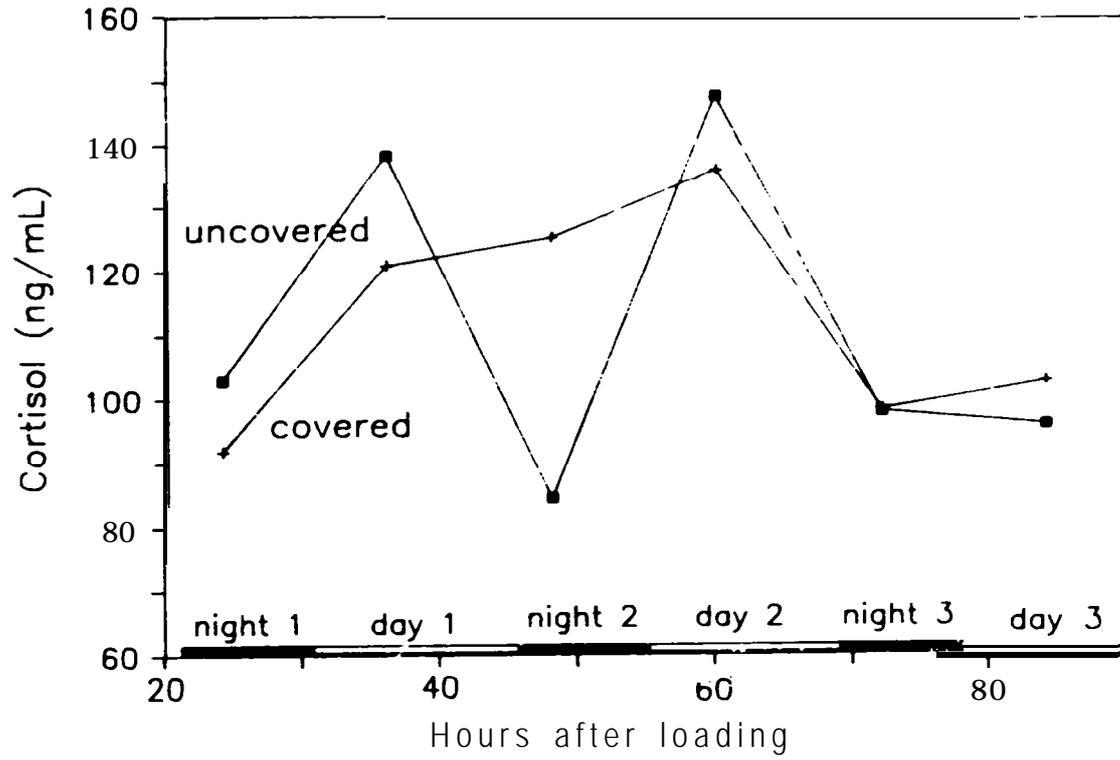


Figure 2. Diel fluctuations in plasma cortisol concentrations in chinook salmon smolts held in darkened and undarkened 168-L tanks.

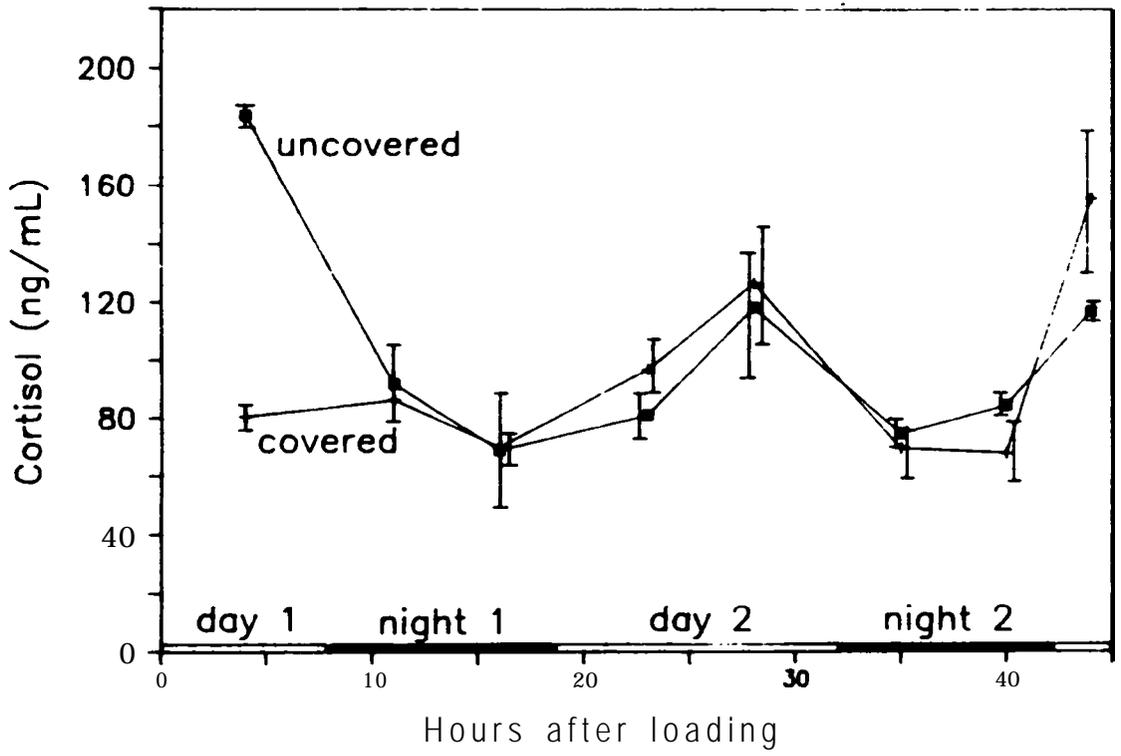


Figure 3. Diel fluctuations in plasma cortisol concentrations in chinook salmon smolts held in covered and uncovered raceways.

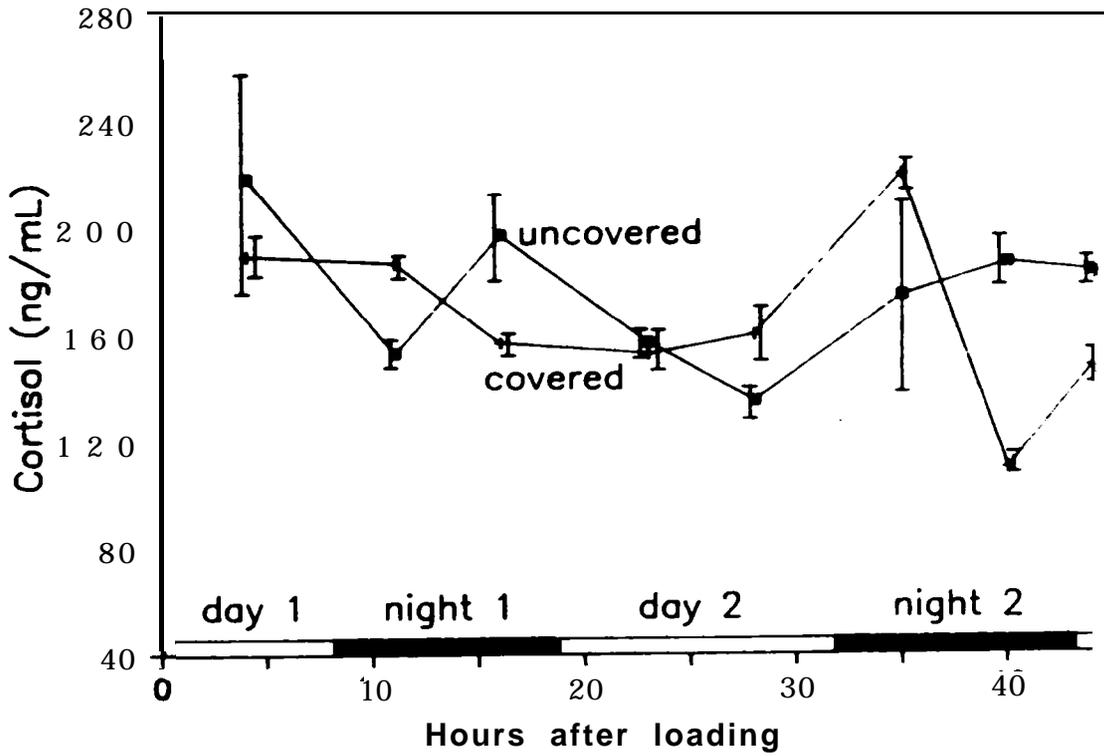


Figure 4. Diel fluctuations in plasma cortisol concentrations in steelhead trout smolts held in covered and uncovered raceways.

Table 9. Mean plasma cortisol concentrations (ng/mL, \pm SE, N=2 trials) in chinook salmon and steelhead trout smolts held in 168-c tanks under four light intensities.

Species	Midday Light Intensity			
	3800 lux	470 lux	4 lux	0.1 lux
Chinook S.	124.0 \pm 54.5	134.7 \pm 38.6	133.2 \pm 36.3	151.8 \pm 34.0
Steel head	214.7 \pm 41.3	222.6 \pm 38.3	258.4 \pm 47.9	204.8 \pm 15.8

Cortisol Concentrations in Isolated Chinook Salmon

Fish that were physically but not visually isolated did not have significantly higher or lower cortisol concentrations than fish that were both physically and visually isolated in either covered or uncovered tanks (Table 10). ANOVA showed that trials were significantly different, but subsequent comparison of means using Tukey's HSD test showed no correlation between plasma cortisol concentrations and the type of isolation. There was no significant difference between fish held in darkened or undarkened tanks (P=0.7).

Mean cortisol concentrations in fish sampled at night (123.5 ng/mL) were significantly lower (P=0.0001) than those sampled during the day (189.1 ng/mL). This is consistent with the results of other tests in which nighttime cortisol concentrations were lower than daytime concentrations.

Table 10. Mean cortisol concentrations (ng/mL; \pm SE, N=12) in chinook salmon smolts held individually in covered and uncovered tanks, visually isolated or not isolated from adjacent smolts.

Visual Isolation	Covered		Uncovered	
	Day	Night	Day	Night
No				
Trial 2	202.3 \pm 10.5	180.7 \pm 10.9	326.1 \pm 19.3	119.2 \pm 15.8
Mean	183.8	120.4 \pm 10.0	175.1 \pm 22.6	73.6 \pm 2.2
			250.6	96.4
Yes				
Trial 4	158.8 \pm 20.6	150.2 \pm 18.0	142.2 \pm 1.0	112.5 \pm 10.4
Mean	146.0	169.2 \pm 37.6	210.0 \pm 31.1	103.6 \pm 4.3
			176.1	108.1

DISCUSSION

Effects of Flume Passage on Cortisol Concentrations

Plasma cortisol concentrations in chinook salmon smolts were not significantly increased by passage through any of the three flumes tested in 1987. To the contrary, in daytime tests cortisol concentrations were consistently lower in fish sampled one hour after flume passage (postpassage samples) than in fish that did not experience flume passage (baseline samples). In earlier tests (Congleton and Ringe 1985), cortisol concentrations were always higher in fish that passed through the flumes than in fish that did not. Also, in the 1985 tests fish that passed through the CMF during the day had significantly higher cortisol concentrations than fish that passed through the BF2 (the BF4 was not tested in 1985). Factors that may have contributed to the differences between 1985 and 1987 results are discussed below.

Baseline cortisol concentrations in chinook salmon smolts were in close agreement in 1985 and 1987 (Fig. 5; differences 2-7 ng/mL). Handling procedures and holding conditions were similar in the two years, so agreement in baseline cortisol concentrations indicates that the two year-classes of smolts responded similarly to the moderate stress of confinement.

Comparison of postpassage cortisol concentrations indicated that nighttime passage through the BF2 and CMF elicited similar cortisol responses in 1985 and 1987 (differences 9-16 ng/mL), again indicating a similar stress response by the two year-classes of smolts. In contrast, daytime passage through both flumes resulted in considerably lower cortisol concentrations in 1987 than in 1985, particularly in tests with the CMF (39 ng/mL lower in 1987 tests with fully covered BF2; 94 ng/mL lower in tests with fully covered CMF).

The major difference in test conditions in 1985 and 1987 was that the flumes and the fish receiving tanks at the downstream end of the flumes were darkened for the 1987 daytime tests. In 1985 the flume interiors were unpainted and the flumes were uncovered; in 1987 the interiors were painted a dark gray-brown inside and the flumes were covered with either perforated plate or opaque plastic. We conclude that these changes are responsible for the lower postpassage cortisol concentrations in 1987. The lowered cortisol response was most pronounced in the CMF, which had a highly reflective interior before painting. Additional evidence of an effect of light on cortisol is provided by the fact that postpassage cortisol concentrations were significantly lower in 1987 daytime tests with fully darkened flumes than in tests with partially darkened flumes. This finding can be attributed solely to the difference in light intensity within the flumes under the two test conditions.

The decline in plasma cortisol concentrations in chinook salmon smolts after passage through the flumes in 1987 daytime tests are thought to have been a consequence of: 1) relatively stressful conditions in the

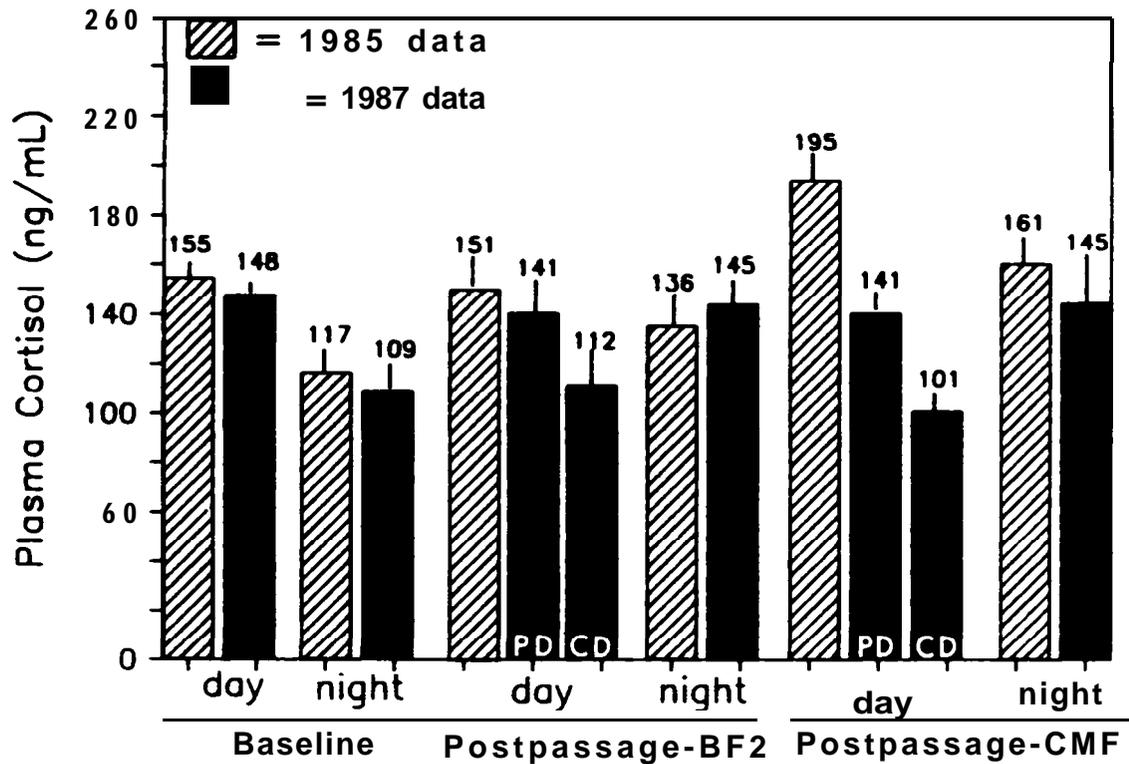


Figure 5. Comparison of mean cortisol concentrations (\pm SE) between baseline and flume postpassage samples in chinook salmon sampled in 1985 and 1987 during the daytime (uncovered in 1985; partially darkened (PD), or completely darkened (CD) in 1987) or nighttime.

holding tanks (possibly because of small volume), 2) the absence of a strong stress response to passage through darkened flumes, and 3) relatively unstressful conditions (large volume, darkness) in the fish receiving tanks.

In contrast to results with chinook salmon smolts, plasma cortisol concentrations in steelhead trout smolts were significantly increased by flume passage in both daytime and nighttime tests in 1987, with the smallest increases measured in fish that passed through the CMF and the largest in fish that passed through the BF2. These results differ from those obtained with steelhead in the 1985 tests, when smolts that passed through the CMF in daytime tests had a significantly greater increase in cortisol than fish that passed through the BF2 (data for both years are summarized in Appendix C). Factors that may have contributed to the differences between 1985 and 1987 results are discussed below.

Baseline cortisol concentrations in steelhead smolts were moderately higher (18-30 ng/mL) in 1987 than in 1985. Comparison of changes in cortisol concentrations following nighttime flume passage indicates smaller (25-46 ng/mL) increases in 1987. Daytime passage through the BF2 resulted in similar elevation of cortisol in the two years, but daytime passage through the CMF resulted in considerably smaller increases in cortisol in 1987 (122 ng/mL smaller in partially darkened flume, 93 ng/mL smaller in fully darkened flume).

The differences between 1985 and 1987 cortisol data for steelhead are minor, except for the much smaller cortisol increase in fish passing through the CMF in 1987 daytime tests. This reduced response to CMF passage indicates that darkening of the CMF and receiving tank effectively reduced the stress response of steelhead smolts to flume passage. However, complete darkening of the CMF did not lower postpassage cortisol concentrations in steelhead trout to a greater extent than did partial darkening, in contrast to findings with chinook salmon.

The reasons for a greater elevation of plasma cortisol **in steelhead** that passed through the baffled flumes in comparison with steelhead that passed through the CMF are unknown. Elevation of cortisol may have been related to the duration of flume passage, which was briefer for the CMF (0.5-2.0 min) than for the baffled flumes (2-10 min).

Descaling of Smolts in Flumes

Indices of descaling (mean percentage of body surface descaled and percentage of descaled fish) indicated no significant increase in descaling after flume passage and no significant effect of flume design. Therefore, descaling was not a problem with any of the flume designs tested.

Behavior of Adult Chinook Salmon in Flumes

The upstream migration of adult spring chinook salmon in the Snake River coincides with the downstream migration of chinook salmon and steelhead trout smolts in late April and May. Some adult salmon become disoriented after passing through fish ladders at the dams and "fall back" into the turbine intakes, eventually entering the juvenile bypass systems at Lower Granite and Little Goose Dams. These "fallback" adults are strong swimmers and could conceivably remain in a low-velocity baffled bypass system for a long time, but the adults we tested did not do so. Loss of the balloon attached to the fish made continuous observation of location impossible in one test, but adults passed quickly (<2.3 min) through the flumes in the remaining 7 tests. Inferences from these observations must be limited, because only four adult chinook salmon were tested; the responses of some adult chinook salmon to a baffled flume could differ from the responses of the few fish we tested.

Passage of Debris Through Flumes

A number of pieces of woody debris longer than 0.8-0.9 m lodged in the BF2 and debris longer than 1.4-1.8 m lodged in the BF4. A large amount of debris 1.4 m and greater in length enters the bypass system at Little Goose Dam in years when river flow is normal or above normal (Sarah Willis, COE, personal communication). Therefore, lodging of debris in a 2-foot or 4-foot wide flume at Little Goose Dam could create a maintenance problem if a debris separator were not installed at the upstream end of the flume. All debris tested passed readily through the CMF, so a debris separator could be installed at either the upstream or downstream end of this flume.

Diel Variation in Plasma Cortisol Concentration and Effects of

Light Intensity

Evidence for a reduced stress response to flume passage in darkened flumes was summarized in the preceding section. This evidence is based on comparison of changes in plasma cortisol following passage through open flumes in 1985 and darkened flumes in 1987, and on the finding that cortisol concentrations in chinook salmon after passage through completely darkened flumes were significantly lower than after passage through partially darkened flumes in 1987. Additional tests were performed in 1987 specifically to determine the effects of light on plasma cortisol concentrations in confined but otherwise undisturbed chinook salmon and steelhead trout smolts.

Plasma cortisol concentrations were significantly higher in daytime than in nighttime samples of chinook salmon smolts held for 48 hours in raceways and for 60 hours in 168-L tanks. This diel cycle was unaffected by light intensity: cortisol concentrations in fish sampled concurrently

from darkened and undarkened tanks and raceways did not differ significantly. A diel cycle in plasma cortisol and glucose concentrations was previously reported in chinook salmon smolts exiting the bypass pipe at the Lower Granite Dam fish holding facility (Congleton et al. 1984).

A diel cycle in plasma cortisol concentrations was not evident in steelhead trout held in raceways. However, cortisol concentrations were consistently and significantly higher in daytime than in nighttime baseline samples of steelhead in 1985 and again in 1987, indicating a probable diel cortisol cycle in this species also.

Diel cycles in plasma cortisol have been previously reported in adult salmonids (Pickering and Pottinger 1983, Nichols and Weisbart 1984), but not in juvenile rainbow trout (Barton et al. 1980), cutthroat trout or chinook salmon (Strange et al. 1977). Rance et al. (1982) reported a diel cortisol cycle in immature rainbow trout (mean weight 253 g) in July, but not in December under a short photoperiod. In all previously reported instances of diel cortisol cycles in salmonid fishes, peak cortisol concentrations have occurred in the nighttime rather than in the daytime. The daytime peak in plasma cortisol in chinook salmon smolts may be related to the initiation of downstream migration in the early hours of darkness, since the diel cortisol maximum in higher vertebrates typically occurs a few hours in advance of the period of peak activity (Moore-Ede et al. 1982).

Diel cycles in plasma corticosteroid concentrations have been studied more extensively than cycles in any other endocrine system in higher vertebrates (Krieger 1979). These cycles are generated by a pacemaker in the central nervous system (endogenous rhythm) rather than solely by response to some periodic external stimulus (exogenous rhythm). Endogenous cycles often have a circadian periodicity slightly longer than 24 hours, but are reset by daily exposure to an environmental stimulus such as the diel light cycle. Therefore, endogenous corticosteroid cycles are "free-running" and continue in animals transferred from a natural photoperiod to constant light or dark, but gradually lose synchrony with the natural light cycle.

In our tests a diel cortisol cycle continued in chinook salmon smolts held in "darkness"; however, true darkness could not be attained under field conditions. Daytime light intensities were 1-4 lux in fully covered raceways and <0.1 lux in tanks with opaque lids. Therefore fish held in "darkness" experienced an attenuated diel light cycle, and further tests under constant light conditions would be necessary to test the hypothesis of a free-running endogenous cortisol cycle.

An alternative explanation for the diel cortisol cycle in chinook salmon smolts is that visual perception of confinement stress might vary on a diel basis (Schreck 1981). Smolts held in 168-L tanks were moderately stressed: cortisol concentrations in fish sampled at night were 25 ng higher than in smolts sampled from raceways at night and 25-80 ng/ml higher than in smolts sampled from gatewells at Lower Granite Dam

(Congleton et al. 1983). However, although cortisol concentrations were lower in smolts confined in raceways than in smolts confined in tanks, the amplitude of the diel fluctuation was larger in smolts confined in raceways (55 vs 35 ng/lml). Continuation of an undampened cortisol cycle in raceways and under very low light intensities argues against a primary role for visual perception of confinement stress in generation of the cycle.

A third alternative explanation for the diel cortisol cycle is that visually mediated agonistic interactions might elevate plasma cortisol concentrations (Ejike and Schreck 1980, Noakes and Leatherland 1977) in the light, but not in darkness. Our data failed to show a significant effect of visual isolation from conspecifics on cortisol concentrations in chinook salmon. A role for agonistic stress can not be firmly rejected, however, because poor agreement between replicated trials reduced the power of the test: rejection of the null hypothesis may have been incorrect.

The cortisol response by smolts of both species to passage through darkened flumes was greater in nighttime tests, when prestress cortisol concentrations were lower, than in daytime tests, when prestress cortisol concentrations were higher. A greater cortisol response in juvenile chinook salmon with lower prestress plasma cortisol concentrations was also reported by Barton et al. (1986). Similarly, in rats the greatest corticosteroid response to a standardized stress occurs at the low point in the diel corticosteroid cycle (Dunn et al. 1972). This inverse relationship between prestress cortisol concentrations and stress-evoked increases could result from adjustments in any one or more of the several interacting processes that control plasma cortisol levels. Sensitivity of the pituitary to CRF stimulation or of interrenal tissue to ACTH stimulation may increase during periods of reduced activity, as may also the quantity of ACTH stored in the pituitary or of cortisol stored in interrenal tissue. Such changes in responsiveness or in quantities of hormones available for release would prime the hypothalamic-pituitary-interrenal axis for an augmented stress response.

Quantification of the stress response by measurement of changes in plasma cortisol concentrations requires that the cortisol response either be unaffected by time of day and other variables or that the effect of these variables be understood (Barton and Schreck 1987). The greater cortisol response to passage through darkened flumes does not imply that nighttime flume passage was more stressful than daytime passage, but rather that the cortisol response varied on a diel basis.

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APPENDIX A
HYDRAULIC ANALYSIS OF FLUMES

INTRODUCTION

This report describes the physical features, discharges, water depths, velocities, and other properties associated with the performance of the three test flumes used in the previously describe biological testing. These parameters were field measured on 26, 27 and 29 June, 1987.

CORRUGATED METAL FLUME (CMF)

Description

Previous field measurements (in August 1985) on this flume indicated a length from beginning *to* the end of sloped channel of 281 feet. The measured elevation difference of 9.5 feet results in an average 3.4-percent slope. The flume alignment has two 53 degree, 30-foot radius curves starting about 85 feet downstream from the head tank. The semi-circular floor and vertical walls of the flume are made of corrugated aluminum with 1/2-inch corrugations on a 2-2/3-inch spacing. The individual sections of the flume were welded together followed by grinding to achieve smooth joints. The flume diameter measured 34.75 inches. Refer to photographs in Figure A9.

Previous tests (1985) in the corrugated metal flume used dewatering screens located at the downstream end of the flume. These screens removed water from the flume before it discharged into the sample tank. During these tests (1987), the dewatering screens were eliminated and all of the flume flow passed into the sample tank. Since all flow passed through the sample tank, it was enlarged from 5-feet wide by 10-feet long (horizontal dimensions) to 10-feet wide by 15-feet long. Also, the interior of the flume was painted a dark gray-brown color and the entire length of the flume was covered with perforated (51-percent open area) aluminum plate.

Discharge

Discharge measurements were obtained by measuring flow in the 30-inch diameter supply pipe. These measurements were made with a manometer flow measurement device; namely, a Cox flow meter. Numerous measurements indicated an average discharge of 29.7 cfs, with flow varying from 28.3 to 30.7 cfs.

Measurements at the weir in the fish sample tank indicated an approximate discharge of 27.5 cfs. However, the nappe of the weir flow was not fully aerated. Therefore, weir flow calculations based on free flow weir equations are not accurate, but do verify the magnitude of the flow meter readings.

Some leakage occurred in the vicinity of the flume head tank. This leakage was minor and was not quantified.

Depth and Velocity

At several locations along the length of the corrugated metal flume, depths and point velocities were measured. At the upstream end of the flume (start of the corrugations), the water depth was 23.0 inches with an average velocity of 6.4 fps. A average normal depth of 17.0 inches was achieved at approximately 20 feet from the transition. The corresponding average velocity for this depth was 9.3 fps.

At the beginning of the shape transition, depth decreased and velocity increased. The decreasing depth and increasing velocity ended with an undular (very weak) hydraulic jump, created by the larger depth of water in the fish sample tank. The upstream Froude number for the jump, F_1 , was approximately 1.25. Specifically, the water depth decreased from 16.25 to 11.9 inches immediately upstream from the jump and the velocity increased to 15.2 fps. The length of the hydraulic jump was approximately 15 feet and ended with an average water depth in the flume of 32.5 inches flowing into the fish sample tank at an average velocity of 3.8 fps.

Figure A1 illustrates the flow profile and velocities at the flume centerline in the lower shape transition of the corrugated metal flume. Figures A2, A3, and A4 show point velocity measurements at selected cross-sections in the flume upstream from the first curve, midway through the first curve, and downstream from the second curve, respectively.

Curve Effects

The 53-degree, 30-foot radius curves of the corrugated metal flume created a water depth difference of about four inches between the water surfaces at the inside and outside of the curve. Refer to Figure A3.

TWO-FOOT WIDE BAFFLED FLUME (BF2)

Description

By previous field measurements in August, 1985, the length of this flume was measured as 113.0 feet from the beginning to the end of the slope. The measured elevation difference of 4.0 feet results in an average 3.5-percent slope. The flume was constructed of plywood (and later lined with fiberglass to reduce leakage during the 1987 tests) with 24-inch by 24-inch cross sectional area. Baffles of fiberglass were bolted onto the flume walls on alternating 2-foot centers. The baffles protrude into the flow area about 8 inches leaving a flow area width of 16 inches at the baffle tip. A 53-degree, 24-foot radius curve occurs about 30 feet downstream from the flume head tank. For the 1987

tests, this flume was also modified by tinting the fiberglass liner the gray-brown color and covering the flume with perforated (51-percent open area) aluminum plate. The flume was operated so that all flow passed through the sample tank. Refer to photographs in Figure A10.

Discharge

The operating discharge for this flume was 4.8 cfs as measured using the Cox flow meter located in the 30-inch diameter supply pipe. Flow varied from 4.6 to 5.1 cfs during measurement of the discharge. Leakage from the flume was minimal at the time of measurement.

Depth and Velocity

The baffled flume maintained a relatively constant water depth of 19.5 inches. The depth was slightly higher at the downstream end of the flume slope due to tailwater effects from the fish sample tank.

Velocity measurements were taken at several locations along the length of this flume. Figures A5 and A6 represent typical cross-sections through the flume with Figure A5 located at the baffle tip and Figure A6 equidistant between baffles. The maximum measured velocities were just downstream from the baffle tip in the center of the channel and were approximately 4 to 4.5 fps. Minimum velocities were directly downstream from each baffle and were near 0 fps. The head drop from the upstream to downstream side of each baffle was approximately 3 inches.

Curve Effects

No specific effects to the flow were attributable to the 53 degree, 24-foot radius curve.

FOUR-FOOT WIDE BAFFLED FLUME (BF4)

Description

This flume replaced the 8.3-percent slope BF2 flume used in the 1985 tests. Field measurements of this flume indicated a length of 119.6 feet from the beginning to the end of the slope. The measured elevation difference of 4.1 feet results in an average 3.4-percent slope. The flume was constructed of concrete with 48-inch by 48-inch cross-sectional area. Baffles of fiberglass were bolted onto the flume walls on alternating 4-foot centers. The baffles protrude into the flow area about 16 inches leaving a flow area width of 32 inches at the baffle tip. This flume does not have a curved alignment. Perforated (51-percent open area) aluminum plate also covered the top of this flume. Refer to photographs in Figure All.

Discharge

Discharge measurements were obtained by measuring flow in the 30-inch diameter supply pipe. Numerous Cox flow meter measurements indicated an average discharge of 24.8 cfs, with flow varying from 24.3 to 25.1 cfs.

Measurements at the weir in the fish sample tank indicated an approximate discharge of 20.6 cfs. However, the nappe of the weir flow was not fully aerated. Therefore, weir flow calculations based on free flow weir equations are not accurate, but do verify the magnitude of the flow meter readings.

Leakage from the flume was minimal at the time of measurement.

Depth and Velocity

The baffled flume maintained a relatively constant water depth of 29.0 inches. However, actual water depth varied slightly at each baffle.

Velocity measurements were taken at several locations along the length of this flume. Figures A7 and A8 represent typical cross-sections through the flume with Figure A7 located at the baffle tip and Figure A8 equidistant between baffles. The maximum measured velocities were downstream from the baffle tip in the center of the channel and were approximately 4.5 to 5.5 fps. Minimum velocities were directly downstream from each baffle and were near 0 fps. The head drop from the upstream to downstream side of each baffle was approximately 5.5 to 6 inches.

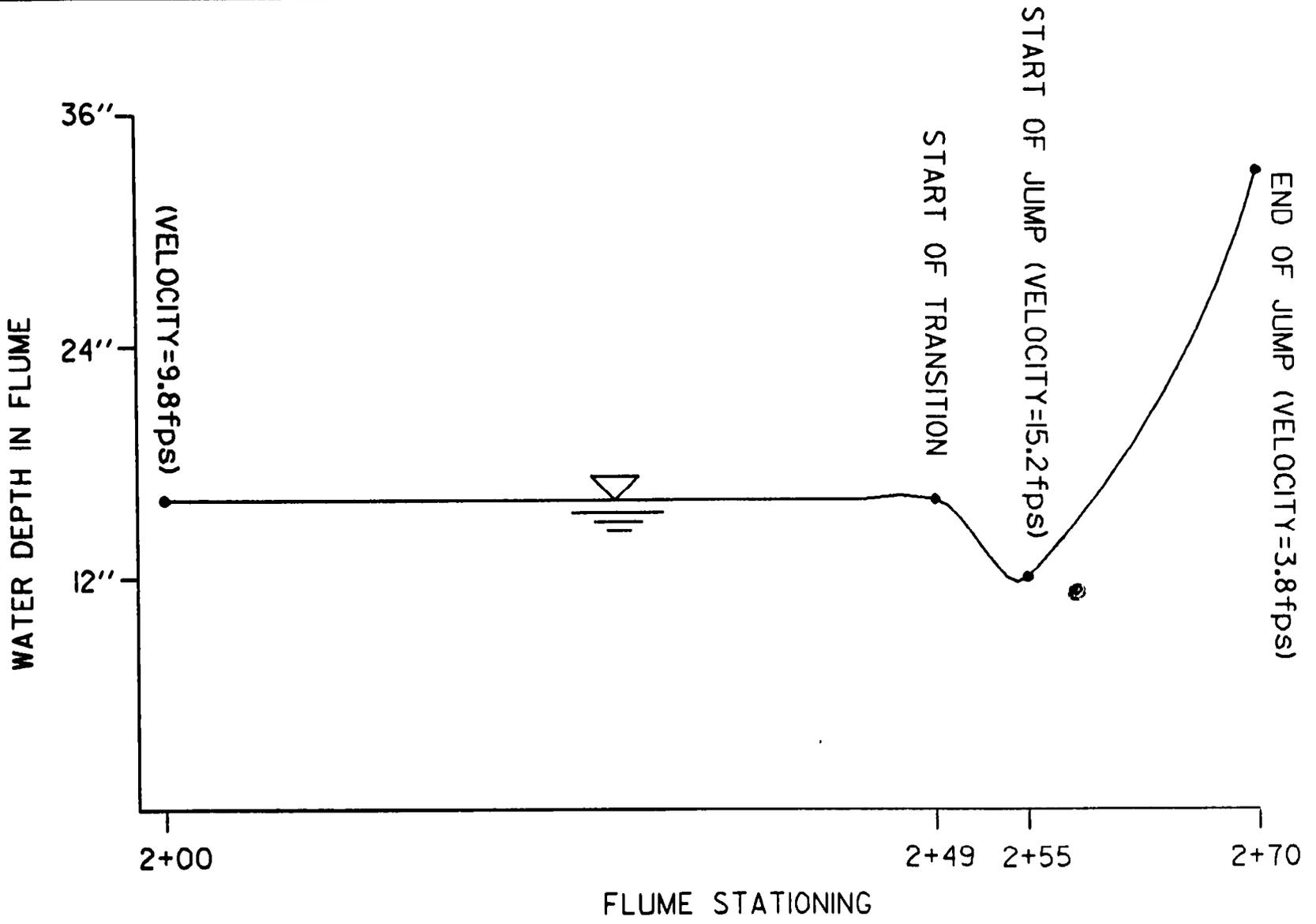
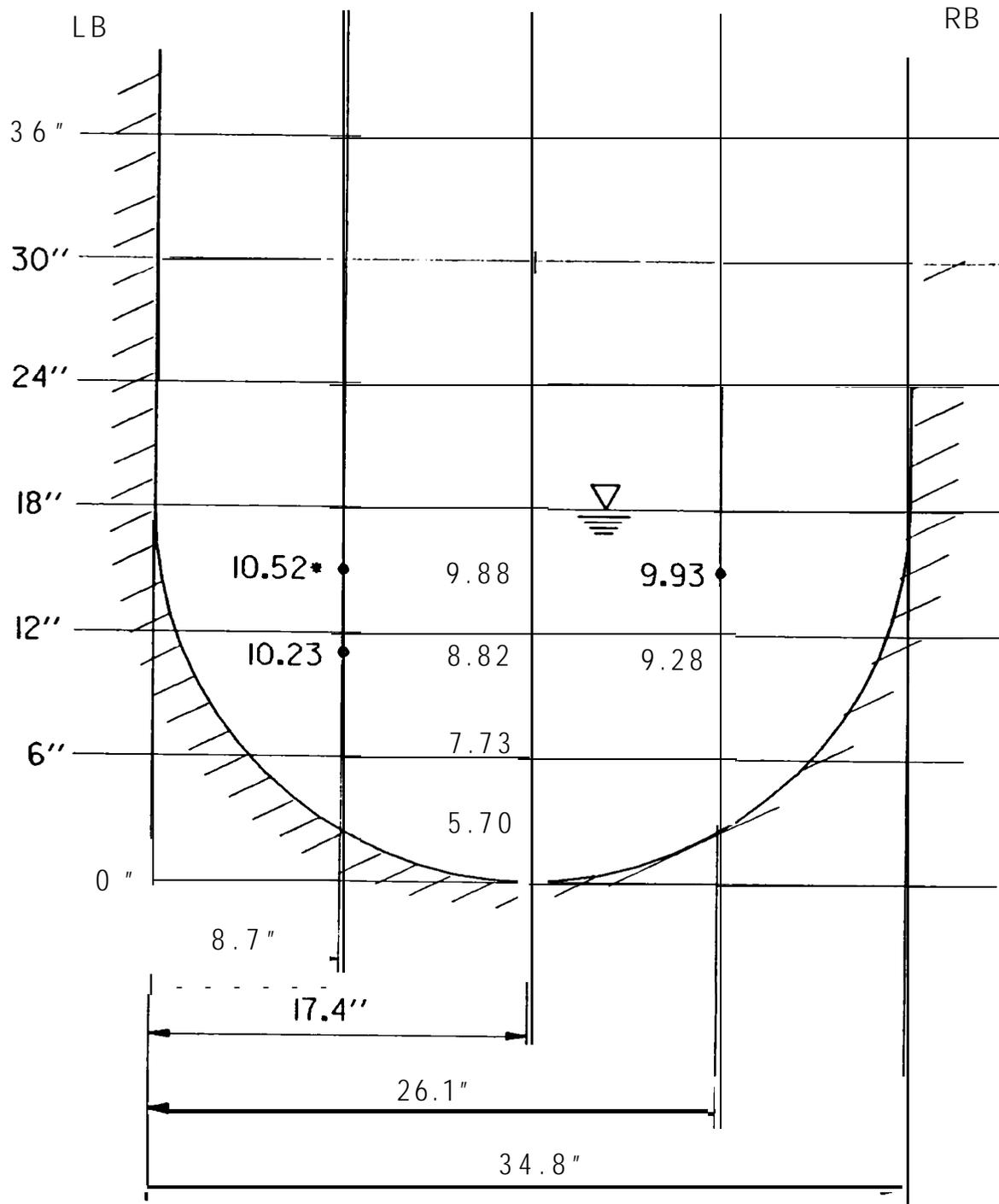
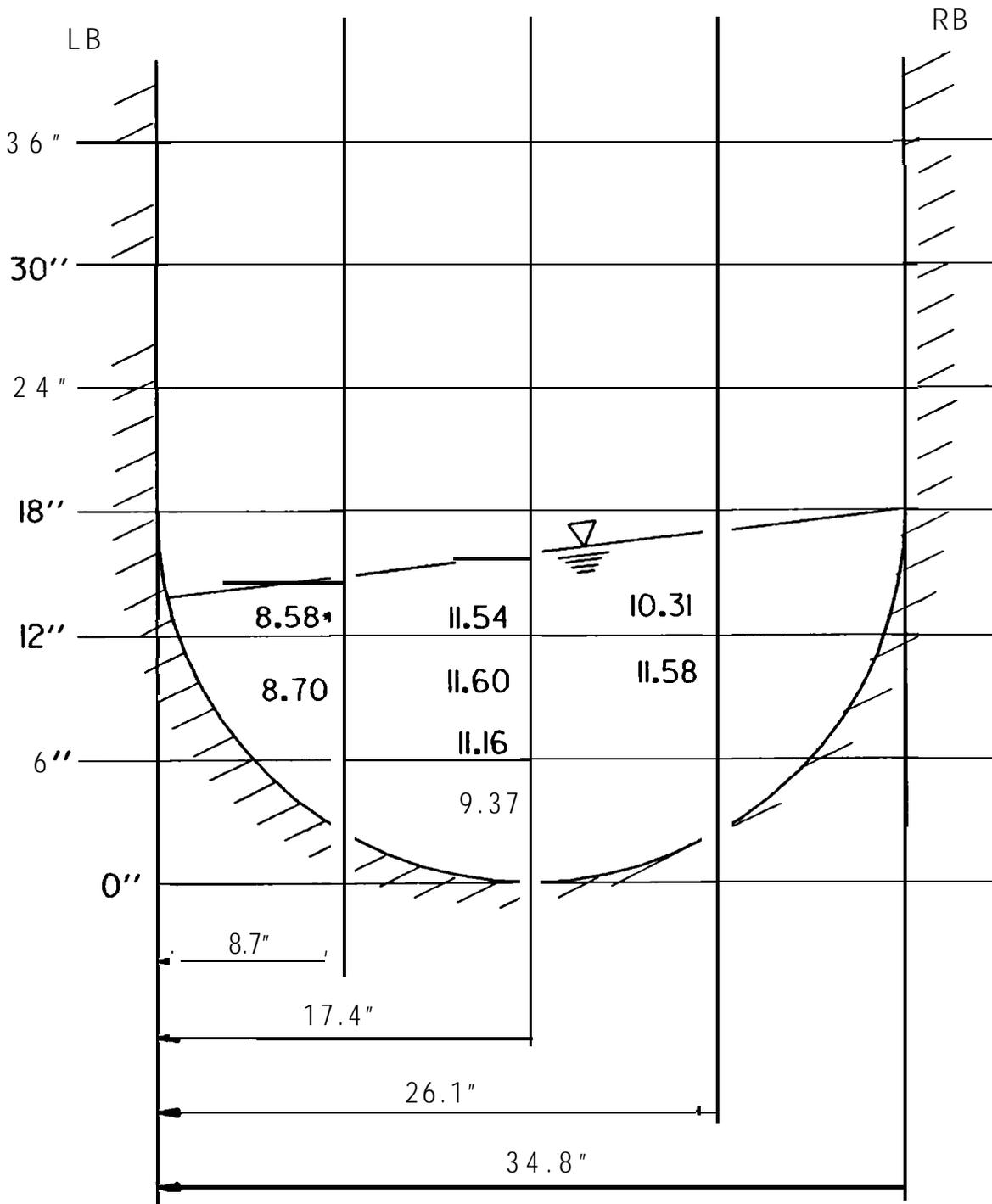


FIGURE A1
 CORRUGATED METAL FLUME
 WATER SURFACE PROFILE



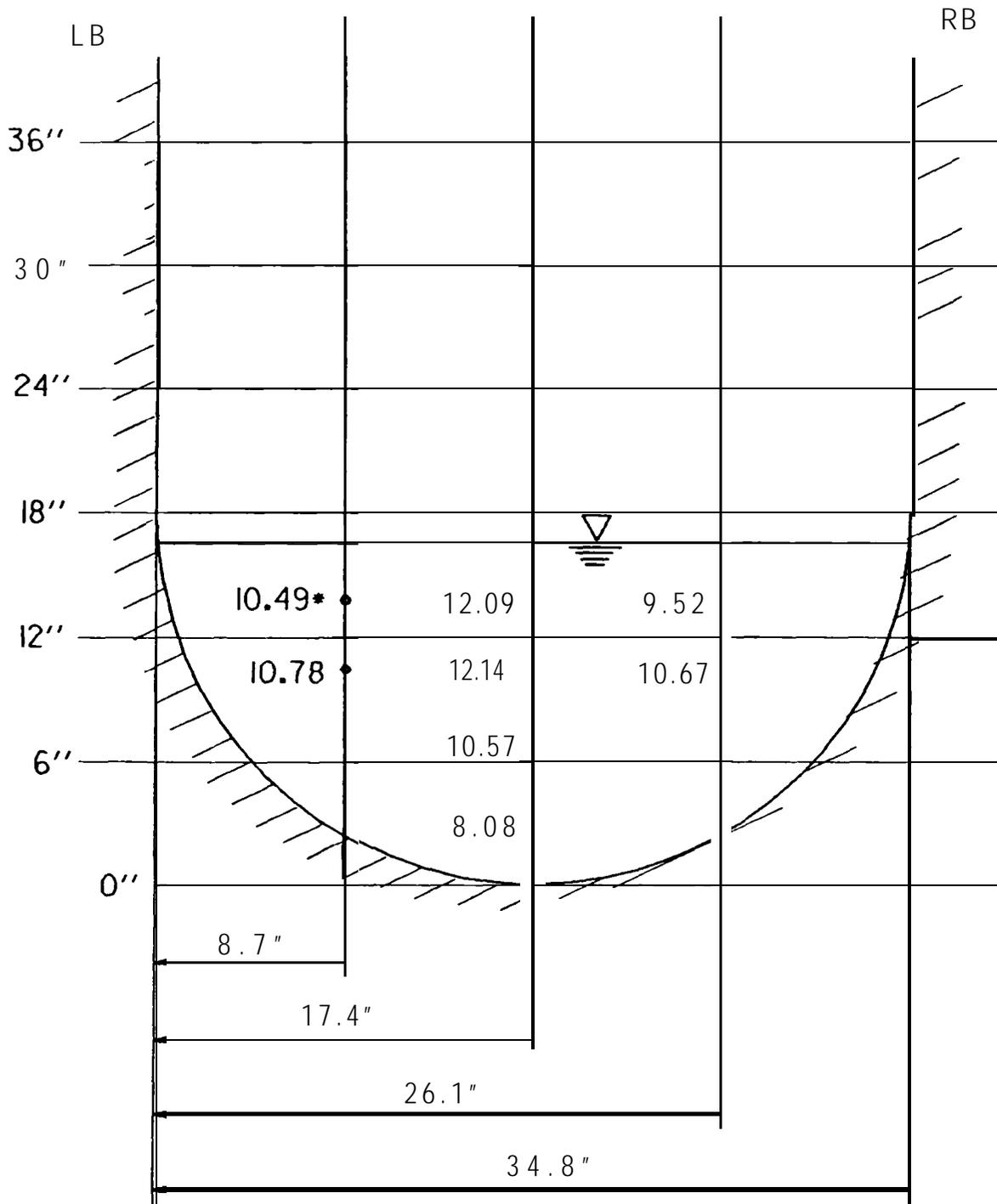
* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A2
 CORRUGATED METAL FLUME
 (3.4 % SLOPE)
 STA 0 + 20.0



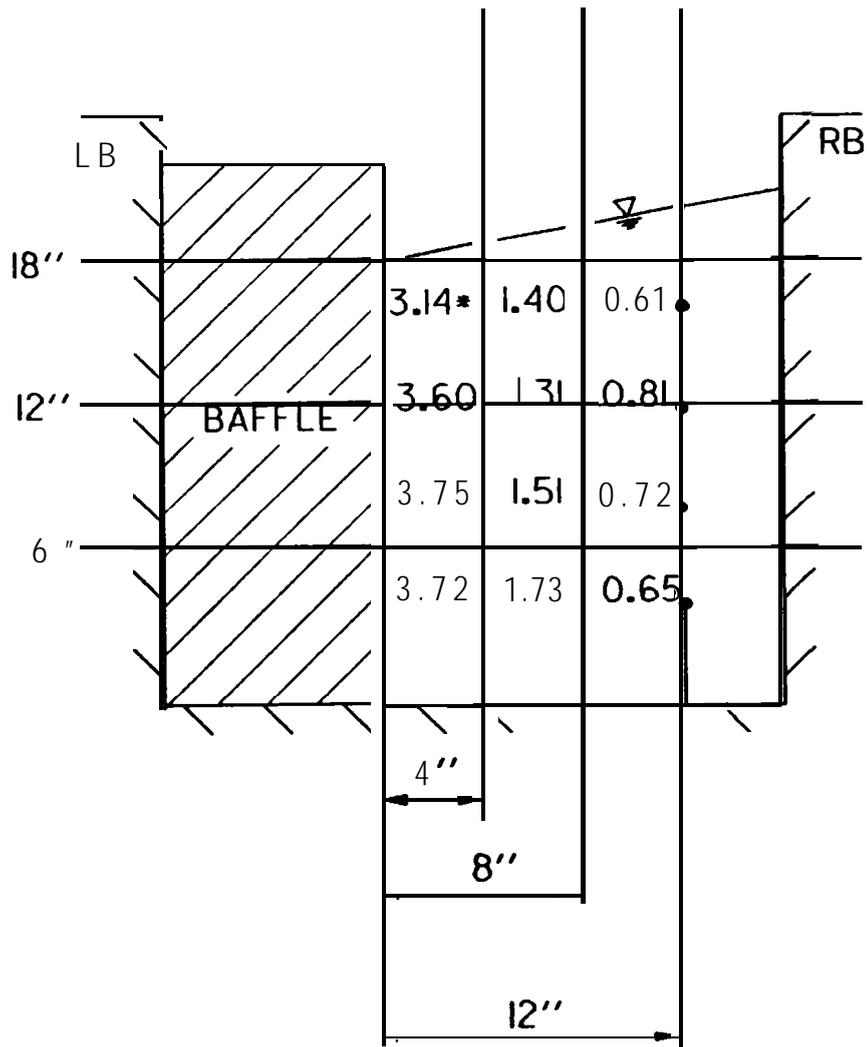
* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A3
 CORRUGATED METAL FLUME
 (3.4 % SLOPE)
 STA 0 + 94.0
 (MIDWAY THROUGH FIRST CURVE)



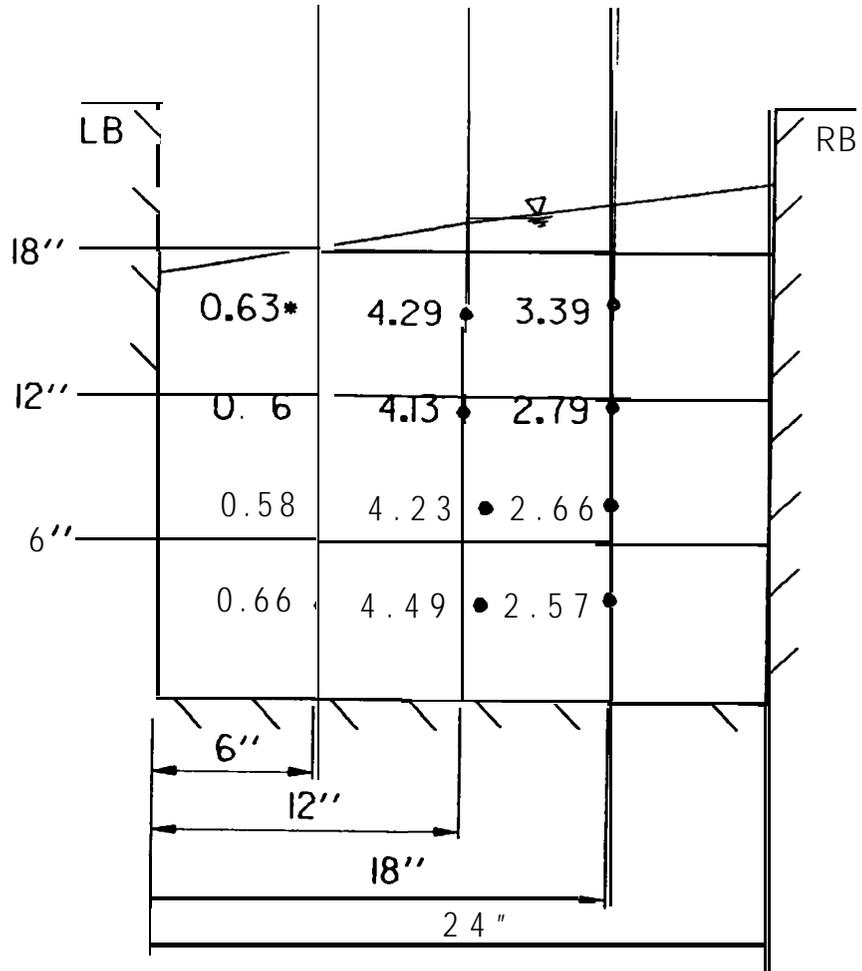
* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A4
 CORRUGATED METAL FLUME
 (3.4 % SLOPE)
 STA 2 + 00.0



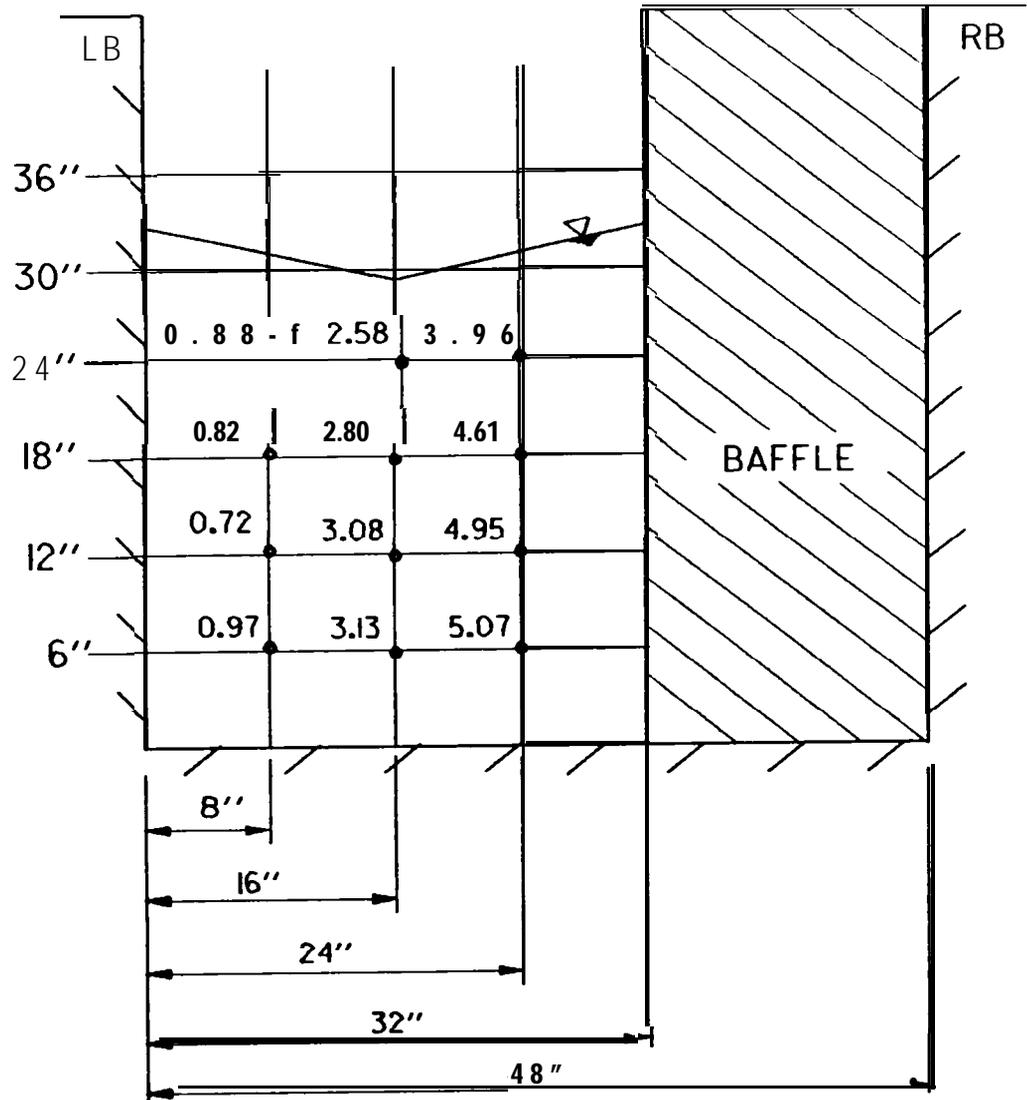
- POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A5
 2' WIDE BAFFLED FLUME
 (3.4 % SLOPE)
 STA. 0 + 70.0



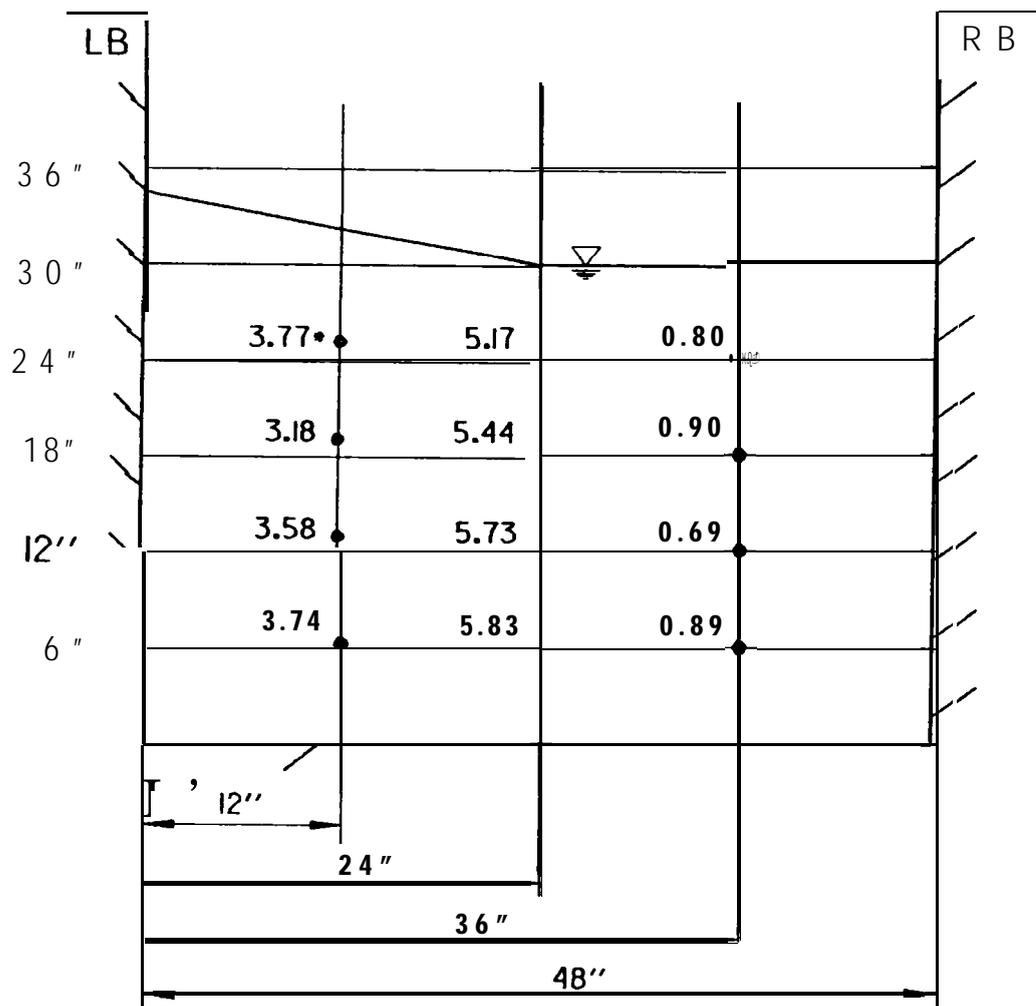
* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A6
 2' WIDE BAFFLED FLUME
 (3.4 % SLOPE)
 STA. 0 + 71.0



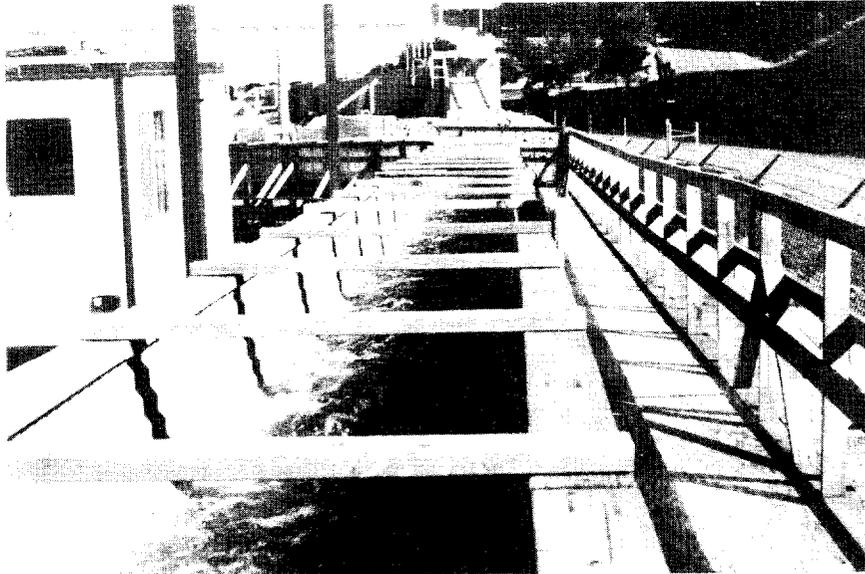
* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A7
 4' WIDE BAFFLED FLUME
 (3.4 % SLOPE)
 STA. 0 + 44.6



* POINTS WITHIN FLOW SECTION REPRESENT VELOCITY IN FEET PER SECOND.

FIGURE A8
4' WIDE BAFFLED FLUME
(3.4 % SLOPE)
STA. 0 + 47.0



LOOKING DOWNSTREAM

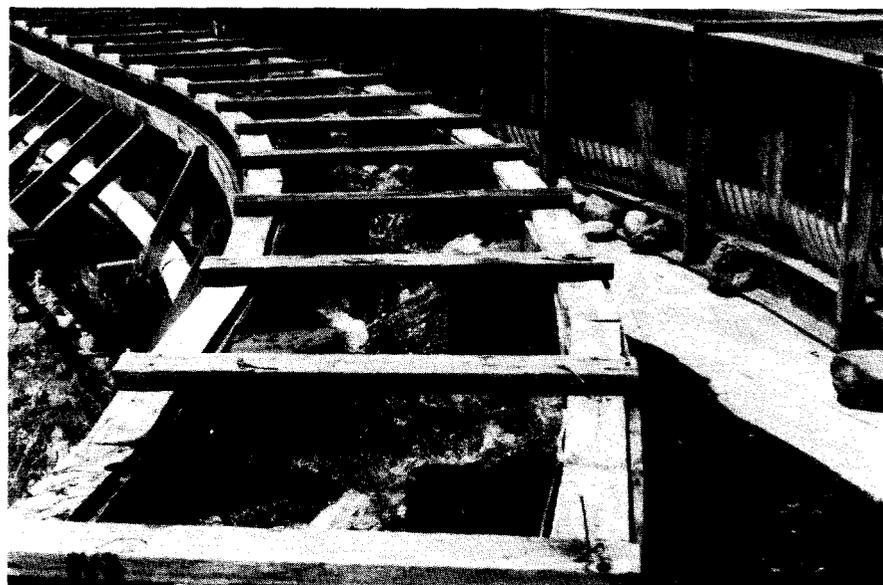


HYDRAULIC JUMP AT SHAPE TRANSITION

FIGURE A9
CORRUGATED METAL FLUME

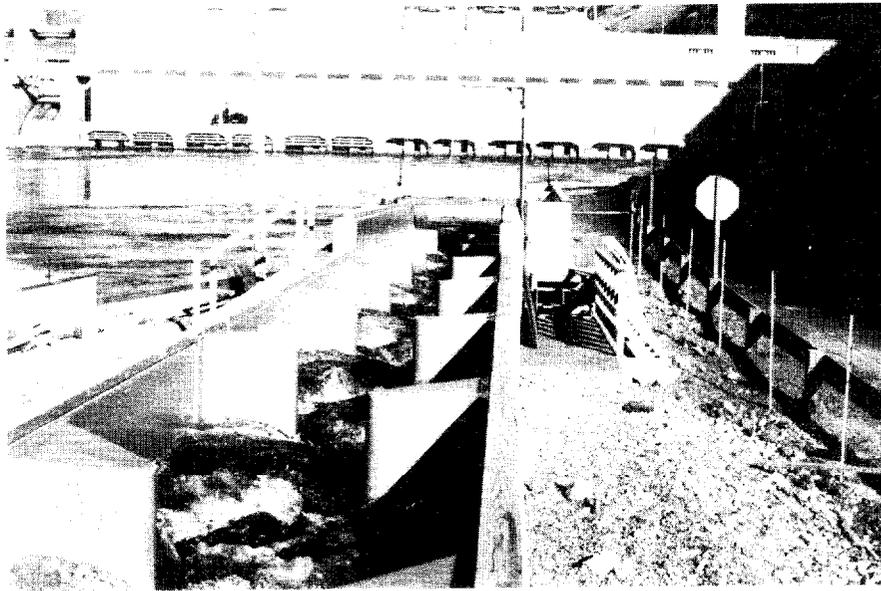


LOOKING UPSTREAM

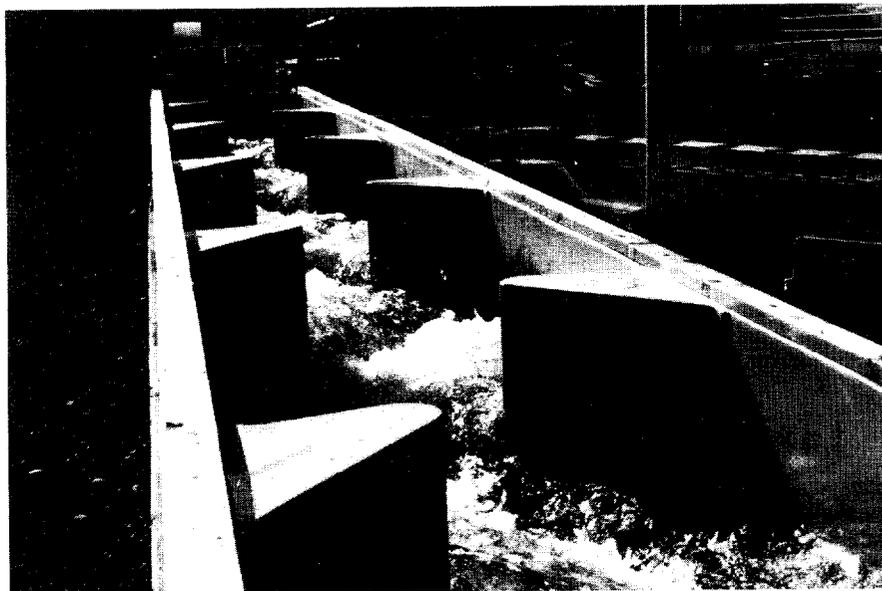


LOOKING UPSTREAM

FIGURE A10
2' WIDE BAFFLED FLUME



LOOKING DOWNSTREAM



LOOKING UPSTREAM

FIGURE AII
4' WIDE BAFFLED FLUME

APPENDIX B

Analysis of Variance Tables for Selected Statistical Tests

1. Comparison of cortisol concentrations in all baseline samples of chinook salmon.

Source	DF	SS	MS	F	P
Light (A)	2	2.13E+04	1.067E+04	13.22	0.0001
Flume(B)	2	1090.1	545.06	.68	0.5184
Rep (C)	6	3.801E+04	6335.9	7.85	0.0001
A*B	4	1.147E+04	2866.5	3.55	0.0206
A*C	12	1.211E+04	1009.6	1.25	0.3077
B*C	12	1.548E+04	1289.8	1.60	0.1585
A*B*C	24	1.937E+04	807.1		
TOTAL	62	1.189E+05			

2. Comparison of cortisol concentrations in chinook salmon between flumes and between light conditions using values calculated by subtracting an average night or day baseline value from each postpassage sample.

Source	DF	SS	MS	F	P
Light (A)	2	4.307E+04	2.154E+04	18.79	0.0000
Flume(B)	2	139.16	69.58	0.06	0.9412
Rep (C)	6	4.808E+04	8013.7	6.99	0.0002
A*B	4	6139.6	1534.9	1.34	0.2843
A*C	12	1.039E+04	865.8	0.76	0.6871
B*C	12	2.153E+04	1794.3	1.57	0.1690
A*B*C	24	2.751E+04	1146.2		
TOTAL	62	1.569E+05			

3. Comparison of cortisol concentrations in chinook salmon passing through darkened or partially darkened flumes during the day.

Source	DF	SS	MS	F	P
Dark vs Part.					
Dark	1	4549.2	4549.2	6.07	0.0299
Flume(B)	2	295.2	147.6	0.20	0.8239
Rep (C)	6	3.679E+04	6132.1	8.18	0.0011
A*B	2	4240.6	2120.3	2.83	0.0986
A*C	6	2501.9	417.0	0.56	0.7571
B*C	12	5705.4	475.4	0.63	0.7793
A*B*C	12	8998.2	749.8		
TOTAL	62	6.308E+04			

4. Comparison of baseline and postpassage cortisol concentrations in chinook salmon.

Source	DF	ss	MS	F	P
Light (A)	2	6820.5	3410.2	3.33	0.0538
Flume(B)	2	286.7	143.4	0.14	0.8702
BL vs PP (C)	1	1694.5	1694.5	1.65	0.2113
Rep (D)	6	8.095E+04	1.349E+04	13.16	0.0000
A*B	4	4921.6	1230.4	1.20	0.3374
A*C	2	2.190E+04	1.095E+04	10.68	0.0005
A*D	12	9916.8	826.4	0.81	0.6418
B*C	2	895.0	447.5	0.44	0.6515
B*D	12	2.081E+04	1734.3	1.69	0.1345
C*D	6	4456.9	742.8	0.72	0.6342
A*B*C	4	1.233E+04	3082.6	3.01	0.0392
A*B*D	24	2.189E+04	911.9	0.89	0.6115
A*C*D	12	1.192E+04	993.0	0.97	0.5035
B*C*D	12	1.495E+04	1245.7	1.22	0.3306
A*B*C*D	23	2.357E+04	1025.0		
TOTAL	124	2.373E+05			

5. Comparison of cortisol concentrations in chinook salmon postpassage samples (no baseline value subtracted).

Source	DF	ss	MS	F	P
Light (A)	2	49584.0	4792.0	4.93	0.0166
Flume(B)	2	184.3	92.2	0.09	0.9099
Rep (C)	6	4.454E+04	7423.5	7.63	0.0001
A*B	4	4491.9	1123.0	1.15	0.3563
A*C	12	8060.7	671.7	0.69	0.7439
B*C	12	1.532E+04	1276.9	1.31	0.2767
A*B*C	24	2.237E+04	972.5		
TOTAL	62	1.045E+05			

6. Comparison of baseline cortisol concentrations in steelhead smolts.

Source	DF	ss	MS	F	P
Light (A)	2	3.107E+04	1.553E+04	8.92	0.0016
Flume(B)	2	7366.8	3683.4	2.11	0.1456
Rep (C)	6	4.770E+04	7949.5	4.56	0.0041
A*B	4	1.220E+04	3049.2	1.75	0.1767
A*C	12	3.130E+04	2608.1	1.50	0.2018
B*C	12	1.770E+04	1475.2	0.85	0.6065
A*B*C	21	3.658E+04	1741.8		
TOTAL	59	1.839E+05			

7. Comparison of baseline and postpassage cortisol concentrations in steelhead smolts.

Source	DF	ss	MS	F	P
BL vs PP(A)	1	8.402E+04	8.402E+04	140.25	0.0000
Flume(B)	2	1229.4	614.7	1.03	0.3878
Rep (C)	6	7.928E+04	1.321E+04	22.05	0.0000
A*B	2	8429.6	4214.8	7.04	0.0095
A*C	6	1.857E+04	3094.8	5.17	0.0077
B*C	12	1.279E+04	1066.0	1.78	0.1658
A*B*C	12	7189.3	599.1		
TOTAL	41	2.115E+05			

8. Comparison of steelhead cortisol concentrations between flumes and light conditions using values derived by subtracting an average daytime or nighttime baseline value from each postpassage sample.

Source	DF	ss	MS	F	P
Light (A)	2	8030.1	4015.0	4.74	0.0184
Flume(B)	2	3.048E+04	1.524E+04	18.01	0.0000
Rep (C)	6	1.105E+04	1.842E+04	21.77	0.0000
A*B	4	7811.5	1952.9	2.31	0.0873
A*C	12	3.375E+04	2812.4	3.32	0.0059
B*C	12	1.6975E+04	1414.5	1.67	0.1373
A*B*C	2	42.031E+04	846.4		
TOTAL	62	2.279E+05			

9. Comparison of cortisol concentrations in steelhead postpassage samples (no baseline value subtracted).

Source	DF	SS	MS	F	P
Light (A)	2	3778.7	1889.4	2.23	0.1291
Flume(B)	2	3.048E+04	1.524E+04	18.01	0.0000
Rep (C)	6	1.105E+05	1.842E+04	21.77	0.0000
A*B	4	7811.5	1952.9	2.31	0.0873
A*C	12	3.375E+04	2812.4	3.32	0.0059
B*C	12	1.697E+04	1414.5	1.67	0.1373
A*B*C	2	42.031E+04	846.4		
TOTAL	62	2.236E+05			

10. Comparison of the number of chinook remaining in each of the three flumes 20 min after release.

Source	DF	SS	MS	F	P
Light (A)	2	1.149e+04	5747.3	1.82	0.1843
Flume(B)	2	4.971E+04	2.485E+04	7.85	0.0024
Rep (C)	6	2.628E+05	4380.5	1.38	0.2612
A*B	4	1.155e+04	2887.5	0.91	0.4727
A*C	12	3.142E+04	2618.0	0.83	0.6232
B*C	12	3.254E+04	2711.4	0.86	0.5972
A*B*C	24	7.596E+04	3164.9		
TOTAL	62	2.389E+05			

11. Comparison of cortisol concentrations in chinook salmon held in darkened and partially darkened tanks and sampled during each of 3 days and nights.

Source	DF	SS	MS	F	P
Night vs Day(A)	1	3657.3	3657.3	29.56	0.0122
Dark vs Partially					
Dark (B)	1	173.0	173.0	1.40	0.3222
Rep (C)	1	912.1	456.1	3.69	0.1556
Day (D)	2	5782.1	2891.1	23.37	0.0148
A*B	2	155.7	155.7	1.26	0.3436
A*C	1	1376.5	688.2	5.56	0.0979
A*D	2	3868.0	1934.0	15.63	0.0259
B*C	2	1811.8	905.9	7.32	0.0701
B*D	2	1095.5	547.7	4.43	0.1273
C*D	4	5685.0	1421.2	11.49	0.0365
A*B*C	2	747.3	373.6	3.02	0.1912
A*B*D	2	2330.0	1165.0	9.42	0.0509
A*C*D	4	1.002E+04	2506.4	20.26	0.0165
B*C*D	4	4430.3	1107.6	8.95	0.0512
A*B*C*D	3	371.2	123.7		
TOTAL	34	4.242E+04			

12. Comparison of cortisol concentrations in chinook salmon held in darkened and partially darkened raceways, sampled during the day or night.

Source	DF	ss	MS	F	P
Day vs Night(A)	1	1.493E+04	1.493E+04	27.44	0.0135
Dark vs Partially					
Dark (B)	1	548.6	548.6	1.01	0.3892
Day (C)	3	4600.4	1533.5	2.82	0.2087
Rep (D)	1	4776.6	4776.6	8.78	0.0594
A*B	1	26.3	26.3	0.05	0.8400
A*C	3	1788.2	596.1	1.10	0.4709
A*D	1	138.1	138.1	0.25	0.6491
B*C	3	5735.1	1911.7	3.51	0.1647
B*D	1	146.0	146.0	0.27	0.6402
C*D	3	1085.4	361.8	0.67	0.6272
A*B*C	3	6471.4	2157.1	3.97	0.1438
A*B*D	1	145.7	145.7	0.27	0.6405
A*C*D	3	2193.5	731.2	1.34	0.4069
B*C*D	3	477.2	159.1	0.29	0.8302
A*B*C*D	3	1631.9	544.0		
TOTAL	31	4.469E+04			

13. Comparison of cortisol concentrations in steelhead smolts held in tanks receiving four different light intensities.

Source	DF	ss	MS	F	P
Light (A)	3	9812.8	3270.9	2.90	0.1240
Rep (B)	1	9.0	9.0	0.01	0.9318
Subrep (C)	2	7612.4	3806.2	3.37	0.1045
A*B	3	2392.8	797.6	0.71	0.5824
A*C	6	1.720E+04	2866.6	2.54	0.1409
B*C	2	350.9	175.5	0.16	0.8595
A*B*C	6	6777.3	1129.5		
TOTAL	23	4.415E+04			

14. Comparison of cortisol concentrations in chinook smolts held individually in compartments within darkened and partially darkened tanks and sampled during the day or night.

Source	DF	ss	MS	F	P
Light vs Dark(A)	1	102.76	102.76	0.15	0.7119
Rep (B)	3	3.201E+04	1.067E+04	15.57	0.0031
Night vs Day (C)	1	5.167E+04	5.167E+04	75.42	0.0001
Tank (D)	2	1587.3	793.7	1.16	0.3755
A*B	3	1.028E+04	3426.8	5.00	0.0452
A*C	1	2.488E+04	2.488E+04	36.31	0.0009
A*D	2	7148.8	3574.4	5.22	0.0487
B*C	3	6.672E+04	2.224E+04	32.46	0.0004
B*D	6	1.137E+04	1894.9	2.77	0.1206
C*D	2	3650.0	1825.0	2.66	0.1486
A*B*C	3	1.171E+04	3902.7	5.70	0.0344
A*B*D	6	9066.5	1511.1	2.21	0.1793
A*C*D	2	1734.7	867.4	1.27	0.3478
B*C*D	6	1.652E+04	2753.1	4.02	0.0573
A*B*C*D	6	4111.0	685.2		
TOTAL	47	2.526E+05			

APPENDIX C

Summary of Plasma Cortisol Concentration Data for 1985 and 1987 Flume Tests

Table 1. Comparison of mean baseline and postpassage plasma cortisol concentrations (ng/mL) in spring chinook salmon smolts used in 1985 and 1987² flume tests.

	Baseline		Postpassage					
			BF2 ³ .			CMF ³ .		
	1985	1987	1985	1987p ⁴ .	1987c ⁴ .	1985	1985p	1985c
Daytime	155	148	151	141	112	195	141	101
Nighttime	111	109	136		145	161		145

Table 2. Comparison of baseline and postpassage plasma cortisol concentrations (ng/mL) in steelhead trout smolts used in 1985¹ and 1987² flume tests.

	Baseline		Postpassage					
			BF2 ³ .			CMF ³ .		
	1985	1987	1985	1987p ⁴ .	1987c ⁴ .	1985	1987p	1987c
Daytime	194	212	274	297	316	336	232	261
Nighttime	144	174	284		289	264		248

Table 3. Comparison of net changes in plasma cortisol concentrations (postpassage - baseline in ng/mL) in chinook salmon and steelhead trout smolts used in **1985**¹ and **1987**² flume tests.

	BF2 ³			CMF ³		
	1985	1987p ⁴	1987c ⁴	1985	1987p	1987c
Chinook Salmon						
Daytime	-4	-7	-36	40	-7	-47
Nighttime	25	36		50	36	
Steelhead Trout						
Daytime	80	85	115	142	20	49
Nighttime	140		104	120	74	

1. Reported in Congleton and Ringe (1985); 4-5 tests completed.
2. Present study; 7 tests completed.
3. **BF2** = baffled flume, 2 feet in width; **CMF** = corrugated metal flume.
4. 1987p = partially darkened flume (perforated metal cover); 1987c = completely darkened flume (opaque cover).