PLASMA CORTISOL LEVELS AND BEHAVIORAL STRESS RESPONSES OF JUVENILE CHINOOK SALMON PASSED THROUGH ARCHIMEDES LIFTS AND AN INTERNAL HELICAL PUMP AT RED BLUFF RESEARCH PUMPING PLANT, SACRAMENTO RIVER, CALIFORNIA

Red Bluff Research Pumping Plant
Report Series: Volume 8

Prepared by:
Edward D. Weber and Sandra M. Borthwick
U.S. Bureau of Reclamation
Red Bluff Fish Passage Program
P.O. Box 159
Red Bluff, CA 96080

July 2000

U.S. Bureau of Reclamation
Mid-Pacific Region
Red Bluff Fish Passage Program
P.O. Box 159
Red Bluff, CA 96080

Disclaimer

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal Government.

The correct citation for this report is:

Weber, E. D., and S. M. Borthwick. 2000. Plasma cortisol levels and behavioral stress responses of juvenile chinook salmon passed through Archimedes lifts and an internal helical pump at Red Bluff Research Pumping Plant, Sacramento River, California. Red Bluff Research Pumping Plant Report Series Volume 8. U.S. Bureau of Reclamation, Red Bluff, CA.

PLASMA CORTISOL LEVELS AND BEHAVIORAL STRESS RESPONSES OF JUVENILE CHINOOK SALMON PASSED THROUGH ARCHIMEDES LIFTS AND AN INTERNAL HELICAL PUMP AT RED BLUFF RESEARCH PUMPING PLANT, SACRAMENTO RIVER, CALIFORNIA

U.S. Bureau of Reclamation
Mid-Pacific Region
Red Bluff Fish Passage Program
P.O. Box 159
Red Bluff, CA 96080

Abstract– We examined plasma cortisol levels and behavioral stress-responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) passed through the Archimedes lifts and internal helical pump at Red Bluff Research Pumping Plant (RBRPP). If juvenile chinook salmon and other fish are significantly stressed by passage through the pumps at RBRPP, latent mortality may occur. In 1998, plasma cortisol was measured for Archimedes-passed salmon at 0, 1, 3, 6, 12, and 24 h after passage. Concentrations did not differ significantly between treatment and control groups indicating no detectable pump effect. In both groups, plasma cortisol concentrations peaked near 200 ng/ml after 1 h and returned to near-baseline after 12 h. In 1999, the Archimedes lifts and internal helical pump were used to examine salmon cortisol levels at 0, 1, 1.5, 3, 6, and 12 h after passage. Because the interaction between treatments and times was significant for both pump types in 1999, we compared treatments at each time. Relatively small pump effects, less than 50 ng/ml net cortisol increase, were observed for the Archimedes lifts 1.5 and 12 h after passage and for the internal helical pump 3 h after passage. A handling-control comparison demonstrated that much of the observed stress response was due to capture, confinement, and transport of fish prior to insertion into pumps. Four behavioral metrics including swimming activity, use of cover, schooling, and vertical position were directly observed following Archimedes lift passage in 1998. Treatment fish did not differ behaviorally from control or reference fish. In general, these results indicate stress resulting from passage through pumps at RBRPP was unlikely to cause primary mortality and probably did not appreciably increase the chance of secondary mortality.

Table of Contents

List of Tables

Table **Page**

List of Figures

Figure **Page**

List of Appendices

Appendix **Page**

Introduction

A major goal of the Red Bluff Research Pumping Plant (RBRPP) biological assessment is to determine if Archimedes and internal helical pumps can be operated with minimal harm to young chinook salmon migrating downstream (Liston and Johnson 1992). If fish are appreciably stressed by pump passage at RBRPP indirect mortality may occur when they return to the river. Other portions of the evaluation have focused on the immediate survival of experimentally introduced hatchery fish (McNabb et. al. 1998, 2000) and entrained wild fish (Borthwick et al. 1999; Objectives B, C, D, E, Liston and Johnson 1992). Although these studies have also observed extended survival by holding fish for 96 h in the RBRPP river water holding facility, the potential exists for secondary mortality that would not be observed in the protected conditions of the laboratory.

One potential source of indirect mortality following pump passage is predation. Varying levels of acute stress have been demonstrated to reduce predator avoidance ability in salmon for up to 4 h after the event (Mesa 1994, Olla et al. 1995). The potential for increased susceptibility to predation after exiting the Archimedes lifts or internal helical pump was addressed in Objective G of the RBRPP evaluation plan (Liston and Johnson 1992). Objective G proposed a study to "assess predator-prey interactions between pump-passed juvenile chinook salmon and Sacramento squawfish (now pikeminnow)." However, the difficulty of collecting reliable experimental data while also recreating realistic in-river conditions prompted the RBRPP interagency fisheries workgroup to modify this study during its December 1997 annual meeting. The workgroup agreed to substitute blood plasma cortisol, a more general stress-response measure, for the predator interaction study. Although plasma cortisol measurements do not provide a direct measure of secondary mortality, they provide a measure of the severity and duration of an elevated physiological state or stress-response. These data allow for an indirect assessment of the risk of latent mortality, not only through predation, but also through other factors associated with physiological stress.

Stress in fish results when environmental factors extend physiological processes beyond the normal range (Morgan and Iwama 1993). Stress may result in direct mortality when physiological tolerance limits are exceeded (Maule et al. 1988, Olla et al. 1995) or indirect mortality, as fish become more vulnerable to disease (Wedemeyer et al. 1976, Maule et al. 1989, Barton and Iwama 1991) and predation (Olla et al. 1992, 1995, Mesa 1994). Stress may also reduce growth rates and reproductive success (Vaughn et al. 1984, Adams et al. 1985).

Elevated blood plasma cortisol concentration is a primary, "fight or flight" response to stress in fish. Cortisol is secreted from the interrenal tissue, located in the anterior portion of the kidney (Donaldson 1981). A primary function of cortisol is to make glucose available for active processes (Leach and Taylor 1982). In general, the magnitude of the plasma-cortisol response reflects the magnitude and duration of the stressor (Barton and Iwama 1991). Cortisol measurements have been used as indicators

of stress due to handling (Strange et al. 1977, Barton et al. 1980, 1986), transport (Maule et al. 1988), confinement (Strange et al. 1978), exposure to toxicants (Donaldson and Dye, 1975), and other environmental factors. Because multiple or prolonged stresses have a cumulative effect on plasma cortisol concentration (Donaldson 1981; Carmichael 1984; Barton et al. 1986), cortisol measurements provide a means of comparing the relative magnitude of stress among treatments. Fish typically experience a rapid rise in cortisol concentration following a stressful event and gradually return to baseline levels over a period of hours to days. The magnitude and duration of cortisol change depends on the type of stress experienced, background environment, and condition of the fish (Strange et al. 1977, Barton and Iwama 1991).

Abnormal behavioral patterns may also indicate sublethal stress in fish. To collect empirical data on the immediate stress-effect of pump passage, we made behavioral observations concurrently with the blood chemistry study during 1998. Behavioral studies have frequently been used to assess the effects of pollutants (Rand 1985) but studies relating behavior to other stressors have rarely been reported. Despite sparse published documentation, relevant behavioral observations can complement physiological studies (Wedemeyer et al. 1990). Mesa (1994) and Sigismondi and Weber (1988) assessed the effects of handling on juvenile chinook salmon using predator avoidance behavior and cover/avoidance of bright light, respectively. Vogel and Marine (1997) examined juvenile chinook salmon predator avoidance following passage through the Tehama-Colusa Canal drum screen bypass. These whole-animal studies, while usually less precise than physiological stress-response indicators, may provide additional information about the effect of a stressor.

To our knowledge, no previous research has been published on the stress-response of salmon to passage through Archimedes lifts or internal helical pumps. We evaluated the plasma cortisol stress-responses of juvenile chinook salmon to Archimedes lift passage during 1998, and to both pump types during 1999. We characterized the juvenile chinook salmon behavioral stress-response to Archimedes lift passage during 1998.

Study Area

The Red Bluff Research Pumping Plant is located southeast of Red Bluff, California, 391 km (243 miles) upriver from the mouth of the Sacramento River. It consists of two Archimedes screw lifts and one internal helical (centrifugal) pump, which divert water directly from the Sacramento River. The Archimedes lifts, manufactured by Wheelebrator/CPC, have 1.2 m (4 ft) diameter intakes. Each has an 11.6 m (38 ft) long, 3.0 m (10 ft) diameter rotating cylinder with three helical flights continuously welded along the length of the inside walls of the cylinder. During this study they operated at 26.5 rpm and delivered water at an average rate of 2.4 m^3/s (84 ft^3/s). The internal helical pump, manufactured by WEMCO-Hidrostal has an intake diameter of 0.90 m (3 ft) and is the largest of its type ever constructed (Frizell and Atkinson 1999). It has a single vane

impeller cast with a rotating conical shroud and operated at 350 rpm, delivering water at an average rate of 2.3 m^3/s (80 ft^3/s).

Each pump discharges into an open channel containing two vertical wedgewire chevron screens (2.4 mm, 0.09 in mesh) with continuously operating brushes (Frizell and Atkinson, 1999; Figure 1). Approximately 90% of the discharged water passes through the screens to the Tehama-Colusa canal forebay while the remaining 10%, along with fish and debris, is diverted into a 46 cm (18 in) wide, curved, open bypass channel. After traveling approximately 13.5 - 31.5 m (45 to 104 ft, depending upon the pump), water can be diverted into a fish evaluation facility, by lowering a wedgewire screen dewatering ramp into the bypass channel, or continue into an underground bypass to the river. During this study, water and fish were diverted to the evaluation facility and directed into either of two 1.3 m^3 (48 ft^3) flow-through holding tanks. Experimental fish were held in these holding tanks until sampled.

Methods

Plasma Cortisol

Juvenile late-fall chinook salmon, *Oncorhynchus tshawytscha*, were obtained from Coleman National Fish Hatchery, Anderson, CA. Fish were transported to the RBRPP well water holding facility, acclimated to laboratory water conditions, and maintained prior to experiments using standardized procedures described by McNabb et al. (1998). Fish ranging from 75 to 87 mm mean fork length and 4.4-7.8 g mean weight, were used in these experiments. To distinguish treatments, experimental fish were marked 7 to 9 days prior to each trial with either upper or lower lobe caudal clips. Fish were then moved to the RBRPP river water holding facility, acclimated to river conditions, and deprived of food 48 h prior to each trial. All trials were performed during mid- to late summer, when there was little variation in river or atmospheric conditions. Water conditions and fish sizes are described for each trial in Appendix 1.

We used a treatment/control design to quantify pump-induced stress in 1998, and added an additional handling control in 1999. Treatment groups consisted of fish injected into a pump insertion tube (McNabb et al. 1998), passed through the pump and vertical screen areas, and collected in one of two associated holding tanks (Figure 1). The control group consisted of fish released into a pump outfall, passed through the vertical screen area, and collected in the other associated holding tank, thereby encountering conditions similar to those experienced by treatment fish with the exception of passing through the pump.

Handling-control fish were gathered into insertion carboys and transported to one of the pump insertion tubes. The handling-control carboys were then lowered into the insertion tube, lifted out, and transported to one of the holding tanks associated with the pump. Handling-control fish were then released into the holding tank and thereafter treated identically to other experimental fish. The handling control was conducted to

approximate the stress-response uniquely due to netting fish from holding tanks and confining fish in insertion carboys. By comparison, the control group quantified the stress-response due to all portions of the study except pump passage; that is, passage through the RBRPP screening facility and open bypass channel, in addition to netting and confinement.

We conducted six trials in 1998, to include three tests with each Archimedes lift in a randomly pre-determined sequence (Appendix 1). In 1999, we repeated the Archimedes experiment and also conducted six trials with the internal helical pump and six handling controls. Trials were generally conducted over a three-day period in 1999 with each pump or handling-control test occurring on a separate day (Appendix 2). We assumed the stress-effect caused by insertion tubes and holding tanks was similar among pumps. Therefore, the pump associated with the insertion tube and holding tank used for each handling-control trial was chosen randomly from either the internal helical pump or the Archimedes lift being utilized for that trial. Pump speeds were held at 350 rpm for the internal helical pump, and 26.5 rpm for the Archimedes lifts throughout all trials.

All trials began two hours before sunrise to minimize the number of fish residualizing upstream of the holding tanks (McNabb et al. 1998). To avoid stressing fish by suddenly turning lights on in the river water holding facility, a row of dim overhead lights was left on over the previous night. For pump trials, one holding tank containing 200 upper or lower caudal clipped fish was randomly assigned to the treatment group prior to each trial. The remaining holding tank containing 200 fish with the opposite caudal clip was assigned to the control group. Handling-control trials were conducted using an identically treated holding tank containing 200 fish with a randomly predetermined caudal clip.

To begin a trial fish were carefully netted from holding tanks. Netting was limited to 3 min to minimize pre-trial stress that might be caused by chasing fish with nets for an extended period. Any remaining fish in the holding tanks (normally <10) were excluded from the study. Netted fish were distributed in approximately equal groups, as determined by visual inspection, into four specially designed insertion carboys containing a mixture of river water, salt, and Kordan's Polyaqua® (McNabb et al. 1998). Carboys were transported to pump insertion tubes or pump outfalls and fish were released according to group, as described above. For treatment groups, all four treatment inserters were emptied into pump insertion tubes within five minutes. Fish were then directed into the flow stream using a crowder as described by McNabb et al. (1998). Control groups were held in carboys for approximately the same amount of time as the treatment groups prior to release into a pump outfall. After traveling through the open channels, treatment or control fish were diverted into one of two holding tanks downstream of each pump (Figure 1). Bypass flows remained diverted into the holding tank for 20 min while the remaining group (treatment or control) was gathered into insertion carboys as described above. After 20 min, water was diverted into the second holding tank and the remaining group was released into the flow stream. Fish were collected in the holding tanks for 20

min before directing flows to the bypass to exclude any fish remaining in the system from either holding tank. A reduced flow (105-125 liters/min) was cycled through the holding tanks to maintain temperature and dissolved oxygen levels for the remainder of the trial.

Twelve fish from each group were netted, euthanized, and sampled for plasma cortisol prior to gathering fish into insertion carboys (time 0), and twelve fish from each group were sampled from the holding tanks at 1, 3, 6, 12, and 24 h after release in 1998. In 1999, groups were sampled at 1, 1.5, 3, 6, and 12 h after release. Fish sampled from holding tanks were quickly netted and immediately euthanized while attempting to minimize disturbance to remaining fish in the tanks. Caudal clips were checked to verify sampled fish were taken from the correct release group.

Sampled fish were euthanized in a chilled (<7° C) river-water solution of 200 mg/L Finquel® buffered with an equal weight of sodium bicarbonate, a solution which does not significantly affect plasma cortisol concentrations (Strange and Schreck 1978). Fish were then transferred to the laboratory where they were weighed, measured, and sampled for blood within 15 min of euthanasia. We collected samples by severing the caudal peduncle and drawing blood into 0.13-µL heparinized capillary tubes. Capillary tubes were sealed using chemically inert sealing clay and centrifuged for five minutes at 17,000 rpm. Twelve fish were sampled from each group and time to insure quality plasma samples were available; of these, ten were analyzed and two were discarded. The first ten samples collected were sent for analysis unless one or more of these did not centrifuge correctly, e.g. platelets were visible in the plasma column. Samples were frozen to –30° C, packed in dry ice, and shipped overnight to BioTech Research and Consulting, Incorporated, Corvallis, Oregon.

BioTech Research and Consulting performed the plasma cortisol assay using enzyme-linked immunosorbent assay (ELISA; Barry et al. 1993). Ten µL samples of plasma were extracted with ethyl ether to remove transcortins. The cortisol in ether was transferred to a second tube and evaporated. The cortisol was then taken up in extraction buffer and used in a standard competitive binding ELISA reaction in 96-well plates. Eight standards in duplicate ranging from 0- to 375-ng/mL cortisol were extracted and used for relating optical density to concentration. All plots of ln concentration (ng/ml) versus optical density gave R^2 values of 0.99 or better.

Behavior

Behavioral studies were conducted concurrently with trials 1-6 in 1998. Observations were conducted in 29-gallon aquaria, which were filled with ambient river water just prior to trials. Aquaria were covered with black cardstock on three sides to minimize visual disturbance to fish. A bright utility light was placed directly over each tank with a piece of plywood providing 50% cover. The bottom 5 cm of the water column was marked with a horizontal line in black permanent marker. Figure 2 illustrates the aquarium setup.

We characterized stressed versus unstressed behavior by direct observation prior to conducting experiments. Unstressed behavior was observed after moving fish into aquaria and allowing them to acclimate for approximately one hour. Stressed behavior was observed by placing salmon in aquaria after repeatedly pouring them back and forth between buckets as described by Mesa (1994). We attempted to select metrics of stress that were: (1) relatively easy to categorize; (2) not confounding; and (3) logically related to predator avoidance.

Initially, three behavioral metrics were selected to evaluate stress: schooling behavior (yes or no), position in the water column (bottom 5 cm or not), and use of cover (in lighted or covered side of the aquarium). We added a fourth metric, activity (stationary or actively swimming), to the experiment after the first two trials to better differentiate behavioral patterns among treatments. Unstressed behavior was characterized by active swimming, schooling, remaining in the covered side of the tank, and swimming in the middle portion of the water column (>5 cm from the bottom). Stressed behaviors were sluggish movement, positioning within 5 cm of the gravel or touching the gravel, no schooling behavior, and remaining in the lighted end of the aquarium. Sigismondi and Weber (1988) noted similar behaviors for stressed juvenile chinook salmon.

Behavior was monitored for three groups of salmon concurrently with the plasma cortisol study: reference, control, and treatment. Seven reference fish were netted from river laboratory holding tanks just prior to beginning cortisol experiments. These fish were placed in buckets of water and carried to an aquarium, where they were carefully released and observed. Control and treatment groups were the same as those used for the plasma cortisol evaluation. Control fish were introduced into the pump outfall, and treatment fish into the pump intake. Seven control fish and seven treatment fish were collected in large nets within five minutes after release as they entered the holding tanks. Netted fish were then gathered into buckets without removing them from the water and placed into observation aquaria. Each group was observed in a separate aquarium.

Observations were made immediately after fish were placed in tanks and every 5 min thereafter, up to 40 min. An observation consisted of evaluating the four behavioral metrics listed above for the majority of fish in the tank. In a few cases, fish did not reach

unstressed behavior within 40 min for the activity and schooling metrics. These groups were categorized as >40 min for statistical analyses. Observations were made consistently by one of two observers throughout the study.

Statistical Analyses

Treatment versus control groups were compared for each pump type and year using two-way, 2x6 factorial analyses of variance (ANOVA) in randomized complete block designs. Factors were group (treatment or control) and time after passage (0, 1, 3, 6, 12, and 24 h in 1998; 0, 1, 1.5, 3, 6, and 12 h in 1999). All ANOVA's were blocked by trial. Cortisol concentration, the dependent variable, was square-root transformed (Kuehl 1994) prior to ANOVA to normalize the data. Unadjusted single-cell *t* tests were used to compare treatment versus control groups at specific times where the interaction between group and time was significant.

Behavioral metrics were compared among treatments using Kruskal-Wallace tests on trials ranked by time (Kuehl 1994). For each trial, the three groups were ranked by time, in five-minute intervals, until fish demonstrated unstressed behavior. If fish exhibited unstressed behavior then reverted back to stressed behavior, the group was ranked by time of the last unstressed behavior. Additional Kruskal-Wallace tests were performed on trials 3-6 ranked by time until fish demonstrated unstressed behavior for all four metrics combined, and on all trials ranked by time until fish demonstrated unstressed behavior for the schooling, cover, and vertical position metrics combined.

Results

Plasma Cortisol

Cortisol concentrations did not differ significantly between treatment and control groups in 1998 (Table 1; Figure 3A), indicating passage through the Archimedes lifts did not appreciably stress fish. Mean plasma cortisol concentrations peaked 1 h after passage for both groups; both exhibited cortisol increases from about 50 ng/mL before passage to peaks of about 175 ng/mL (Figure 3). Cortisol concentrations decreased non-linearly to near-baseline levels between 1 and 12 h after passage but remained significantly higher than time 0 concentrations at 12 h (*t*-test, P=0.003). Cortisol levels increased slightly between 12 and 24 h suggesting fish experienced some additional stress during confinement in holding tanks.

For the 1999 Archimedes lift trials, the interaction between time and group was significant (Table 1) indicating the patterns of treatment and control group responses were different over time. Therefore, it was necessary to make statistical comparisons at each time individually (Kuehl 1994). Cortisol concentrations increased from about 50 ng/ml to near 225 ng/ml 1 h after passage and declined to near 100 ng/ml 12 h after passage for both treatment and control groups (Figure 3b). Single-cell contrasts indicated

the Archimedes' treatment group had significantly higher cortisol levels than the control group at times 1.5 (27 ng/ml difference, P=0.016) and 12 (28 ng/ml difference, P=0.011) but groups were similar at times 1, 3, and 6.

Treatment and control groups associated with the internal helical pump exhibited similar cortisol responses to the 1999 Archimedes-passed groups (Figure 3c). Cortisol levels peaked near 250 ng/ml 1 h after passage and declined to near 100 ng/ml 12 h after passage. The interaction between time and group was also significant (Table 1). Single-cell contrasts indicated helical-pump treatment and control groups differed significantly at time 3 (41 ng/ml difference, P=0.009), but were otherwise similar.

The handling-control indicated much of the stress-response observed for both pump types was due to the netting, confinement, and transport necessary to conduct the experiment; however, the recovery rates of handling-control fish suggest a portion of the observed stress-response may have been due to passage through the screening facilities to the holding tanks (Figure 3B,C). Cortisol levels for handling-control fish peaked after 1 h near the same level as those in the treatment and control groups from both pump types. However, cortisol levels in handling-control fish generally declined faster between 1 and 6 h than those of treatment or control fish. Since handling-control trials were not conducted simultaneously with treatment and control groups, no direct statistical comparison was made (Appendix 2).

Behavior

On average, reference, control, and treatment groups demonstrated at least one stressed behavior for 35-40 min (Table 2). Fish from all groups generally positioned themselves near or touching the bottom gravel and showed little movement for about 10 min following introduction into aquaria. As fish recovered they moved into the center of the water column and the covered end of the tank, usually in less than 15 min. Fish generally did not resume normal swimming and schooling activity until late in the observation period (Table 2).

We did not detect significant differences among reference, control, and treatment groups for any single behavioral metric or for all metrics in combination (Table 2). The time period in which reference fish exhibited stressed behavior indicated handling contributed importantly to the total observed stress-response and had a larger effect on the analysis than small differences between treatment and control groups. The pattern of time-to-unstressed behavior fit the scientific hypothesis for activity and vertical position metrics, as well as for all metrics combined (Table 2). That is, reference fish took the least amount of time to reach unstressed behaviors and treatment fish the most. The schooling and cover behavioral metrics, however, did not meet this pattern.

Discussion

The relatively small differences in cortisol concentration observed between treatment and control groups in this experiment suggest juvenile salmon are not at risk of immediate stress-induced mortality after passage through the Archimedes lifts or internal helical pump. Elevating plasma cortisol concentration is an adaptive response to stress that optimizes the chance of survival by mobilizing metabolites for action (Munck et al. 1984). However, repeated or chronic stress can result in loss of homeostasis and death (Barton et al. 1986, Maule et al.1988, Olla et al. 1995). Cortisol levels are strictly comparable only in controlled experimental situations because responses differ among individuals (Strange et al. 1978), genetic groups (Heath et al. 1993), and runs (Maule et al. 1988). Nevertheless, previous research has demonstrated some consistency. Cortisol levels in juvenile chinook salmon that have been stressed to death have usually been reported to be 400 to 500 ng/ml (e.g. Strange et al. 1978, Barton et al. 1986, Maule et al. 1988). Our treatment fish exhibited cortisol concentrations well below this lethal range, despite treatments that included some background experimental stress, as demonstrated by the controls. Therefore, we believe that most juvenile salmon should be able to recover from some additional stress following passage through Archimedes lifts or internal helical pumps.

The net pump effects, the difference between controls and treatments, never exceeded 50 ng/ml of cortisol for either type of pump. Although cortisol concentrations of juvenile salmon have not been quantified well in natural settings, salmon may commonly encounter this level of stress in hatcheries or laboratories. For example, Strange and Schreck (1978) reported yearling chinook salmon cortisol levels of 75 ng/ml after fish were transferred between tanks, and 50 ng/ml after fish were anesthetized then transferred. Barton and Iwama (1991) summarized 26 studies involving chinook salmon and various stressors. Pre-stress cortisol levels ranged from 5 to 200 ng/ml but most were between 10 and 50 ng/ml. This stress was presumably due to laboratory activity prior to experiments and anesthetization to obtain samples. Activities such as transferring fish between tanks do not generally result in high immediate or secondary mortality. Therefore, we believe the similar stress-effect of pump passage is relatively safe for juvenile salmon. On the other hand, our estimates of net pump effects should be interpreted with caution. Although multiple stressors evoke cumulative cortisol stress-responses (Barton et al. 1986), the relationship has not been demonstrated to be linear. Experimental net pump effects are, at best, an approximation of the absolute pump effects that would occur in the absence of background experimental stress.

To our knowledge, no published literature quantifying the secondary ecological effects of a single acute stress such as pump passage exists. We speculate the additional risk of secondary mortality to chinook salmon after passage is low because the magnitude and duration of pump effects were small in this experiment. However, brief acute stresses have been reported to cause immunosuppression by interfering with lymphocyte function (Barton and Iwama 1991). Salmonids typically recover from a single acute stress within

a relatively short period (<24 h) but to what extent pump passage would increase the incidence of disease in bypassed salmon returned to the Sacramento River is unknown.

Although acute stress has been demonstrated to reduce predator avoidance ability in salmon, cortisol levels are not correlated with predator avoidance. Mesa (1994) reported juvenile chinook salmon subjected to stress from handling or agitation regained baseline ability to avoid Northern pikeminnow *Ptychocheilus oregonensis* after 1 h although plasma cortisol concentrations peaked between 1 and 2 h (in the range of 75 to 200 ng/ml) and did not return to baseline for up to 24 h. Likewise, Olla et al. (1995) reported juvenile coho salmon regained the ability to avoid capture by lingcod *Ophiodon elongatus* within 24 h after multiple-handling stress but cortisol concentrations remained significantly higher than baseline. The poor correlation between cortisol concentration and predator avoidance makes sense given the physiological role of cortisol. Elevated plasma cortisol indicates fish have experienced stress because it is a direct physiological response to an environmental stressor; the purpose of the stress-response is to maximize the chance of surviving the perceived emergency. Thus, elevating plasma cortisol, within a range, is a positive adaptation that does not necessarily correlate with the initial negative effects of a stressor on behavioral performance. Assuming the stress caused by pump passage was less than that caused by handling in the Mesa (1994) experiment because the net effect on cortisol was lower, we estimate any reduction in predator avoidance would be less than one hour.

The behavioral observation study provided limited evidence that Archimedes passage did not affect performance. Fish that passed through the Archimedes lift were not appreciably more stressed than reference fish, which were only gathered into buckets and released into observation aquaria. However, the background stress caused by moving fish into observation tanks was responsible for much of the observed behavioral response as indicated by the reference group (Table 2). We could not remove this larger-than-expected background stress because fish could not accurately be observed in the RBRPP holding tanks. Because we could not characterize short-term pump effects on behavior in the absence of background handling stress, we discontinued this portion of the study in 1999. Our behavioral data suggest that if an Archimedes pump-induced predator-avoidance deficit exists, it is relatively subtle and usually overcome within 40 min.

By indirect comparison, the Archimedes lifts and internal helical pump affected fish similarly in that they exhibited similar response curves and few significant treatment effects. Initially, we intended to make direct statistical comparisons among pump types and handling control, blocking by trial. Since trials for each pump type and a handling control were conducted over three-day periods using the same lot of fish, and under stable environmental conditions, we assumed day to day variation would be minimal. Conditions were also very similar among trials, yet block effects were highly significant (Table 1). Because these results suggested daily environmental or handling stress might vary significantly, we avoided direct statistical comparisons between pump types, and between each pump type and the handling control.

Because our inferences concerning the fish-friendliness of these pumps were tied to the null hypothesis (no difference between treatment and control groups), we used large sample sizes to conduct statistical tests with unusually high power. In this case, a type II error, accepting a false null hypothesis of no difference among treatment and control groups, would be more serious than a type I error. An undetected but real treatment effect would mean operating pumps under the assumption that they were not negatively impacting fish while they actually were. A type I error, in this case, would mean erring on the side of caution. The high power of the tests may have resulted in experimental error being detected as significant interactions in 1999. However, we are confident that no real treatment effects existed for those tests where we failed to reject the null hypothesis. *A posteriori* tests of power (Kuehl 1994) indicated all cortisol ANOVA's had greater than 0.99 power to detect mean differences $\leq$5 ng/ml among groups.

The handling control provided evidence that capture and confinement of experimental fish in insertion carboys was responsible for much of the observed responses in treatment and control groups (Figure 3). A plasma cortisol stress-response of similar magnitude and duration to our observations has been reported in juvenile chinook salmon exposed to other types of brief handling and confinement (Barton et al. 1986, Barton and Iwama 1992, Sharp et al. 1998). Handling-control fish appeared to recover more quickly between 1.5 and 6 h after release than treatment or control fish from either pump type (Figure 3). These results suggest there was a stress-response to passage through the screening facility and open bypass channel to the holding tanks at RBRPP.

If passage between the pumps and holding tanks stressed fish, cortisol levels in fish passing through the entire plant and bypass system may be higher than those observed in this experiment. This study was designed to evaluate stress due to passage through Archimedes lifts or helical pumps, not RBRPP as a whole. Stress caused by passage through RBRPP may vary with river conditions and channel settings within the plant. Maule et al. (1988) reported juvenile chinook salmon cortisol concentrations varied with the volume of water in a fish collection system at McNary Dam. River stage, as well as water temperature, turbidity, and debris load vary considerably throughout the year at RBRPP, potentially altering the amount of stress associated with fish passage. Furthermore, fish may respond differently to passage through different sized pumps or pumps operating at different speeds than those tested here. We operated pumps at the operational speeds used by RBRPP during spring and fall water deliveries; however, many pump size and speed configurations are possible for future pumping plant designs. Accurate estimates of the stress-response to passage through any pumping plant will be site and condition specific.

Recommendations

1. Further trials examining cortisol response to pump passage do not appear warranted at RBRPP. The risk of secondary mortality to juvenile salmon following pump passage, as measured by plasma cortisol response, appears low. The large sample sizes used in this study allowed us to conduct statistical tests with unusually high power. Barring the development of methods to reduce background stress experienced by control fish, collecting additional cortisol data would not be warranted.

2. If further physiological studies are conducted at USBR facilities, they should be directly linked to some measure of fish behavior or performance. Although such studies would be difficult to design, measures of physiological change are of limited use without some measure of the ecological consequences of these changes.

3. Managers should be aware that the stress-response to passage through pumping facilities is likely site-specific and account for these differences when evaluating permanent facilities

Acknowledgements

We would like to thank various people who have been involved with this study from its inception through the completion of this report. Special thanks go to Dr. Charles Liston, the Bureau's Research Director for the RBRPP evaluation program. Charles was responsible for developing the overall research program of which this study was a part. Dr. Cal McNabb, consultant to the Bureau, was involved in the formative stages of the study and provided guidance throughout its development. Dr. Louis Helfrich of the Bureau's Fisheries Applications Research Group in Denver developed a study plan from which the behavioral observation component of this study was derived. We thank Max Stodolski, Red Bluff Fish Passage Program Manager, for supporting and facilitating our research. Dr. Scott Foote and Kim True of the U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, and Dr. Joseph Cech from UC Davis provided advice on the blood chemistry component of the study. Darryl Hayes, George Heise, Ralph Hinton, Steve Hirtzel, John Johnson, Chris Mobley, Harry Rectenwald, Jim Smith, Paul Ward, Marcin Whitman, and David Vogel, who served on the Research Pumping Plant Interagency Fisheries Work Group and Rich Johnson and Craig Martin with the U.S. Fish and Wildlife Service, North Central Valley Fish and Wildlife Office (NCVFWO), were helpful in reviewing study plans and providing comments during our annual interagency meetings. Roger Shudes, Jon Rueth, and other staff at Coleman National Fish Hatchery provided juvenile chinook salmon used in the study and the feed used by juveniles in our holding facilities. Scott Hamelburg from NCVFWO also facilitated our acquisition of salmon. Special thanks go to Jeremy Brasch, Richard Corwin, Rick Crouch, Marie-Christine Daems, Mike Hilton, Sharon Nowak, Christy Skelton, Michael Stroing, Susan Hornsby, Drea Traeumer, and Zachary Wilson who comprise the Bureau's on-site staff of

biological technicians. These people worked diligently while conducting field and laboratory work for this study and the 1997 pilot study. Richard Corwin deserves special recognition for taking the lead in overseeing the laboratory procedures and ensuring that samples were properly packaged for shipment to the contractor each week. We also thank the Bureau's Operations and Maintenance staff at Red Bluff for facilitating plant operations and for construction of equipment during the research and development phase of this study. Special thanks to Dr. Helfrich, Dr. Liston, Dr. McNabb, Craig Martin, and Robert Null for reviewing an earlier draft of this report and providing insightful and constructive comments. Funding for this project was provided by Reclamation's Mid-Pacific Region, supplemented by Reclamation's Research and Technology Development Program, Study Number AE CO-98.016 (WATR).

Literature Cited

Adams, S. M., J. E. Breck, and R. B. McLean. 1985. Cumulative stress-induced mortality of gizzard shad in a southeastern U.S. reservoir. Environmental Biology of Fishes 13:103-112.

Barry, T. P. A., F. Lapp, T. B. Kayes, and J. A. Malison. 1993. Validation of ELISA for measuring cortisol in fish and comparison of stress responses of rainbow trout and lake trout. Aquaculture 117:351-363.

Barton, B. A., and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3-26.

Barton, B. A., R. E. Peter, and C. R. Paulencu. 1980. Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Canadian Journal of Fisheries and Aquatic Sciences 37:805-811.

Barton, B. A., C. B. Schreck, and L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society 115:245-251.

Borthwick, S. M., R. R. Corwin, and C. R. Liston. 1999. Investigations of fish entrainment by Archimedes and internal helical pumps at the Red Bluff Research Pumping Plant, Sacramento River, California: February 1997 – June 1998. Red Bluff Research Pumping Plant Report Series, Volume 7. U.S. Bureau of Reclamation, Denver, CO.

Carmichael, G. J. 1984. Long distance truck transport of intensively reared largemouth bass. Progressive Fish-Culturist 46:111-115.

Donaldson, E. M. 1981. The pituitary-interrenal axis as an indicator of stress in fish. Pages 11-48 *in* A. D. Pickering, editor. Stress and fish. Academic Press, London.

Donaldson, E. M., and H. M. Dye. 1975. Corticosteroid concentrations in sockeye salmon *Oncorhynchus nerka* exposed to low concentrations of copper. Journal of the Fisheries Research Board of Canada 32:533-539.

Frizell, K. W., and S. P. Atkinson. 1999. Engineering evaluation of the Red Bluff Pumping Plant on the Sacramento River in Northern California: 1995-1998. Red Bluff Research Pumping Plant Report Series, Volume 6. U.S. Bureau of Reclamation, Denver Colorado.

Heath, D. D., N. J. Bernier, J. W. Heath, and G. K. Iwama. 1993. Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry. Canadian Journal of Fisheries and Aquatic Sciences 50:435-442.

Kuehl, R. O. 1994. Statistical principles of research design and analysis. Wadsworth Publishing Company, Belmont, California.

Leach, G. J., and M. H. Taylor. 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. General and Comparative Endocrinology 48:76-83.

Liston, C. R., and P. L. Johnson. 1992. Biological and engineering evaluation plan for a pilot pumping plant at Red Bluff Diversion Dam on the Sacramento River, California. Bureau of Reclamation, Research and Laboratory Services Division, Denver.

Maule, A. G., C. B. Schreck, C. S. Bradford, and B. A. Barton. 1988. Physiological effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia River. Transactions of the American Fisheries Society 117:245-261.

Maule, A. G., R. A. Tripp, S. L. Kaattari, and C. B. Schreck. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology 120:135-142.

McNabb, C. D., C. R. Liston, and S. M. Borthwick. 1998. In-plant biological evaluation of the Red Bluff Research Pumping Plant on the Sacramento River in Northern California: 1995 and 1996. Red Bluff Research Pumping Plant Report Series, Volume 3. U.S. Bureau of Reclamation, Denver, CO.

McNabb, C. D., S. M. Borthwick, and C. R. Liston. 2000. Experimental results from passing juvenile chinook salmon through Archimedes lifts and an internal helical pump at Red Bluff Research Pumping Plant, Upper Sacramento River, California. Red Bluff Research Pumping Plant Report Series, Volume 9. U.S. Bureau of Reclamation, Denver, CO.

Mesa, M. G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. Transactions of the American Fisheries Society 123:786-793.

Morgan, J. D., and G. K. Iwama. 1993. Stress assessment manual. University of British Columbia, Department of Animal Science, Vancouver, B.C., Canada.

Munck, A. P., P. M. Guyre, and N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relations to pharmacological actions. Endocrine Reviews 5:25-44.

Olla, B. L., M. W. Davis, and C. B. Schreck. 1992. Comparison of predator avoidance capabilities with corticosteroid levels induced by stress in juvenile coho salmon. Transactions of the American Fisheries Society 121:544-547.

Olla, B. L., M. W. Davis, and C. B. Schreck. 1995. Stress-induced impairment of predator evasion and non-predator mortality in Pacific salmon. Aquaculture Research 26:393-398.

Rand, G. M. 1985. Behavior. Pages 221-263 in G. M. Rand and S. R. Petrocelli, editors. Fundamentals of aquatic toxicology. Methods and applications. Hemisphere, Washington, D. C.

Sharp, C. S., D. A. Thompson, and H. L. Blankenship. 1998. Effects of routine handling and tagging procedures on physiogical stress responses in juvenile chinook salmon. The Progressive Fish Culturist 60:81-87.

Sigismondi, L. A., and L. J. Weber. 1988. Changes in avoidance response time of juvenile chinook salmon exposed to multiple acute handling stresses. Transactions of the American Fisheries Society 117:196-201.

Strange, R. J. and C. B. Schreck. 1978. Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). Journal of the Fisheries Research Board of Canada 35:345-349.

Strange, R. J., C. B. Schreck, and R. D. Ewing. 1978. Cortisol concentrations in confined juvenile chinook salmon (*Oncorhynchus tshawytscha*). Transactions of the American Fisheries Society 107:812-819.

Strange, R. J., C. B. Schreck, and J. T. Golden. 1977. Corticoid stress response to handling and temperature in salmonids. Transactions of the American Fisheries Society 106:213-218.

Vaughn, D. S., R. M. Yoshiyama, J. E. Breck, and D. L. DeAngelis. 1984. Modeling approaches for assessing the effects of stress on fish populations. Pages 259-278 *in* V.W. Cairns, P. V. Hodson, and J. O. Nriagu, editors. Contaminant effects on fisheries. Wiley, Toronto.

Vogel, D. A. and K. R. Marine. 1997. Fish passage and stress effects on juvenile chinook salmon physiology and predator avoidance abilities. Natural Resource Scientists, Incorporated, Red Bluff, California.

Wedemeyer, G. A., F. P. Meyer, L. Smith. 1976. Environmental stress and fish diseases. T.F.H. Publications, Neptune City, New Jersey.

Wedemeyer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and acclimation. Pages 451-489 *in* Shreck, C. B. and P. B. Moyle, editors. Methods For Fish Biology. American Fisheries Society, Bethesda, Maryland.

Table 1. ANOVA's for (square-root transformed) plasma cortisol concentrations of juvenile chinook salmon passing through the Archimedes lifts or helical pump at Red Bluff Research Pumping Plant.

Archimedes lifts 1998

Source of Variation	df	*F*	*P*
Group (treatment or control)	1	0.109	0.741
Time	5	106.343	<0.001
Trial	5	3.799	0.002
Group x Time Interaction	5	2.117	0.062
Error	690		

Archimedes lifts 1999

Source of Variation	df	*F*	*P*
Group (treatment or control)	1	2.533	0.112
Time	5	189.573	<0.000
Trial	5	37.113	<0.000
Group x Time Interaction	5	2.671	0.021
Error	703		

Helical pump 1999

Source of Variation	df	*F*	*P*
Group (treatment or control)	1	0.188	0.665
Time	5	179.751	<0.000
Trial	5	13.308	<0.000
Group x Time Interaction	5	3.645	0.003
Error	693		

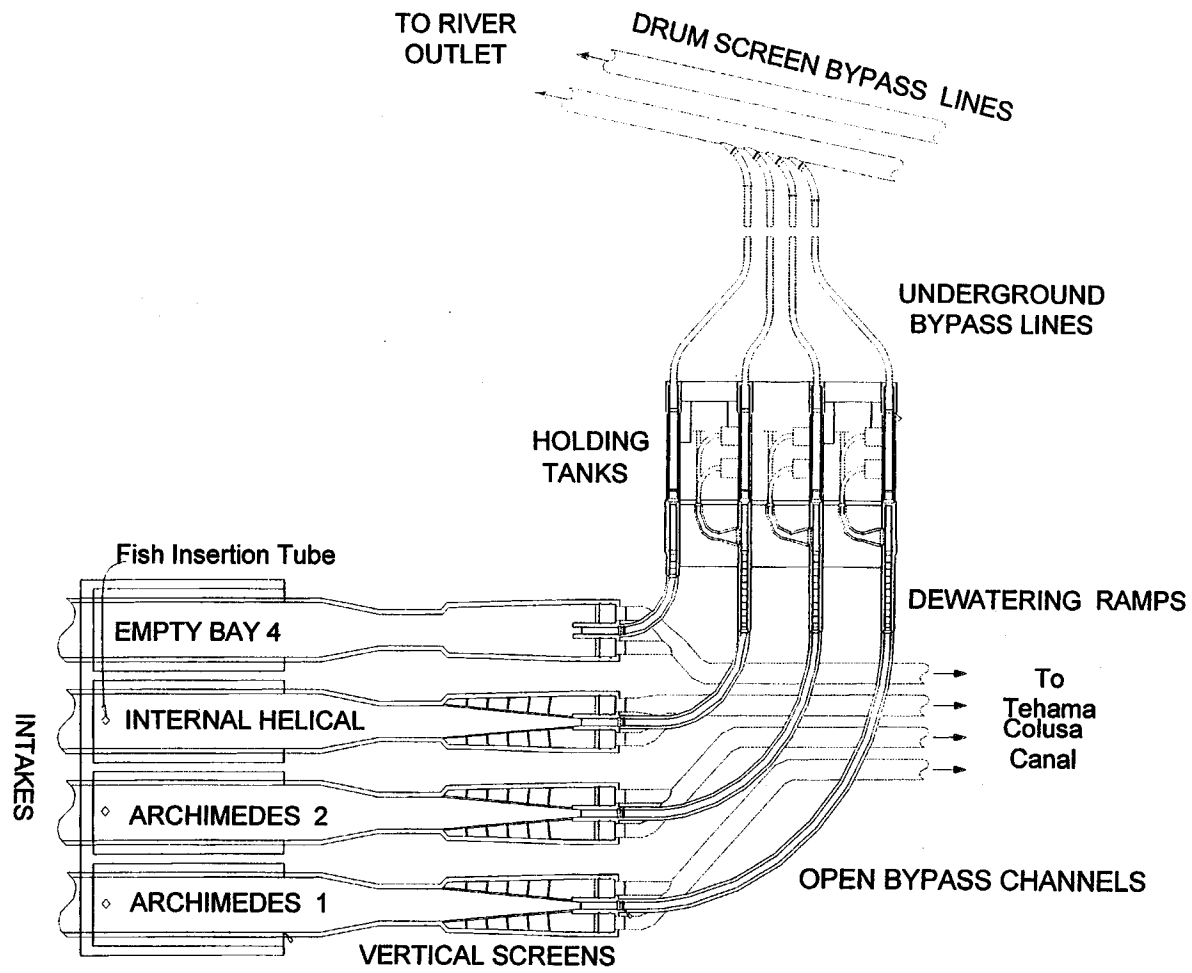


Figure 1. Schematic diagram of Red Bluff Research Pumping Plant illustrating relative positions of pumps, vertical screen areas, and holding tanks where fish were collected and held during the experiment.

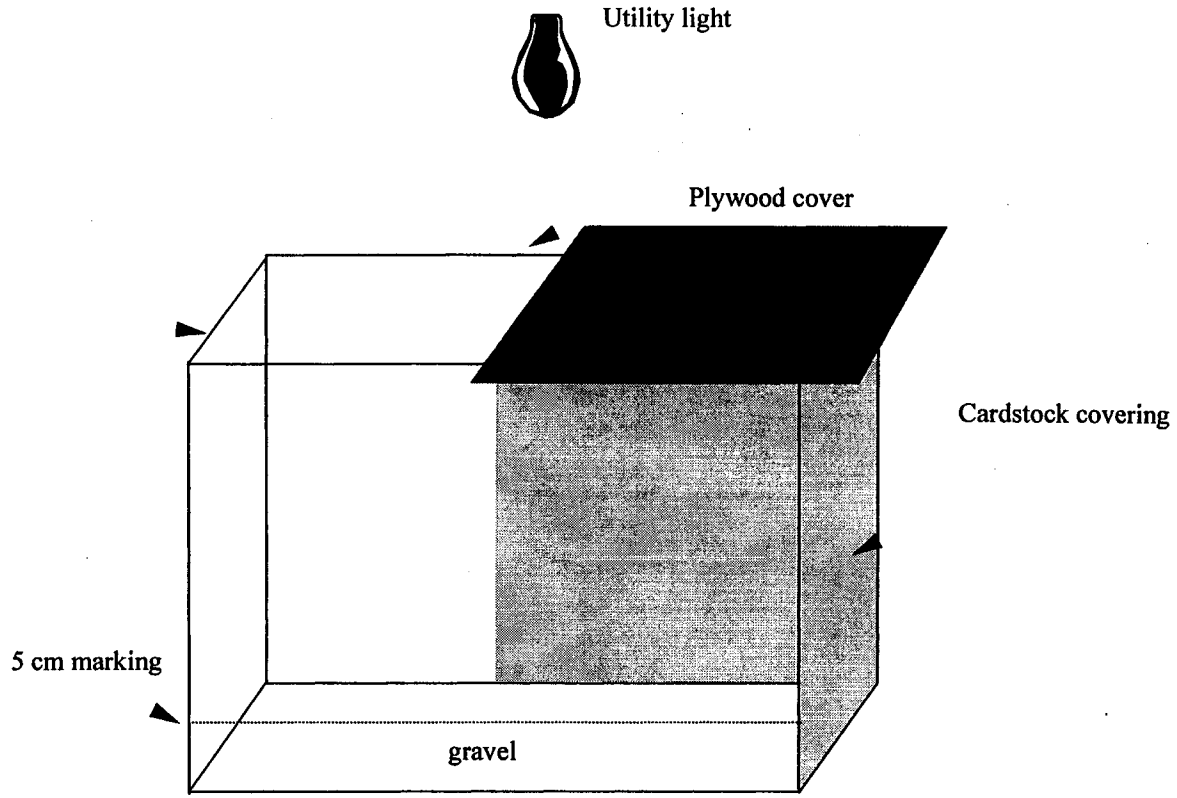
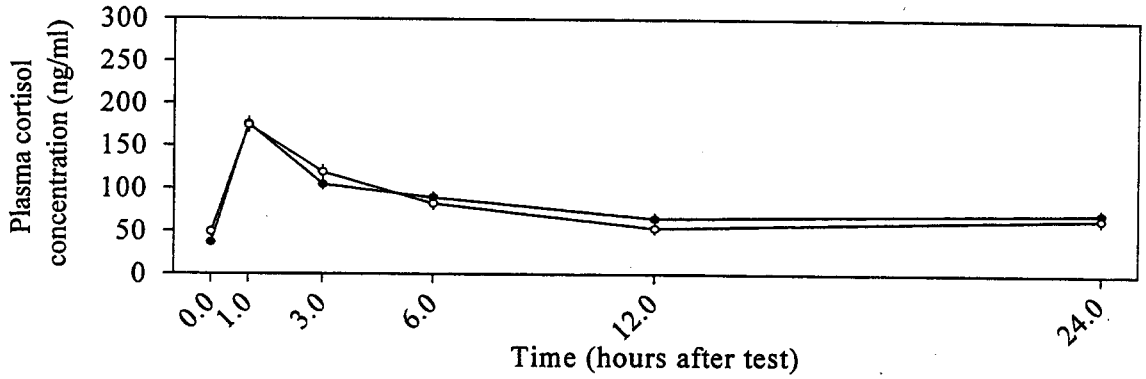
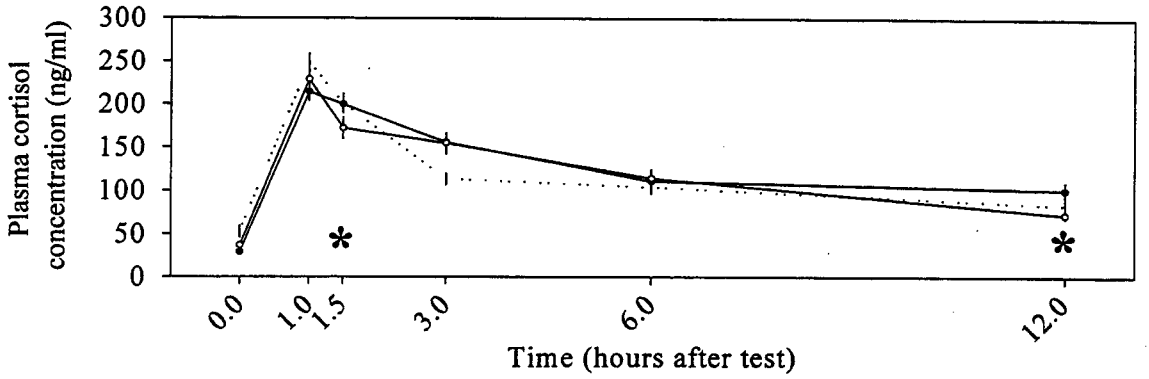


Figure 2. Aquarium setup used for behavioral observations.

A. Archimedes pumps, 1998



B. Archimedes pumps, 1999



C. Internal helical pump, 1999

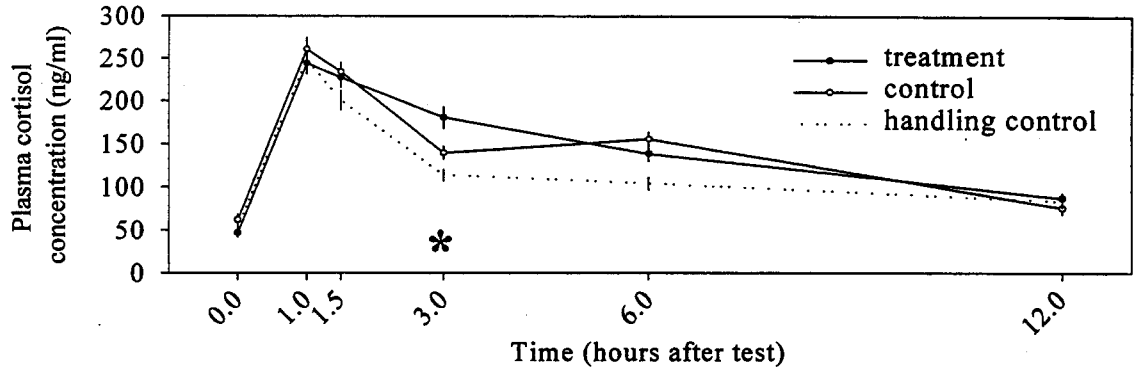


Figure 3. Mean plasma cortisol concentrations (ng/ml; ±SE) at 0, 1, 1.5, 3, 6, 12, and 24 h after passage through (A) Archimedes lifts during 1998 trials, and 0, 1, 1.5, 3, 6, and 12 h after passage through (B) Archimedes lifts or (C) internal helical pump during 1999 trials at Red Bluff Research Pumping Plant. Asterisks indicate significant ($P \leq 0.05$) differences between treatment and control groups.

Appendix 1. Trial dates, pump used, pump discharge, mean sizes of chinook salmon tested, and water quality for 1998 Red Bluff Research Pumping Plant plasma cortisol experiments

				Chinook salmon			Water			
Trial	Date	Pump	Pump discharge (cfs)	Lot[1]	Length (mm; SD)	Weight (g; SD)	Temp (°C)	Turbidity (NTU)	DO[2] (%)	River stage (ft)
1	8/5/98	Archimedes-2	86.5	6/3/98	75 (4)	4.4 (0.9)	14.4	4.8	99.4	241.32
2	8/11/98	Archimedes-2	86.6	6/3/98	78 (5)	4.9 (1.0)	13.9	4.4	102.0	241.12
3	8/18/98	Archimedes-1	87.3	8/7/98	77 (5)	4.9 (1.0	14.0	4.8	98.2	240.94
4	8/20/98	Archimedes-1	88.7	8/7/98	79 (7)	5.5 (1.3)	13.8	4.8	93.7	240.96
5	8/26/98	Archimedes-2	87.4	8/7/98	79 (6)	5.2 (1.3)	13:9	4.1	99.7	240.79
6	9/2/98	Archimedes-1	89.6	8/7/98	80 (5)	5.5 (1.1)	14.0	4.6	100.4	240.67

[1] Date fish were obtained from Coleman National Fish Hatchery.

[2] Dissolved Oxygen.

Appendix 2. Trial dates, pump used, pump discharge, mean sizes of chinook salmon tested, and water quality for 1999 Red Bluff Research Pumping Plant plasma cortisol experiments

				Chinook salmon			Water			
Trial	Date	Pump[1]	Pump discharge (cfs)	Lot[2]	Length (mm; SD)	Weight (g; SD)	Temp (°C)	Turbidity (NTU)	DO[3] (%)	River stage (ft)
7	7/21/99	Archimedes-1	73.6	7/15/99	85(6)	7.1 (1.7)	13.9	4.3	100.	240.40
7	7/22/99	Helical	83.8	7/15/99	85 (6)	7.4 (1.7)	14.0	5.6	95.1	240.34
7	7/23/99	Handling (H)	83.3	7/15/99	86 (8)	7.5 (2.2)	14.1	4.1	106.	240.28
8	7/27/99	Helical	82.2	7/15/99	85 (7)	7.1 (2.0)	14.6	4.6	96.2	240.24
8	7/28/99	Handling(A1)	83.0	7/15/99	86 (7)	7.4 (1.9)	14.1	4.3	96.6	240.13
8	7/29/99	Achimedes-1	82.0	7/15/99	87 (7)	7.8 (2.3)	14.2	4.3	96.7	240.09
9	8/3/99	Helical	80.0	7/31/99	82 (7)	6.4 (1.9)	14.4	4.0	93.9	240.11
9	8/4/99	Handling(H)	81.3	7/31/99	85 (7)	7.1 (1.9)	14.4	4.7	98.2	240.13
9	8/5/99	Archimedes-2	80.6	7/31/99	87 (9)	7.6 (2.7)	14.6	4.4	91.5	239.93
10	8/10/99	Archimedes-1	78.2	7/31/99	80 (7)	5.5 (1.5)	14.2	4.7	96.1	239.59
10	8/11/99	Handling(A1)	79.2	7/31/99	84 (7)	6.7 (2.0)	13.6	4.7	96.2	239.25
10	8/12/99	Helical	78.5	7/31/99	80 (7)	6.0 (1.5)	14.4	5.0	92.4	239.33
11	8/17/99	Handling(A2)	81.6	7/31/99	81 (6)	6.3 (1.4)	13.9	6.1	89.7	239.13
11	8/18/99	Helical	78.0	7/31/99	83 (7)	7.0 (2.1)	13.6	6.7	94.2	239.0
11	8/20/99	Archimedes-2	80.9	7/31/99	84 (8)	7.0 (1.9)	13.7	8.0	95.8	239.0
12	8/24/99	Archimedes-2	82.3	8/17/99	80 (7)	5.3 (1.4)	13.8	3.1	97.2	239.03
12	8/25/99	Handling(H)	78.3	8/17/99	80 (6)	5.3 (1.2)	13.6	3.3	93.5	239.03
12	8/26/99	Helical	79.0	8/17/99	80 (7)	5.4 (1.6)	13.4	3.5	95.8	239.01

[1] Parentheses indicate pump associated with holding tanks (A1 = Archimedes 1, A2 = Archimedes 2, H = helical).
[2] Date fish were obtained from Coleman National Fish Hatchery.
[3] Dissolved oxygen.

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)		N
1	8/5/98	A2	0	control	40	(9)	10
1	8/5/98	A2	0	treatment	73	(13)	10
1	8/5/98	A2	1	control	244	(22)	10
1	8/5/98	A2	1	treatment	175	(21)	10
1	8/5/98	A2	3	control	113	(15)	10
1	8/5/98	A2	3	treatment	143	(16)	10
1	8/5/98	A2	6	control	52	(5)	10
1	8/5/98	A2	6	treatment	139	(20)	10
1	8/5/98	A2	12	control	29	(3)	10
1	8/5/98	A2	12	treatment	94	(15)	10
1	8/5/98	A2	24	control	79	(14)	10
1	8/5/98	A2	24	treatment	77	(19)	10
2	8/11/98	A2	0	control	33	(7)	10
2	8/11/98	A2	0	treatment	27	(8)	10
2	8/11/98	A2	1	control	140	(17)	10
2	8/11/98	A2	1	treatment	141	(20)	10
2	8/11/98	A2	3	control	135	(13)	10
2	8/11/98	A2	3	treatment	71	(7)	10
2	8/11/98	A2	6	control	79	(16)	10
2	8/11/98	A2	6	treatment	70	(11)	10
2	8/11/98	A2	12	control	111	(17)	10
2	8/11/98	A2	12	treatment	85	(20)	10
2	8/11/98	A2	24	control	28	(5)	10
2	8/11/98	A2	24	treatment	72	(14)	10
3	8/18/98	A1	0	control	104	(16)	10
3	8/18/98	A1	0	treatment	9	(1)	10
3	8/18/98	A1	1	control	128	(11)	10
3	8/18/98	A1	1	treatment	201	(18)	10
3	8/18/98	A1	3	control	109	(20)	10
3	8/18/98	A1	3	treatment	105	(10)	10
3	8/18/98	A1	6	control	50	(7)	10
3	8/18/98	A1	6	treatment	51	(7)	10
3	8/18/98	A1	12	control	58	(14)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)		N
3	8/18/98	A1	12	treatment	49	(8)	10
3	8/18/98	A1	24	control	51	(11)	10
3	8/18/98	A1	24	treatment	32	(7)	8
4	8/20/98	A1	0	control	15	(3)	10
4	8/20/98	A1	0	treatment	31	(9)	10
4	8/20/98	A1	1	control	188	(23)	10
4	8/20/98	A1	1	treatment	233	(24)	10
4	8/20/98	A1	3	control	74	(16)	10
4	8/20/98	A1	3	treatment	109	(18)	10
4	8/20/98	A1	6	control	98	(21)	10
4	8/20/98	A1	6	treatment	74	(13)	10
4	8/20/98	A1	12	control	27	(6)	10
4	8/20/98	A1	12	treatment	broken tubes-no sample		
4	8/20/98	A1	24	control	128	(19)	10
4	8/20/98	A1	24	treatment	113	(11)	10
5	8/26/98	A2	0	control	49	(8)	10
5	8/26/98	A2	0	treatment	26	(7)	10
5	8/26/98	A2	1	control	186	(21)	10
5	8/26/98	A2	1	treatment	166	(9)	10
5	8/26/98	A2	3	control	112	(17)	10
5	8/26/98	A2	3	treatment	94	(16)	10
5	8/26/98	A2	6	control	137	(19)	10
5	8/26/98	A2	6	treatment	119	(8)	10
5	8/26/98	A2	12	control	39	(4)	10
5	8/26/98	A2	12	treatment	45	(6)	10
5	8/26/98	A2	24	control	55	(12)	10
5	8/26/98	A2	24	treatment	87	(14)	10
6	9/2/98	A1	0	control	54	(11)	10
6	9/2/98	A1	0	treatment	56	(10)	10
6	9/2/98	A1	1	control	159	(13)	10
6	9/2/98	A1	1	treatment	138	(14)	10
6	9/2/98	A1	3	control	170	(24)	10
6	9/2/98	A1	3	treatment	106	(9)	10
6	9/2/98	A1	6	control	80	(8)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)	N
6	9/2/98	A1	6	treatment	85 (8)	10
6	9/2/98	A1	12	control	59 (16)	10
6	9/2/98	A1	12	treatment	54 (8)	10
6	9/2/98	A1	24	control	50 (10)	10
6	9/2/98	A1	24	treatment	39 (10)	8
7	07/21/99	A1	0	control	47 (15)	10
7	07/21/99	A1	0	treatment	25 (4)	10
7	07/21/99	A1	1	control	199 (24)	10
7	07/21/99	A1	1	treatment	199 (19)	10
7	07/21/99	A1	1.5	control	99 (15)	10
7	07/21/99	A1	1.5	treatment	196 (15)	10
7	07/21/99	A1	3	control	144 (19)	10
7	07/21/99	A1	3	treatment	223 (18)	10
7	07/21/99	A1	6	control	91 (12)	10
7	07/21/99	A1	6	treatment	237 (28)	10
7	07/21/99	A1	12	control	84 (11)	10
7	07/21/99	A1	12	treatment	76 (16)	10
7	07/22/99	H	0	control	83 (16)	10
7	07/22/99	H	0	treatment	50 (11)	10
7	07/22/99	H	1	control	215 (37)	10
7	07/22/99	H	1	treatment	117 (13)	10
7	07/22/99	H	1.5	control	196 (28)	10
7	07/22/99	H	1.5	treatment	162 (21)	10
7	07/22/99	H	3	control	106 (14)	10
7	07/22/99	H	3	treatment	212 (26)	10
7	07/22/99	H	6	control	197 (21)	10
7	07/22/99	H	6	treatment	157 (23)	10
7	07/22/99	H	12	control	79 (18)	10
7	07/22/99	H	12	treatment	96 (9)	10
7	07/23/99	H	0	handling-control	40 (7)	10
7	07/23/99	H	1	handling-control	332 (23)	10
7	07/23/99	H	1.5	handling-control	251 (36)	10
7	07/23/99	H	3	handling-control	90 (10)	10
7	07/23/99	H	6	handling-control	72 (6)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)		N
7	07/23/99	H	12	handling-control	87	(14)	10
8	07/27/99	H	0	control	78	(19)	10
8	07/27/99	H	0	treatment	60	(19)	10
8	07/27/99	H	1	control	214	(15)	10
8	07/27/99	H	1	treatment	280	(16)	10
8	07/27/99	H	1.5	control	282	(30)	10
8	07/27/99	H	1.5	treatment	267	(23)	10
8	07/27/99	H	3	control	131	(11)	10
8	07/27/99	H	3	treatment	253	(41)	10
8	07/27/99	H	6	control	157	(13)	10
8	07/27/99	H	6	treatment	202	(16)	10
8	07/27/99	H	12	control	65	(12)	10
8	07/27/99	H	12	treatment	74	(15)	10
8	07/28/99	A1	0	handling-control	31	(8)	10
8	07/28/99	A1	1	handling-control	286	(20)	10
8	07/28/99	A1	1.5	handling-control	260	(30)	10
8	07/28/99	A1	3	handling-control	110	(10)	10
8	07/28/99	A1	6	handling-control	107	(9)	10
8	07/28/99	A1	12	handling-control	78	(19)	10
8	07/29/99	A1	0	control	20	(5)	10
8	07/29/99	A1	0	treatment	24	(4)	10
8	07/29/99	A1	1	control	291	(23)	10
8	07/29/99	A1	1	treatment	253	(26)	10
8	07/29/99	A1	1.5	control	229	(29)	10
8	07/29/99	A1	1.5	treatment	208	(24)	10
8	07/29/99	A1	3	control	156	(12)	10
8	07/29/99	A1	3	treatment	152	(23)	10
8	07/29/99	A1	6	control	108	(15)	10
8	07/29/99	A1	6	treatment	108	(14)	10
8	07/29/99	A1	12	control	81	(10)	10
8	07/29/99	A1	12	treatment	95	(16)	10
9	08/03/99	H	0	control	73	(14)	10
9	08/03/99	H	0	treatment	10	(2)	10
9	08/03/99	H	1	control	404	(41)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)	N
9	08/03/99	H	1	treatment	329 (31)	10
9	08/03/99	H	1.5	control	265 (21)	10
9	08/03/99	H	1.5	treatment	334 (21)	10
9	08/03/99	H	3	control	193 (16)	10
9	08/03/99	H	3	treatment	216 (27)	10
9	08/03/99	H	6	control	192 (14)	10
9	08/03/99	H	6	treatment	113 (18)	10
9	08/03/99	H	12	control	122 (19)	10
9	08/03/99	H	12	treatment	64 (14)	10
9	08/04/99	H	0	handling-control	45 (12)	10
9	08/04/99	H	1	handling-control	262 (20)	10
9	08/04/99	H	1.5	handling-control	179 (19)	10
9	08/04/99	H	3	handling-control	129 (21)	10
9	08/04/99	H	6	handling-control	173 (23)	10
9	08/04/99	H	12	handling-control	83 (16)	10
9	08/05/99	A2	0	control	47 (7)	10
9	08/05/99	A2	0	treatment	43 (10)	10
9	08/05/99	A2	1	control	437 (31)	10
9	08/05/99	A2	1	treatment	269 (15)	10
9	08/05/99	A2	1.5	control	287 (37)	10
9	08/05/99	A2	1.5	treatment	235 (19)	10
9	08/05/99	A2	3	control	261 (45)	10
9	08/05/99	A2	3	treatment	169 (14)	10
9	08/05/99	A2	6	control	244 (36)	10
9	08/05/99	A2	6	treatment	89 (13)	10
9	08/05/99	A2	12	control	102 (9)	10
9	08/05/99	A2	12	treatment	202 (25)	10
10	08/10/99	A1	0	control	29 (7)	10
10	08/10/99	A1	0	treatment	19 (5)	10
10	08/10/99	A1	1	control	181 (18)	10
10	08/10/99	A1	1	treatment	209 (23)	10
10	08/10/99	A1	1.5	control	151 (18)	10
10	08/10/99	A1	1.5	treatment	185 (19)	10
10	08/10/99	A1	3	control	61 (8)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H=helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)	N
10	08/10/99	A1	3	treatment	72 (11)	10
10	08/10/99	A1	6	control	89 (9)	10
10	08/10/99	A1	6	treatment	62 (10)	10
10	08/10/99	A1	12	control	45 (5)	10
10	08/10/99	A1	12	treatment	99 (18)	10
10	08/11/99	A1	0	handling-control	31 (10)	10
10	08/11/99	A1	1	handling-control	219 (21)	10
10	08/11/99	A1	1.5	handling-control	204 (22)	10
10	08/11/99	A1	3	handling-control	163 (18)	10
10	08/11/99	A1	6	handling-control	97 (11)	10
10	08/11/99	A1	12	handling-control	125 (13)	10
10	08/12/99	H	0	control	29 (5)	10
10	08/12/99	H	0	treatment	26 (5)	10
10	08/12/99	H	1	control	242 (28)	10
10	08/12/99	H	1	treatment	261 (16)	10
10	08/12/99	H	1.5	control	172 (27)	10
10	08/12/99	H	1.5	treatment	162 (24)	10
10	08/12/99	H	3	control	103 (14)	10
10	08/12/99	H	3	treatment	129 (25)	10
10	08/12/99	H	6	control	142 (13)	10
10	08/12/99	H	6	treatment	114 (22)	10
10	08/12/99	H	12	control	72 (13)	10
10	08/12/99	H	12	treatment	90 (21)	10
11	08/17/99	A2	0	handling-control	143 (16)	10
11	08/17/99	A2	1	handling-control	158 (13)	10
11	08/17/99	A2	1.5	handling-control	114 (11)	10
11	08/17/99	A2	3	handling-control	80 (13)	10
11	08/17/99	A2	6	handling-control	123 (16)	10
11	08/17/99	A2	12	handling-control	99 (19)	10
11	08/18/99	H	0	control	77 (20)	10
11	08/18/99	H	0	treatment	72 (15)	10
11	08/18/99	H	1	control	250 (17)	10
11	08/18/99	H	1	treatment	280 (19)	10
11	08/18/99	H	1.5	control	240 (17)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)		N
11	08/18/99	H	1.5	treatment	208	(26)	10
11	08/18/99	H	3	control	204	(12)	10
11	08/18/99	H	3	treatment	115	(16)	10
11	08/18/99	H	6	control	181	(15)	10
11	08/18/99	H	6	treatment	125	(20)	10
11	08/18/99	H	12	control	broken tubes-no sample		
11	08/18/99	H	12	treatment	107	(15)	10
11	08/20/99	A2	0	control	34	(9)	10
11	08/20/99	A2	0	treatment	31	(6)	10
11	08/20/99	A2	1	control	109	(16)	10
11	08/20/99	A2	1	treatment	110	(12)	10
11	08/20/99	A2	1.5	control	131	(21)	10
11	08/20/99	A2	1.5	treatment	184	(18)	10
11	08/20/99	A2	3	control	103	(19)	10
11	08/20/99	A2	3	treatment	128	(12)	10
11	08/20/99	A2	6	control	90	(7)	10
11	08/20/99	A2	6	treatment	89	(5)	10
11	08/20/99	A2	12	control	56	(7)	10
11	08/20/99	A2	12	treatment	76	(11)	10
12	08/24/99	A2	0	control	43	(7)	10
12	08/24/99	A2	0	treatment	31	(4)	10
12	08/24/99	A2	1	control	155	(12)	10
12	08/24/99	A2	1	treatment	244	(20)	10
12	08/24/99	A2	1.5	control	137	(18)	10
12	08/24/99	A2	1.5	treatment	190	(16)	10
12	08/24/99	A2	3	control	203	(18)	10
12	08/24/99	A2	3	treatment	191	(19)	10
12	08/24/99	A2	6	control	64	(5)	10
12	08/24/99	A2	6	treatment	82	(8)	10
12	08/24/99	A2	12	control	68	(10)	10
12	08/24/99	A2	12	treatment	58	(11)	10
12	08/25/99	H	0	handling-control	24	(5)	10
12	08/25/99	H	1	handling-control	232	(21)	10
12	08/25/99	H	1.5	handling-control	195	(15)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical

Appendix 3. Mean plasma cortisol concentrations by group and time for chinook salmon passed through pumps at Red Bluff Research Pumping Plant. Continued.

Trial	Date	Pump[1]	Time after release (h)	Group	Cortisol (ng/ml; SE)		N
12	08/25/99	H	3	handling-control	108	(13)	10
12	08/25/99	H	6	handling-control	50	(4)	10
12	08/25/99	H	12	handling-control	26	(5)	10
12	08/26/99	H	0	control	32	(5)	10
12	08/26/99	H	0	treatment	62	(6)	10
12	08/26/99	H	1	control	240	(15)	10
12	08/26/99	H	1	treatment	198	(28)	10
12	08/26/99	H	1.5	control	250	(20)	10
12	08/26/99	H	1.5	treatment	230	(14)	10
12	08/26/99	H	3	control	100	(15)	10
12	08/26/99	H	3	treatment	159	(24)	10
12	08/26/99	H	6	control	65	(7)	10
12	08/26/99	H	6	treatment	120	(18)	10
12	08/26/99	H	12	control	38	(6)	10
12	08/26/99	H	12	treatment	90	(10)	10

[1] A1 = Archimedes 1; A2 = Archimedes 2; H = helical