Annual Progress Report

Lower Snake River Compensation Plan
Confederated Tribes of the Umatilla Indian Reservation
Evaluation Studies for 1991

Peter T. Lofy

and

Michael L. Mclean

Department of Natural Resources, Fisheries Program
Confederated Tribes of the Umatilla Indian Reservation
P.O. Box 638 Pendleton, OR 97801

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Abstract

We conducted experiments concomitant with ongoing Oregon Department of Fish and Wildlife (ODFW) acclimation facility evaluations to determine if smoltification and stress indices differed between acclimated and non-acclimated treatments. Summer steelhead juveniles (*Oncorhynchus mykiss*) approximately 1-year-old were sampled at the Wallowa, Big Canyon Creek and Little Sheep Creek acclimation facilities and Irrigon Hatchery in northeast Oregon from 12 March to 28 April, 1991. Gill Na^+K^+- ATPase activity (ATPase) and skin guanine concentrations were measured to index smoltification at four different dates during the experiment, depending upon dates for acclimation for the acclimated treatment: once before transfer of the test fish to the acclimation facilities (from 12 March to 25 March), twice during acclimation (from 25 March to 5 April; then from 9 April to 12 April), and once within 2 days of release (from 21 April and 26 April). Plasma cortisol concentrations and plasma chloride concentrations were measured as stress indices. Sampling for stress indices occurred for each treatment at seven different times: 8 hours before release, at release, and 1, 4, 12, 24 and 48 hours from fish which were retained after the rest of the acclimated or non-acclimated cohorts had been released. A stress challenge was administered to fish that were retained after release of the cohort.

There was generally little difference in smoltification indices between acclimated and non-acclimated treatments. No seasonal trend in ATPase was observed over the four dates. Both treatments were generally similar to one another at each of the four sampling dates. Both acclimated and non-acclimated treatments had increased skin guanine concentrations during the experiment and both treatments were generally similar to each other at each of the four sampling dates.

Differences in stress indices between acclimated and non-acclimated treatments were observed at both the Big Canyon Creek and Little Sheep Creek Facilities. Differences between treatments in plasma chloride samples were observed as early as 8 hours before release of the rest of the

cohort. After a stress challenge, plasma cortisol concentrations and plasma chloride concentrations generally differed between treatments starting 1 or 4 hours after the stressor was applied, continuing for 12 to 24 hours after the stressor, depending upon the facility. Increases in plasma cortisol concentrations and decreases in plasma chloride concentrations in the acclimated treatments after those times resulted in no differences between treatments thereafter. Neither acclimated nor non-acclimated treatments recovered from the stress challenge within 48 hours. Plasma chloride concentrations of the acclimated treatment at the Wallowa Facility were generally lower than those of the Big Canyon Facility. Plasma cortisol concentrations of the two acclimated treatments at the Wallowa and Big Canyon facilities were generally not significantly different from one another.

We assisted the Oregon Department of Fish and Wildlife in sampling adults and juveniles at Lower Snake River Compensation Plan hatchery and satellite facilities in Oregon. In addition, assistance with spawning ground surveys in the Grande Ronde and Imnaha river basins, data summarization and analysis, scale pattern studies and hatchery evaluation was provided.

Smoltification and Stress Studies

Introduction

Although limited data suggested that acclimation of juvenile summer steelhead juveniles before release may increase the probability of survival to adulthood (Messmer et al. 1992), the mechanisms that produce this advantage have not been investigated. These experiments were undertaken to determine if two of the potential benefits of acclimation that might produce a survival advantage for the acclimated juvenile salmonids, decreased stress and increased smoltification (compared to juveniles retained at the hatchery), might result from acclimation of juvenile steelhead(*Oncorhynchus mykiss*).

As part of the Lower Snake River Compensation Plan (LSRCP), satellite facilities that rear and release juvenile steelhead and chinook salmon (*O. tshawtscha*) have been built in Oregon, Washington and Idaho to mitigate for the losses of anadromous salmonids caused by the construction and operation of the four lower Snake River dams. The Wallowa Acclimation Facility (Wallowa Facility) has been operated in Northeast Oregon under the LSRCP since 1987. Three additional facilities, the Big Canyon Creek Acclimation/Adult Collection Facility (Big Canyon Facility), Little Sheep Creek Acclimation/Adult Collection Facility (Little Sheep Facility), and the Imnaha River Acclimation/Adult Collection Facility, were built and have been operated in the Grande Ronde and Imnaha river basins since then.

Experiments to determine the effect of accimation on juvenile migration performance and survival of juvenile summer steelhead to adulthood began with spring releases of acclimated and non-acclimated treatments from 1988 to 1990 at the Wallowa Facility. Juveniles were cold-branded the February before release so that travel time, migration timing, and juvenile survival to the collection site at Lower Granite Dam could be indexed. They were also coded-wire-tagged the fall before release to estimate juvenile-to-adult survival rate for each treatment (Carmichael et al. 1990; Messmer et al. 1991a; Messmer et al. 1991b; Messmer et al. 1992). Preliminary data from acclimated versus non-acclimated treatments suggested that survival rates of acclimated

treatments were equal to or greater than those of non-acclimated treatments (Messmer et al. 1992). Higher juvenile-to-adult survival for the acclimated fish may have been due to reduced stress or more smolted juveniles at release.

Stress around the time of release may be an important factor in survival of juvenile steelhead released in the Grande Ronde River basin because Irrigon Hatchery is outside the basin and juveniles must be transported by truck before being released at facilities or elsewhere in the basin. Handling and restriction of movement have been shown to produce an acute stress response (a change from some baseline level after a stressor has been applied) in salmonids (Barton et al. 1980). Therefore, the crowding and loading associated with the transportation are probably stressors. Acclimated fish had time to recover from transportation for weeks in the acclimation facility, while non-acclimated fish had no such opportunity. We determined stress indices of both the acclimated and non-acclimated treatments within 48 hours before loading of the non-acclimated fish and at release to determine potential differences.

Release from a facility or a truck may involve movement through narrow passageways or tubes before juvenile steelhead reach the stream. Thus, fish would be exposed to additional stressors immediately before release. Multiple stressors have been shown to produce an increased stress response (Barton et al. 1986), suggesting stress responses may be additive or synergistic. Multiple stressors in our experiments, such as those probably encountered by the non-acclimated treatments in association with transport and at release, may elicit a greater stress response than single stressor events. We therefore subjected both treatments to a stress challenge to determine differences in stress levels after additional stressors.

We used plasma cortisol concentrations and plasma chloride concentrations as stress indices. Plasma cortisol concentrations have long been used as a stress index in fishes, with an increase in plasma cortisol concentrations being observed in response to acute stressors (Schreck 1981). A reduction in plasma chloride concentrations has also been used as an index of response to an acute stressor in juvenile steelhead and coho salmon (Wedemeyer 1972).

Faster migration of juvenile anadromous salmonids in the Columbia and Snake rivers may result in increased survival to adulthood because of such factors as decreased exposure time to some sources of mortality associated with freshwater residence (e.g. risk of predation by northern squawfish and avian predators at mainstem Columbia and Snake river dams). Raymond (1979) found a decrease in the survival rate of natural and hatchery steelhead and hatchery chinook salmon associated with an increase in the length of time that juvenile salmonids required to migrate from the upper Snake River to Little Goose, Ice Harbor, and The Dalles dams during 1966 to 1975. Presumably hatchery juvenile anadromous salmonids that are more advanced in the smoltification process might be more likely to begin the seaward migration at release, while those that are not as advanced might take longer before initiating migration. Thus smoltification status may be useful in predicting readiness to migrate and, consequently, be related to probability of survival.

We used both gill Na^+K^+adenosine triphosphatase activity (ATPase) and concentrations of guanine in the skin as smoltification indices. Increases in both gill ATPase and silvering in the body have been used as indicators of smoltification. Large increases in ATPase have been observed in juvenile anadromous salmonids in response to salt water (Zaugg and McLain 1970). And increases in gill ATPase in hatchery steelhead have been observed to coincide with increased readiness to leave raceways (Zaugg and Wagner 1973). Guanine in the skin of juvenile anadromous salmonids has been used as a smoltification index because it is the main purine that causes silvering in the skin and scales during the smolt transformation (Vanstone and Markert 1968).

Differences between facilities may also produce differences in stress and smoltification of juvenile steelhead. The dimensions and particularly the environmental conditions at the two acclimation facilities on the Grande Ronde River differ from one another. Recurrent fish losses after initial "hauling mortality" at the Wallowa Facility in prior years prompted an in-depth project to monitor water quality and fish health in the springs of 1988 and 1989. Mortality of juvenile steelhead and general poor fish health was attributed to poor water quality at the facility (Groberg and Spangler 1989; Oregon Department of Environmental Quality 1989). Therefore, acclimation

at the Wallowa Facility may have affected juvenile steelhead differently than acclimation at facilities with good water quality (e.g. Big Canyon Facility). Thus, we compared physiological indices of stress and smoltification of juvenile steelhead acclimated at the Wallowa Facility to those acclimated at the Big Canyon Facility in 1991.

Methods

Fish Groups

Juvenile summer steelhead sampled for this study were from groups already being used to evaluate potential differences in juvenile migration performance, survival to adulthood and contribution to fisheries. Acclimated and non-acclimated treatments were released at LSRCP acclimation facilities in Northeast Oregon. Acclimated treatments were transported and held in acclimation facilities for 24 to 39 days before release. Non-acclimated treatments were transported directly from Irrigon Hatchery near Irrigon, Oregon, and released near the acclimation facility on the same day as release commenced for acclimated fish (Table 1). Both treatments were loaded into transport trucks by crowding into the downstream end of the raceway into a funnel. The funnel was connected by hose to a Nielsen fish pump which pumped the fish and water to a separator above the transport truck compartments. Transfer (acclimated) or release (non-acclimated) treatments were removed from the truck by gravity through a 6-inch (inside diameter) pipe. All experiments were conducted in 1991 with fish about a year old (from the 1990 broods) that were reared to about 91 grams (g).

Table 1. Transfer and first date of release (acclimated) or transport/release dates (non-acclimated) for juvenile steelhead released at the Grande Ronde River acclimation facilities.

Facility	Treatment	Transfer date	Release date
Big Canyon	Acclimated	3/15	4/26
	Non-acclimated	--	4/26
Little Sheep	Acclimated	3/13	4/23
	Non-acclimated	--	4/23
Wallowa	Acclimated	3/26	4/21

Experiments to evaluate potential differences in stress and smoltification between acclimated and non-acclimated treatments were conducted at two acclimation facilities. The Wallowa Hatchery stock was used at the Big Canyon Facility located on Deer Creek, a tributary to the Wallowa

River (Figure 1). Stress and smoltification indices for the Big Canyon Facility acclimated treatment were compared to the non-acclimated treatment retained at Irrigon Hatchery until release at the Big Canyon Facility (Table 1). The endemically-derived Little Sheep Creek stock was used at the Little Sheep Facility located on Little Sheep Creek, a tributary of the Imnaha River (Figure 1). Stress and smoltification indices of the Little Sheep Creek stock acclimated at the Little Sheep Facility were compared to the non-acclimated treatment retained at Irrigon Hatchery until release at the Little Sheep Facility (Table 1).

We sampled an acclimated treatment (Wallowa Hatchery stock) at the Wallowa Facility located at Wallowa Hatchery, on Spring Creek, a tributary of the Wallowa River (Figure 1). All planned experimental releases of non-acclimated treatments at the Wallowa Facility were completed in the spring of 1989. There was no non-acclimated treatment released at the Wallowa Facility in 1991. Comparison of smoltification indices between acclimated and non-acclimated treatments was accomplished by comparing the Wallowa Hatchery stock acclimated at the Wallowa Facility to the same stock retained at Irrigon Hatchery for release as the non-acclimated treatment at the Big Canyon Facility. Both treatments were reared to about the same target size and scheduled for approximately the same release date. A comparison between the two different acclimation facilities in the Grande Ronde River basin was accomplished with both stress and smoltification indices using the Wallowa Hatchery stock acclimated at the Wallowa Facility and the Wallowa Hatchery stock acclimated at the Big Canyon Facility (Appendix Table A-1 and A-2).

Juvenile steelhead from the Wallowa Facility and the Big Canyon Facility were a mixture of progeny of the Wallowa Hatchery stock adults returning to these facilities. Steelhead released from the Little Sheep Facility were progeny of a mixture of naturally-produced and hatchery fish that returned to that facility. All Little Sheep Facility hatchery stock adults that returned in 1990 were progeny of wild endemic stock parents. All broodstock were spawned in 1990 at either the Little Sheep Facility (Little Sheep stock) or the Wallowa Hatchery and Big Canyon Facility (Wallowa stock). Eyed eggs were incubated at Wallowa Hatchery, then transferred to, and hatched and reared at Irrigon Hatchery. Juveniles from each experimental treatment were cold-

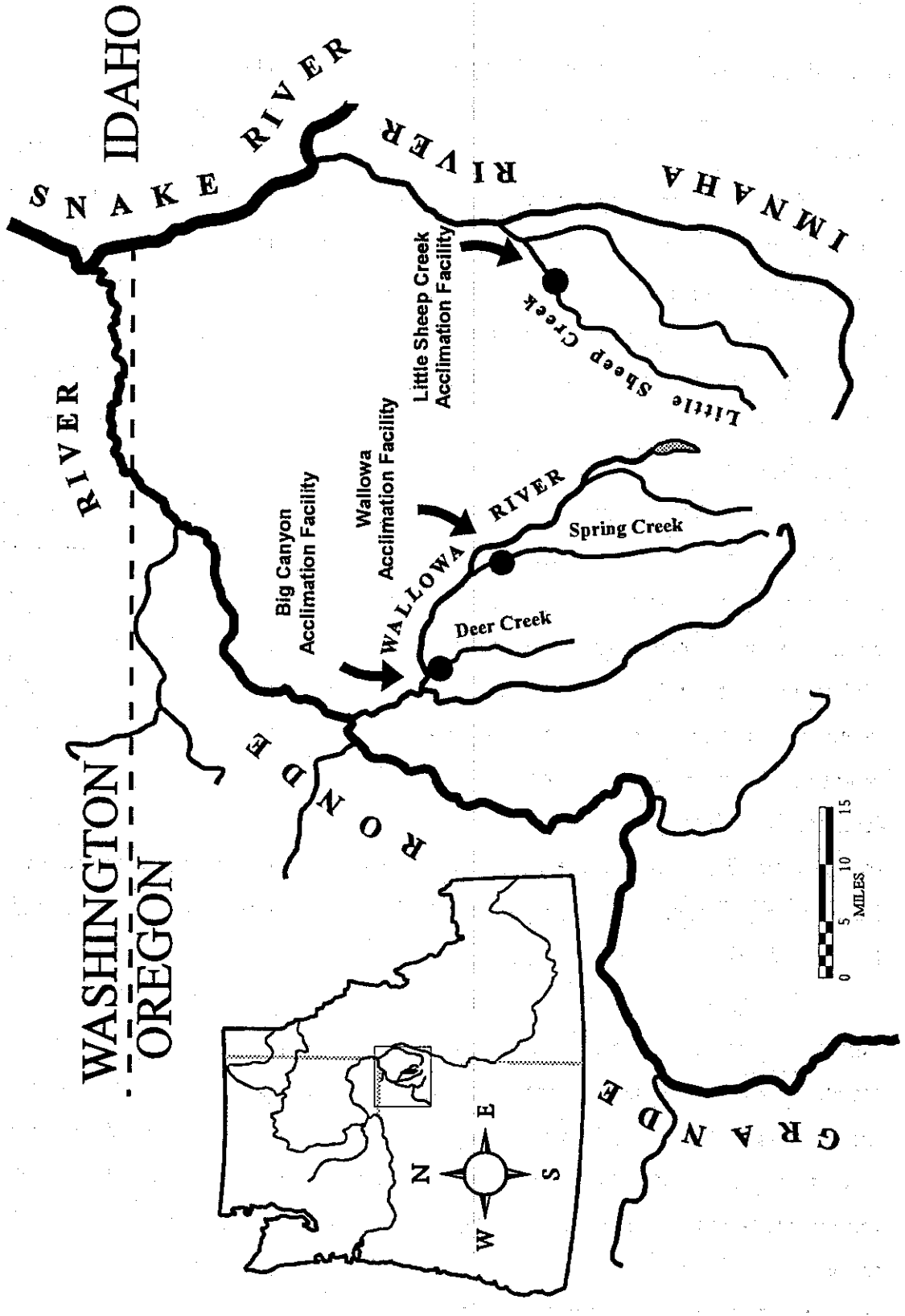

Figure 1. Location of acclimation facilities on the Grande Ronde and Imnaha river basins.

branded (February) and coded-wire-tagged (the previous fall) as part of the acclimation evaluation experimental design (Messmer et al. 1991b; Messmer et al. 1992).

The growth schedules at Irrigon Hatchery for the acclimated treatment were designed to achieve a size near the proposed size at release (5.0 fish per pound, ~91 g) before transfer, with the assumption that there would be little or no growth at the acclimation facility. The non-acclimated treatment was placed on a slightly slower growth schedule at Irrigon Hatchery from the beginning of feeding which was designed to produce fish that were 91 g at the time of release.

General Sampling Protocol

Fish at all acclimation facilities and those samples at Irrigon Hatchery were taken off feed for 36 to 48 hours before sampling. Fish were removed from the transport trucks (for non-acclimated treatments at release), hatchery raceways or acclimation ponds with a dip net. They were then either placed directly into anaesthetic and sampled immediately, or administered a stress challenge, placed in a container at the facility or the hatchery, and sampled later. Fork length (to the nearest millimeter (mm)) and weight (to the nearest 0.1 g) were recorded before physiological samples were taken.

Collection methods were slightly different at the hatchery compared to the acclimation facilities. Herding, by having one person walking along either side of the raceway, was necessary at Irrigon Hatchery because the fish were easily able to avoid capture. In acclimation ponds, fish were not observed avoiding capture as often, presumably because turbidity of the water and outside height of the pond sides allowed approach by samplers. In instances where fish at Irrigon Hatchery destined for an acclimation facility were held in several raceways, a percentage of our sample was removed from each raceway according to the proportion of the treatment in that raceway. Fish were generally removed from at least 3 different locations in the raceway or acclimation pond or were removed from each of the five compartments in the two transport trucks.

Sorting for cold-branded fish that were part of the acclimation evaluation was necessary for acclimated fish once they left the hatchery. Fish were netted from the pond or raceways in groups of no more than 6 or 7 fish. Fish were taken after equilibrium was lost and they were breathing at the bottom of the bucket. For fish that were not killed immediately (i.e. those administered a stress challenge and held for sampling later), exhaustion from that procedure served to slow the fish to the extent that they could easily be handled and sorted for brands without anaesthetic. To retain individuals of the appropriate brand groups, the net was suspended in a bucket of recovery water, and the fish were inspected for the appropriate brands (in less than 5 seconds). When acclimated fish were sorted for brands at the acclimation facility, non-acclimated fish taken from hatchery raceways or the transportation trucks were handled as if to inspect for brands to equalize the potential affect of inspection.

Stress Index Sampling

Because rearing conditions differ between acclimation facilities and Irrigon Hatchery, and between different acclimation facilities, stressors (conditions that cause a stress response) may result in differences in stress (as indicated by levels of physiological parameters), or stress response (as indicated by changes in the levels of physiological parameters). We investigated physiological indicators of stress before release (when each group was at its respective facility, prior to transport for the non-acclimated treatments), at release (after the non-acclimated treatment had been loaded onto the transport truck and hauled to the release site), and after the release of the cohort had occurred (a portion of the release group was retained for later sampling, and the rest of the fish from the treatment had been released). Fish taken after release of the rest of the cohort would be taken when they would have been required to cope with environmental conditions and additional stressors different from those within the hatchery).

Two physiological parameters were used as indices of stress, plasma cortisol concentration (nannograms per liter, ng/L) and plasma chloride concentration (milliequivalents per liter, mEq/L). Diel fluctuations in blood plasma cortisol concentrations have been observed in some salmonids (Zelnik and Goldspink 1981; Pickering and Pottinger 1983), but not others (Barton et al. 1980; Strange et al. 1977). Fish collected for comparisons between treatments or facilities

were taken (removed from the raceway) and sampled (tissue or blood removed) as close to one another chronologically as was logistically possible (usually within 2 hours) to control for potential diel fluctuations. Stress indices were monitored for both acclimated and non-acclimated fish at the Big Canyon and Little Sheep facilities. At the Wallowa Facility only the acclimated fish were available for sampling (Appendix Table A-1).

Differences between acclimated and non-acclimated treatments at the Little Sheep and Big Canyon facilities or between the two acclimated treatments at the Wallowa and Big Canyon facilities were tested under three different handling procedures: "baseline" (early in the morning; no stress challenge), "pre-stressed" (without a stress challenge) and "post-challenge" (after a stress challenge had been administered). The "baseline" group was taken near 0700 hours on the day of (acclimated treatment) or the day before (non-acclimated treatment) release and sampled immediately. The "pre-stressed" group was taken and sampled immediately a few minutes after the post-challenged groups were administered the stress challenge. Fish in the "post-challenged" groups were taken and administered a standardized stress challenge (held out of the water in a net for 30 seconds) (Barton et al. 1985). They were then placed in a covered, floated 30-gallon plastic trash can with ~452 cm^2 screening on each of two sides to allow water circulation. Fish were sampled 1, 4, 12, 24, or 48 hours later. Fish were administered the stress challenge and placed in the trash cans in the holding areas in the reverse order in which they were to be sampled to minimize the disturbance to groups that would be sampled later (i.e. the group that was to be sampled 48 hours later was taken first). When post-challenge groups were sampled, the water was drained to a depth of 18 centimeters through the screens on the sides of the trash can. Fish were poured from the trash cans, straining off the remainder of the water, directly into the bucket with anaesthetic. This avoided the potential stressor of chasing fish with a net and resulted in the fish being placed into the anaesthetic as soon as possible.

Fish for the pre-stressed and post-challenged samples were removed from the two transportation trucks first, then from the acclimation facility. The trucks arrived about 30 or 90 minutes apart and were sampled in the order of arrival. Five non-acclimated fish were taken from each compartment in each truck, administered the stress challenge, and one was placed into each of

five buckets (for each of the five post-challenge groups). All fish from each bucket were transferred into 1 of 10 trash cans (5 for each truck). Then one fish was dipped from each compartment for the pre-stressed sample, placed directly into anaesthetic and sampled. Fish from the acclimation facility were then administered the stress challenge and placed in the garbage cans (post-challenged samples) or anaesthetized and sampled immediately (pre-stressed samples). At the Big Canyon Facility the trash cans were held in a pond adjacent to the acclimation pond. At the Little Sheep and Wallowa facilities the containers were held in sections of the adult holding ponds separated from adults. The last group of fish taken was always the pre-stressed sample which was placed directly into the bucket of anaesthetic and sampled thereafter. Sample size for groups was generally 10 fish (Appendix Table A-1).

The stage during release at the acclimation facility when fish were taken from the pond for stress sampling differed depending upon the facility. At the Big Canyon Facility, acclimated fish were taken after the water level had been drawn down and about half of the fish were visually estimated to have been crowded out. The other facilities were drawn down very slowly, either overnight (Little Sheep Facility) or over a period of 2 days (Wallowa Facility). Therefore acclimated fish were taken from these facilities relatively early in the release process to assure that sampling occurred was at about the same time of day as the non-acclimated fish.

Blood plasma was sampled from fish that were anaesthetized in approximately 10 liters of MS-222 (tricaine methanesulfonate, 150 milligrams per liter (mg/L)) until equilibrium was lost. Fork length and weight were recorded and the fish was wrapped in a paper towel. The caudal peduncle was severed and blood was taken using a 250-microliter ammonium-heparinized capillary tube which was placed against the caudal vasculature, mainly the dorsal aorta. Blood was then aspirated into an ice-cooled 0.4 ml microcentrifuge tube. Tubes were centrifuged at 1720 *g* using an IEC microcentrifuge Model Micro-MB, with a #837 rotor at ambient temperature for 4 minutes within about 0.5 hours after the first fish in the group was bled. Plasma was pipetted off into another ice-cooled 0.4 ml microcentrifuge tube, sealed and stored in liquid nitrogen (-196°C) until transfer to an ultra-low freezer (-70°C) for storage. Samples were transported to the USFWS (United States Fish and Wildlife Service) laboratory (Cook, WA)

packed in dry ice. We analyzed plasma for chloride concentration using a 10-μl sample in a Haake-Buchler Digital Chloridometer. Samples were then refrozen and transported on dry ice to Biotech, Inc. (Corvallis, OR) within 48 hours. Plasma cortisol analyses were conducted at Biotech Inc. following the procedures of Redding et al. (1984).

In addition to sampling around the time of release, we took pre-stress and post-challenge samples before transfer of the acclimated treatment. Pre-transfer groups (PT) were sampled no more than 2 days prior to transfer of the acclimated fish to acclimation facilities. Fish for the pre-transfer, pre-stressed samples were placed directly into MS-222 and sampled after equilibrium loss. Post-challenged fish were sampled about 1 hour after the stress challenge. Sample sizes were generally 20 fish (Appendix Table A-1).

Smoltification Index Sampling

Two physiological parameters were used as indices of smoltification for these experiments, gill ATPase (micromoles of ATP hydrolyzed per milligram of protein per hour, μmole P_i/mg protein/h) and guanine concentrations in the skin (milligrams per square millimeter, mg/mm^2). Physiological indices of smoltification were monitored for both acclimated and non-acclimated treatments at the Big Canyon and Little Sheep Facilities. Samples of only acclimated fish were taken at the Wallowa Facility. Samples were taken at four dates during rearing from 12 March through 23 April for the Little Sheep Facility, 14 March through 26 April for the Big Canyon Facility, and 25 March through 21 April for the Wallowa Facility (Appendix Table A-2). Sample dates were described as: pre-transfer (PT), when both acclimated and non-acclimated treatments were still at the hatchery within 2 days prior to transfer of the acclimated treatment; one-third acclimated (1/3), when fish were approximately one-third of the way through acclimation (after acclimation about 8 to 13 days, depending upon the facility); two-thirds acclimated (2/3), when fish were approximately two-thirds of the way through acclimation (after acclimation about 16 to 26 days); and within two days prior to release (RE) (Table 1). Sample sizes were generally 20 for each treatment (Appendix Table A-2).

fish were immobilized, gill filaments from the 2nd and 3rd gill arches on the left side of the fish were removed and placed in a fixative solution of sucrose, Na_2 EDTA and imidazole. The capped sample was shaken to coat the tissue with fixative, then the sample was frozen in liquid nitrogen at -196°C until being transferred to an ultra-low freezer (-70°C) for storage until analysis. The samples were delivered on dry ice to the USFWS laboratory (Cook, WA). Personnel at the laboratory analyzed them following the method of Zaugg (1982).

The skin samples were taken from these same fish after gill samples were removed. Whole fish were frozen using liquid nitrogen. A circular plug of skin on the left side of the fish immediately ventral to the lateral line bisected by the anterior insertion of the dorsal fin (Figure 2) was cut with a standard #9 cork borer (~201 mm^2) and removed with tweezers. Any adhering muscle tissue was removed from the skin, then the sample was placed in a vial which was frozen in liquid nitrogen. When a #9 cork borer would not produce a symmetrical circle (because the fish was too small), a #7 cork borer was used (~154 mm^2). Samples were transported in liquid nitrogen and transferred into an ultra-low freezer (-70° C). Analyses by the USFWS laboratory (Cook, WA) followed those described by Staley (1984).

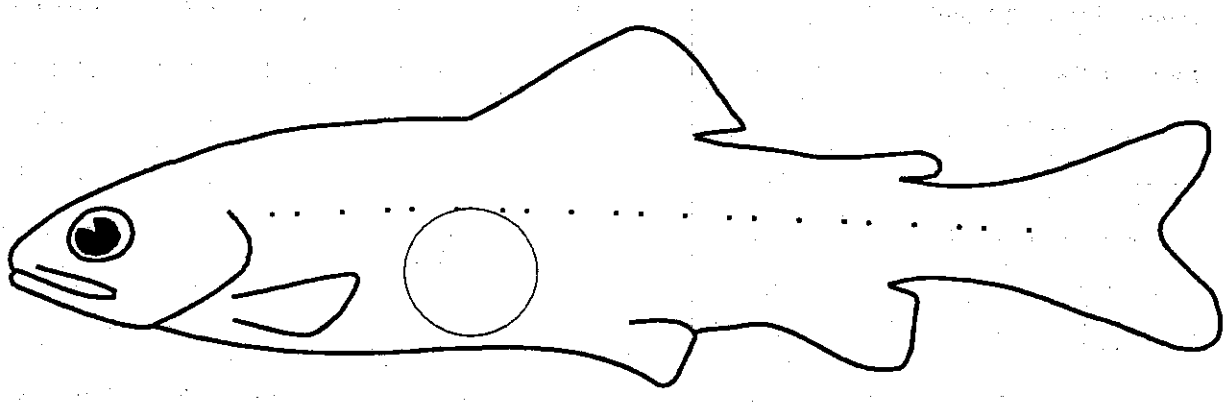

Figure 2. Area on juvenile steelhead where skin samples were taken.

Statistical Analyses

Bartlett's Test for homogeneity of variances of all four physiological indices revealed significant differences among variances. We transformed data to make variances homogeneous. Transformations yielded homogeneous variances for both the skin guanine [y = ln(100*guanine)] and ATPase [y= ln(ATPase)] data. Transformed smoltification data were analyzed using analysis of variance (ANOVA). A Tukey post-hoc test was performed for comparisons between treatments at each of the four dates and among dates within each treatment. Data distributions were illustrated as back-transformed means $\pm$ 95% confidence intervals which were asymmetric.

We used distribution-free tests for all comparisons of stress indices because arcsine and log transformations of plasma cortisol and plasma chloride data did not produce homogeneous variances. Mann-Whitney tests were used to compare treatments at individual sample times. We used the Kruskal-Wallis Test and Dunn's post-hoc comparison between the baseline (Time -8) and individual post-challenge times (Times 1, 4, 12, 24 and 48) to determine when recovery had occurred. Recovery was defined as stress-challenged groups that returned to and remained at or below baseline concentrations for plasma cortisol, or at or above baseline concentrations for plasma chloride. Differences between treatments were tested using $\alpha \leq 0.05$. Because we considered a Type II error more serious in determining when stress indices had returned to baseline concentrations, and because a higher α is normally used for multiple simultaneous comparisons (Daniel 1978), we used $\alpha \leq 0.30$ for comparisons between baseline samples and individual post-challenged samples. We illustrated these data as medians and interquartile ranges.

Results

Stress indices

Baseline plasma cortisol concentrations of acclimated and non-acclimated treatments were not significantly different from one another at both Little Sheep ($p \leq 0.257$) and Big Canyon ($p \leq 1.000$) facilities (Figures 3B and 3A). Plasma cortisol concentration of pre-stressed samples of the acclimated treatment was lower than that of the non-acclimated treatment at the Little Sheep Facility ($p \leq 0.028$) (Figure 3B), but there was no difference between the acclimated and non-acclimated treatments at the Big Canyon Facility ($p \leq 0.070$) (Figure 3A). After the stress challenge, plasma cortisol concentrations of acclimated treatments at the Little Sheep Facility were generally lower than the non-acclimated treatments (Time 1, $p \leq 0.023$; Time 4, $p \leq 0.199$; Time 12, $p \leq 0.000$) until 24 hours after the stress challenge. At that time an apparent increase in plasma cortisol concentration resulted in the acclimated treatment having higher plasma cortisol concentration than the non-acclimated treatment ($p \leq 0.014$). We saw no difference between treatments thereafter ($p \leq 0.597$) (Figure 3B). After the stress challenge at the Big Canyon Facility the acclimated treatment had lower plasma cortisol concentration than the non-acclimated treatment from 4 to 24 hours after the stress challenge ($p \leq 0.023$). An apparent increase in plasma cortisol concentration in the acclimated treatment between 24 and 48 hours after the stress challenge resulted in no significant difference between treatments 48 hours after the stress challenge ($p \leq 0.650$) (Figure 3A).

Differences in plasma cortisol concentration between baseline samples and stress-challenged samples (1 to 48 hours after the stress challenge) within a treatment occurred relatively consistently for both treatments at both the Big Canyon and Little Sheep facilities throughout the post-challenge period (Figures 3A and 3B). With the exception of the pre-stress sample ($p \leq 0.002$), there were no differences in plasma cortisol concentrations between the acclimated treatments for the Big Canyon and Wallowa facilities (Figure 3C).

Plasma chloride concentrations of the acclimated treatments were higher those of the non-acclimated treatments at both the baseline ($p \leq 0.028$) and pre-stress ($p \leq 0.001$) sampling at both

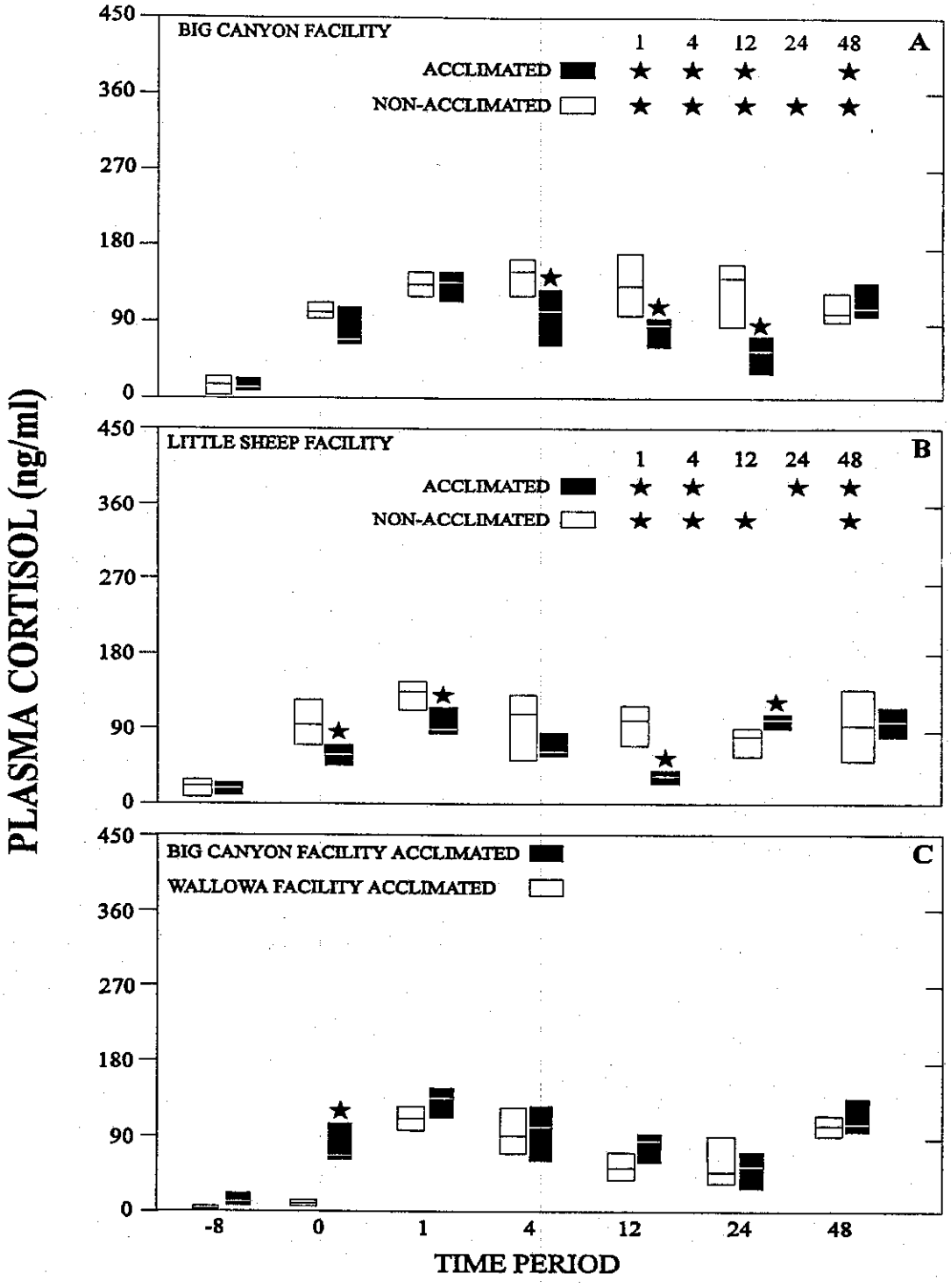

Figure 3. Medians and interquartile ranges of plasma cortisol concentrations of acclimated and non-acclimated juvenile summer steelhead at Northeast Oregon acclimation facilities and Irrigon Hatchery in 1991. Stars above medians indicate significant differences between treatments ($\alpha \leq 0.05$) at that time. Stars to the right of the legend indicate significant differences ($\alpha \leq 0.30$) between that time period and baseline samples (-8) within a treatment.

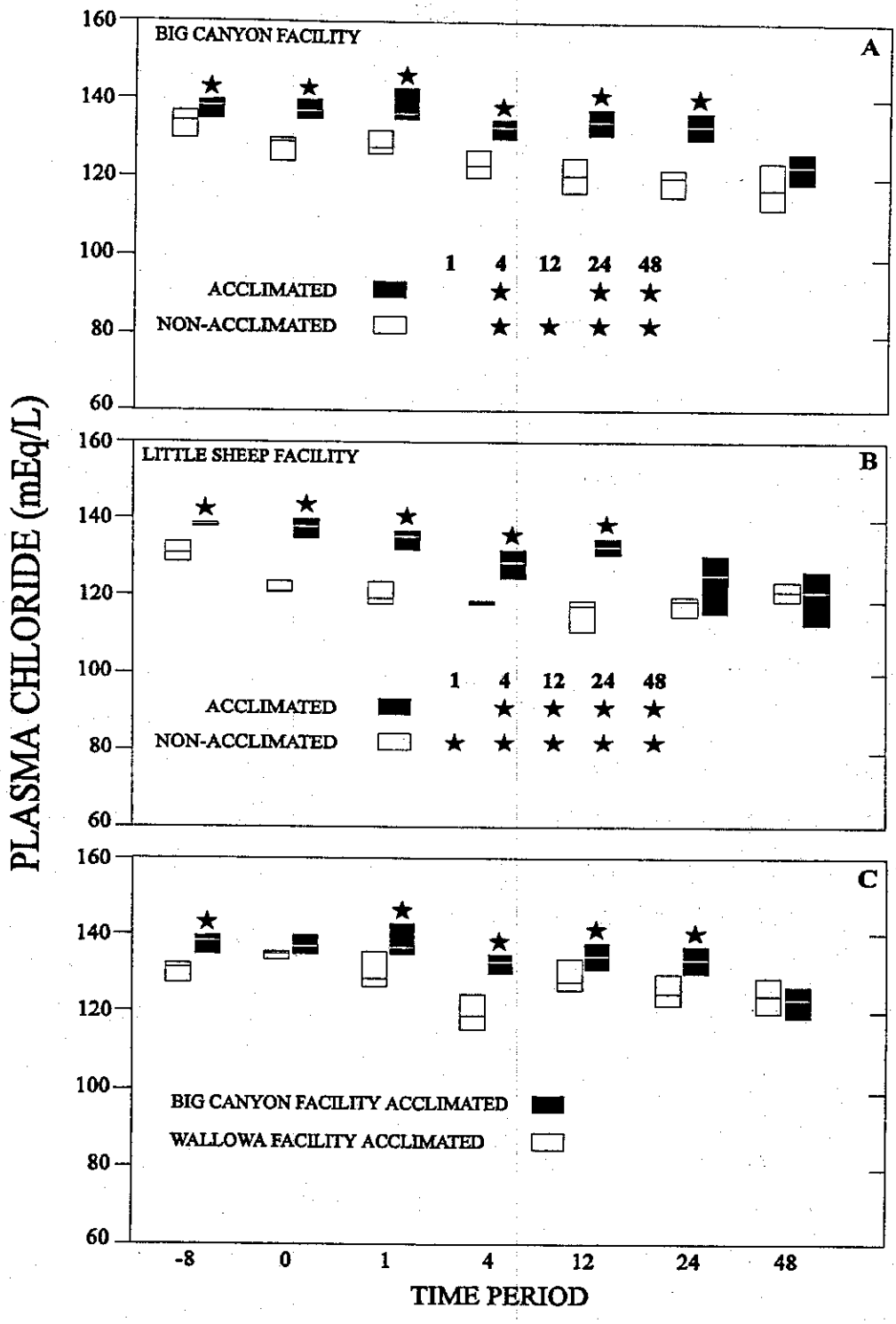

Figure 4. Medians and interquartile ranges of plasma chloride concentrations of acclimated and non-acclimated juvenile summer steelhead at Northeast Oregon acclimation facilities and Irrigon Hatchery in 1991. Stars above medians indicate significant differences between treatments ($\alpha \leq 0.05$) at that time. Stars to the right of the legend indicate significant differences ($\alpha \leq 0.30$) between that time period and baseline samples (-8) within a treatment.

Big Canyon and Little Sheep facilities (Figures 4A and 4B). Plasma chloride concentration of the acclimated treatments continued to be higher than the non-acclimated treatments during the post-challenge period through 12 hours after the stress challenge at the Little Sheep Facility ($p \leq 0.001$) and through 24 hours after the stress challenge at the Big Canyon Facility ($p \leq 0.011$). Consistent with timing of apparent changes in plasma cortisol concentrations in the acclimated treatments, decreases in plasma chloride concentration 48 hours after the stress challenge at the Big Canyon Facility and 24 hours after the stress challenge at the Little Sheep Facility resulted in no significant differences between treatments at those times or thereafter (Figures 4A and 4B). The timing of decreases in plasma chloride concentrations were consistent with the timing of increases in plasma cortisol concentrations.

Consistent with plasma cortisol data, plasma chloride data suggested that recovery did not occur. Differences in plasma chloride concentrations between baseline samples and stress-challenged samples were not observed until later in the experiment than occurred with plasma cortisol concentrations. Typically significant differences were first observed at 4 hours after the stress challenge and continued through 48 hours after the stress challenge (Figures 4A and 4B). With the exceptions of Time 0 and Time 48, plasma chloride concentrations of acclimated treatments at the Big Canyon Facility were higher than those at the Wallowa Facility (Figure 4C).

Results for all statistical comparisons for plasma cortisol concentrations and plasma chloride concentrations are in Appendix Tables A-3 and A-4.

Smoltification Indices

There were no significant differences between treatments in ATPase at the PT dates for any facility (Figures 5A, 5B and 5C). Thereafter, the only difference noted between treatments was at the Big Canyon Facility where the acclimated treatment showed significantly higher levels of ATPase than the non-acclimated treatment at the 1/3 and RE sample dates ($p \leq 0.032$; $p \leq 0.033$) (Figure 5A).

Of all of the comparisons of ATPase among sample dates within treatments, the only difference noted was within the Little Sheep non-acclimated treatment, where the activity on the 2/3 sample date was lower than that at the 1/3 date (Appendix Table A-6). The only instance where there were differences in mean ATPase between the Wallowa and Big Canyon acclimated treatments was at the 1/3 date when ATPase at the Big Canyon Facility was higher than that at the Walllowa Facility ($p \leq 0.020$) (Figure 5D).

Few differences in skin guanine between acclimated and non-acclimated treatments were observed. There were no significant differences in skin guanine concentration at the pretransfer dates between treatments for any facility (Figure 6A, 6B and 6C). However, the acclimated treatments at the Big Canyon and Wallowa ($p \leq 0.020$) facilities had significantly higher skin guanine on the 1/3 dates than the non-acclimated treatments (Figures 6A and 6C). No significant differences between treatments were observed thereafter. From the pretransfer dates to the release dates, skin guanine increased in every treatment ($p \leq 0.001$). Increases in skin guanine above that at pretransfer within a treatment were first observed as early as the 1/3 date with the acclimated treatment at the Wallowa Facility ($p \leq 0.010$), the 2/3 dates for both treatments at the Little Sheep Facility ($p \leq 0.001$), without any significant increases until the RE dates for both treatments at Big Canyon Facility ($p \leq 0.001$) (Appendix Table A-2).

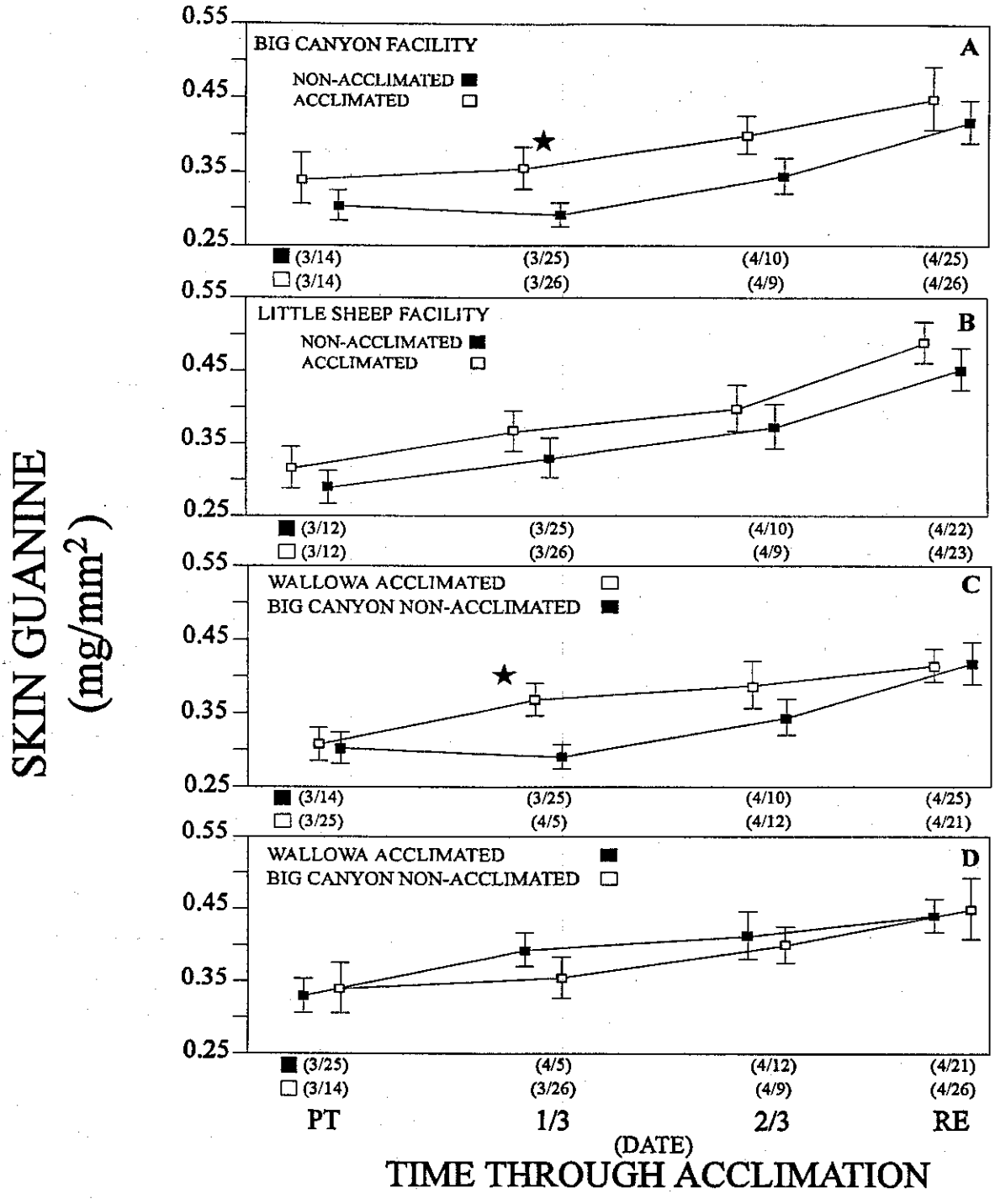

Figure 5. Mean skin guanine concentrations and 95% confidence intervals (bars) for acclimated and non-acclimated juvenile summer steelhead at Northeast Oregon acclimation facilities and Irrigon Hatchery in 1991. Stars above means indicate significant differences ($\alpha \leq 0.05$) at that date between treatments. PT = pre-transfer; ⅓ = after ⅓ of the acclimation time; ⅔ = after ⅔ of the acclimation time; RE = within 2 days of release.

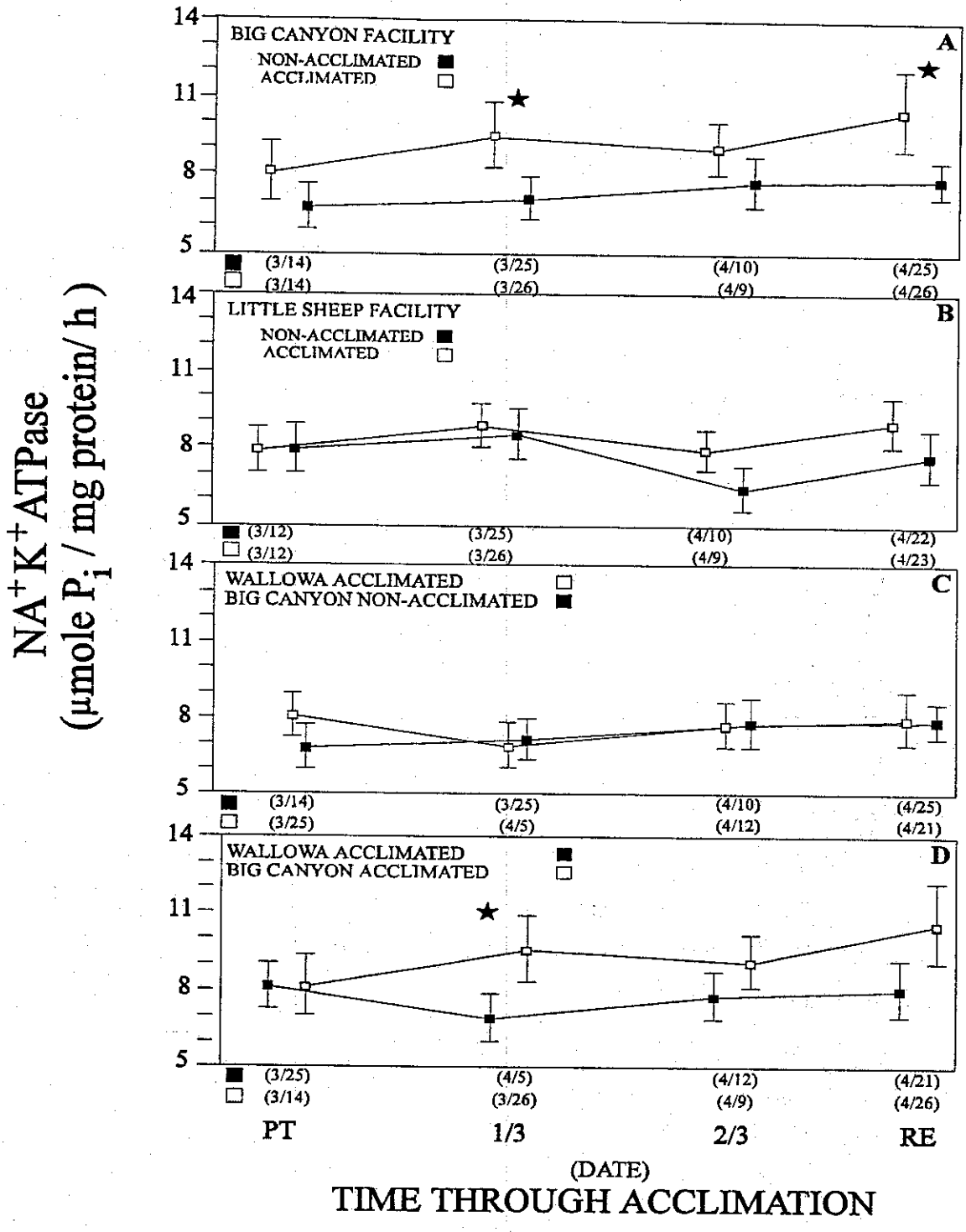

Figure 6. Mean gill Na^+K^+-ATPase activities and 95% confidence intervals (bars) for acclimated and non-acclimated juvenile summer steelhead at Northeast Oregon acclimation facilities and Irrigon Hatchery in 1991. Stars above means indicate significant differences ($\alpha \leq 0.05$) at that date between treatments. PT = pre-transfer; ⅓ = after ⅓ of the acclimation time; ⅔ = after ⅔ of the acclimation time; RE = within 2 days of release.

Discussion

Stress indices

Acclimation appeared to have produced lower stress in juvenile summer steelhead acclimated at LSRCP facilities in 1991 compared to non-acclimated fish. Consistently higher plasma chloride concentrations of the two acclimated treatments compared to non-acclimated treatments at baseline sampling suggested that differences in stress between treatments may have been evident before transport. Low plasma chloride concentration (hyperchloremia) has been used as an indicator of acute rather than chronic stress in juvenile salmonids (Wedemeyer 1972). Hyperchloremia has, however, also been used as an indicator of chronic stress for adult Atlantic salmon exposed to acidic water (Brown et al. 1990). If hyperchloremia of the non-acclimated juveniles was indicative of greater chronic stress than acclimated fish, juvenile summer steelhead at Oregon LSRCP acclimation facilities may have been exposed for a shorter time to stressors or perhaps less intense stressors compared to the non-acclimated treatments.

Because crowding and handling associated with transport have been shown to be stressful (Barton et al. 1980), we expected differences between treatments, if they occurred, to be most evident as a result of the differences between the two treatments in the length of time between transport and release. We investigated evidence for differences between acclimated and non-acclimated treatments in stress level after transport using three criteria: 1) stress indices at the time of release, 2) stress indices after a stress challenge, and 3) the length of time each treatment required to recover. We found evidence for differences in stress between treatments in the first two of the three criteria.

Our data indicated that acclimated treatments were less stressed than non-acclimated treatments at the time of release. Stress levels of the acclimated treatments were lower than those of the non-acclimated treatments in three of the four comparisons (two facilities, two indices). Plasma chloride concentrations in the acclimated treatments at both facilities were higher than those in the non-acclimated treatments. With plasma cortisol concentrations, a similar effect was seen at the Little Sheep Facility, where values of the acclimated treatment were lower than those of

the non-acclimated treatment at the time of release. No difference between treatments was noted at the release time at the Big Canyon Facility. At this facility, a relatively large increase in plasma cortisol concentration in the acclimated treatment from baseline to the time of release resulted in no differences between the two treatments until later in the experiment. This increase in plasma cortisol concentration may have been due in part to crowding of the acclimated treatment out of the raceways at the Big Canyon Facility.

Acclimated treatments continued to be less stressed during the early part of the post-challenge period. Differences between the acclimated and non-acclimated treatments after the stress challenge were evident at both facilities with both stress indices. Plasma chloride concentrations of the acclimated treatments were consistently higher than those of the non-acclimated treatments until at least 12 hours after the stress challenge. Likewise plasma cortisol concentrations of the acclimated treatments were lower than those of the non-acclimated treatments until at least 12 hours after the stress challenge. There were no differences in stress between the acclimated and non-acclimated treatments at the Big Canyon Facility an hour after release of the cohort (similar to release). Differences between treatments were not evident until 3 hours later (4 hours after the stress challenge).

Because none of the groups appeared to have recovered from the stress challenge within the timeframe of this experiment, we could not compare the length of time it took to recover between acclimated and non-acclimated treatments. However, two pieces of data indicated that the acclimated treatments may have had the capacity to recover more quickly. First, the stress responses, as indicated by an increases in plasma cortisol concentrations or decreases in plasma chloride concentrations, appeared to have been, in general, smaller in the acclimated treatments compared to the non-acclimated treatments. A smaller stress response would mean a smaller change was necessary to return to baseline levels, possibly occurring in a shorter period of time. Secondly, of the 4 treatments that had a transitory increase (plasma chloride concentration) or decrease (plasma cortisol concentration) to baseline concentrations, three of the four were the acclimated treatments. This would suggest that it may have been more likely that had we observed recovery, it might have occurred first in the acclimated treatment. In the case of the

Little Sheep Facility, where a transitory return to baseline was observed in both treatments, it was still observed earlier in the acclimated treatment.

Although there was a general lack of difference between the acclimated treatments at the Wallowa Facility and Big Canyon Facility in plasma cortisol concentrations, consistently lower plasma chloride concentrations at the Wallowa Facility may have indicated a chronic stress problem at that facility. No epidemic outbreak of Wallowa Acclimation Pond Syndrome occurred in 1991. However, there have been a few fish at the Wallowa Facility each year that have exhibited the classic symptoms: lesions in body musculature, opaque eyes that are often eroded, and hemorrhaged gastrointestinal tract (W. Groberg, ODFW, personal communication), suggesting that the syndrome was present, but at less than epidemic levels.

Smoltification

Acclimation of juvenile steelhead at LSRCP facilities did not appear to increase the smoltification process compared to non-acclimated fish in 1991. The occasional differences between treatments in ATPase appeared to have been due to fluctuations in that index within treatments rather than consistent differences between treatments. This was indicated by data that showed no consistent change in ATPase in either treatment during the length of the experiment. Although there was a consistent increase in skin guanine of the acclimated fish during over the experiment, a similar increase was observed in the non-acclimated treatment, indicating that the increase was not related to acclimation.

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Appendix Table A-1. Summary of information for juvenile summer steelhead that were sampled for stress indices at Northeast Oregon Hatchery facilities in 1991.

Acclimation Facility	Date	Time Period[a]	Stressor Applied	Acclimated n	Acclimated Time, h	Non-acclimated n[b]	Non-acclimated Time, h
Little Sheep	3/12	PT-0	No	21	1212-1235	21	0907-1044
	3/12	PT-0	Yes	20	1335-1352	20	1055-1121
	4/23[c]	-8	No	10	0724-0741	10	0706-0724
	4/23	0	No	10	1633-1644	10	1458-1534
	4/23	1	Yes	10	1733-1744	10	1557-1654
	4/23	4	Yes	10	2044-2056	10	1853-1934
	4/24	12	Yes	10[d]	0443-0455	10	0255-0332
	4/24	24	Yes	10	1645-1655	10	1455-1535
	4/25	48	Yes	10	1644-1656	10	1504-1635
Big Canyon	3/14	PT-0	No	21	0939-1009	21	1136-1204
	3/14	PT-0	Yes	21	1032-1104	20	1239-1310
	4/26[c]	-8	No	10	0718-0734	10	0710-0736
	4/26	0	No	10	1510-1517	10	1346-1430
	4/26	1	Yes	10	1553-1558	10	1440-1535
	4/26	4	Yes	10	1853-1905	10	1743-1829
	4/27	12	Yes	10	0256-0307	10	0142-0232
	4/27	24	Yes	10	1455-1505	10	1343-1431
	4/28	48	Yes	10	1313-1319	10	1347-1435
Wallowa	3/25	PT-0	No	20	1022-1112		
	3/25	PT-0	20	Yes	1313-1319		
	4/21	-8	No	10	0716-0748		
	4/21	0	No	10	1439-1452		
	4/21	1	Yes	10	1525-1537		
	4/21	4	Yes	10	1824-1838		
	4/22	12	Yes	10	0224-0235		
	4/22	24	Yes	10	1428-1437		
	4/23	48	Yes	10	1203-1213		

a Unchallenged groups: PT-0 = pre-transfer; -8 = approximately 8 hours before release; 0 = at release. Stress-challenged groups: PT-1 = pre-transfer, one hour after challenge; Times 1 to 48, 1 to 48 hours after stress challenge.

b Five fish were removed from each truck. Sample times cover both trucks which arrived about 30 or 90 minutes apart.

c Sample date was the previous day for the non-acclimated fish.

d Sample size for the plasma chloride analysis was 9.

Appendix Table A-2. Summary of information for juvenile summer steelhead that were sampled for smoltification indices at Northeast Oregon Hatchery facilities in 1991.

Acclimation Facility	Date	n	Time Period[a]	Treatment[b]	Time
Little Sheep	3/12	21	PT	Acc	1212-1235
	3/26	20	1/3	Acc	1340-1506
	4/09	21[c]	2/3	Acc	1249-1413
	4/23	20[d]	RE	Acc	0724-0759
	3/12	21	PT	Non	0907-1044
	3/25	19[c]	1/3	Non	1552-1619
	4/10	20[d]	2/3	Non	1211-1333
	4/22	20[d]	RE	Non	0706-0742
Big Canyon	3/14	21	PT	Acc	0939-1009
	3/26	19	1/3	Acc	0843-1041
	4/09	20	2/3	Acc	0840-1011
	4/26	20	RE	Acc	0718-0748
	3/14	21[c]	PT	Non	1136-1204
	3/25	20	1/3	Non	1444-1513
	4/10	20	2/3	Non	0957-1135
	4/25	20	RE	Non	0710-0750
Wallowa	3/25	20[d]	PT	Acc	1022-1112
	4/05	20	1/3	Acc	1224-1338
	4/12	21[e]	2/3	Acc	1113-1252
	4/21	20[d]	RE	Acc	0716-0821

a PT = Pretransfer; 1/3 = one third of the way through acclimation; 2/3 = two-thirds of the way through acclimation; RE = within 2 days prior to release.

b Acc = acclimated treatment, Non = Non-acclimated treatment.

c Guanine sample was 20.

d Guanine sample was 19.

e Guanine sample was 18.

Appendix Table A-3. Probability values for results of Mann-Whitney Tests for differences in plasma cortisol and plasma chloride concentrations between acclimated and non-acclimated treatments for each time for the Little Sheep and Big Canyon facilities and between acclimated treatments at the Wallowa and Big Canyon facilities. Significant values are in bold.

Time[a]	Little Sheep		Big Canyon		Wallowa/ Big Canyon[b]	
Period	Cortisol	Chloride	Cortisol	Chloride	Cortisol	Chloride
PT-0	**0.003**	0.585	0.753	0.211	0.137	**0.001**
PT-1	**0.013**	0.149	0.938	0.425	0.620	0.091
-8	1.000	**0.001**	0.257	**0.028**	0.112	**0.001**
0	**0.028**	**0.000**	0.070	**0.000**	**0.002**	0.073
1	**0.023**	**0.000**	0.940	**0.001**	0.121	**0.001**
4	0.199	**0.000**	**0.023**	**0.011**	0.940	**0.001**
12	**0.000**	**0.000**	**0.001**	**0.000**	0.070	**0.017**
24	**0.014**	0.057	**0.001**	**0.000**	0.935	**0.005**
48	0.597	0.909	0.650	0.120	0.650	0.567

[a] Unchallenged groups: PT-0 = pre-transfer; -8 = approximately 8 hours before release; 0 = at release. Stress-challenged groups: PT-1 = pre-transfer, one hour after challenge; Times 1 to 48, 1 to 48 hours after stress challenge.

[b] Comparison between the acclimated treatments.

Appendix Table A-4. Calculated Z values for Dunn's multiple comparison's test for differences in plasma cortisol concentration and plasma chloride concentration between unchallenged baseline samples (-8) and post-challenge samples (1-48) within treatments. Significant values ($\alpha \leq 0.30$) are in bold.

Index[b]	Facility	Treatment[c]	Time[a] 1	4	12	24	48
Cor	Big Canyon	Acc	**52.60**	**37.30**	**28.60**	16.40	**44.70**
		Non	**40.05**	**42.80**	**39.70**	**36.75**	**26.80**
	Little Sheep	Acc	**40.00**	**23.90**	5.40	**43.50**	**41.80**
		Non	**40.70**	**25.80**	**23.10**	14.50	**24.70**
Chl	Big Canyon	Acc	0.30	**23.20**	12.05	18.50	**41.20**
		Non	15.15	**29.90**	**37.20**	**44.00**	**39.45**
	Little Sheep	Acc	11.50	**27.40**	**19.17**	**35.35**	**41.65**
		Non	**26.85**	**35.35**	**40.40**	**33.95**	**23.50**

[a] Times 1 to 48 were 1 to 48 hours after the stress challenge.
[b] Cor = plasma cortisol concentration; Chl = plasma chloride concentration.
[c] Acc = acclimated; Non = non-acclimated.
Note Critical values: 17.84 $\alpha \leq 0.30$; 21.79 $\alpha \leq 0.10$; 24.01 $\alpha \leq 0.05$.

Appendix Table A-5. Probability values for results of Tukey post-hoc HSD comparisons for differences in mean gill Na^+K^+-ATPase activity and skin guanine concentrations between acclimated and non-acclimated treatments for Little Sheep and Big Canyon facilities and between acclimated treatments at the Wallowa and Big Canyon facilities. Significant values are in bold.

Time Period[a]	Little Sheep Facility		Big Canyon Facility		Wallowa/Big Canyon[b]	
	ATPase	Guanine	ATPase	Guanine	ATPase	Guanine
PT	1.000	0.737	0.456	0.439	1.000	1.000
1/3	1.000	0.552	**0.032**	**0.012**	**0.020**	0.793
2/3	0.169	0.942	0.682	0.134	0.846	1.000
RE	0.556	0.879	**0.033**	0.911	0.103	1.000

[a] PT = Pre-transfer, 1/3 = 1/3 of the way through acclimation, 2/3 = 2/3 of the way through acclimation and RE = at release.

[b] Comparison between the acclimated treatments.

Appendix Table A-6. Matrix of probability values for the Tukey post-hoc HSD comparison results for differences among sample dates in gill Na^+K^+-ATPase activity and skin guanine concentrations within treatments. Significant values are in bold.

Facility, Treatment	Time Period[a]	ATPase			Guanine		
		PT	1/3	2/3	PT	1/3	2/3
Big Canyon, Acclimated	1/3	0.633			0.955		
	2/3	0.916	0.999		0.061	0.395	
	RE	0.071	0.962	0.729	**0.000**	**0.001**	0.437
Big Canyon, Non-acclimated	1/3	0.999			0.996		
	2/3	0.788	0.979		0.291	0.051	
	RE	0.681	0.949	1.000	**0.000**	**0.000**	**0.013**
Little Sheep, Acclimated	1/3	0.849			0.121		
	2/3	1.000	0.861		**0.001**	0.824	
	RE	0.756	1.000	0.770	**0.000**	**0.000**	**0.007**
Little Sheep, Non-Acclimated	1/3	0.991			0.248		
	2/3	0.147	**0.016**		**0.000**	0.377	
	RE	1.000	0.922	0.358	**0.000**	**0.000**	**0.017**
Wallowa, Acclimated	1/3	0.555			**0.010**		
	2/3	0.999	0.865		**0.000**	0.971	
	RE	1.000	0.673	1.000	**0.000**	0.290	0.876

[a] PT = Pre-transfer; 1/3 = 1/3 of the way through acclimation; 2/3 = 2/3 of the way through acclimation and RE = at release.

Assistance Provided To Cooperators

We provided assistance to Oregon Department of Fish and Wildlife in 1991 for ongoing hatchery evaluation research. Project personnel completed extensive spawning ground surveys for spring chinook salmon (*Oncorhynchus tshawytscha*) in the Grande Ronde and Imnaha river basins. We provided assistance in pre-release sampling of juvenile summer steelhead (*Oncorhynchus mykiss*) at Irrigon Hatchery and the Little Sheep Creek and Big Canyon Creek acclimation facilities and spring chinook salmon at Lookingglass Hatchery and the Imnaha River Acclimation Facilities. In addition, project personnel provided assistance in sampling adult spring chinook salmon and summer steelhead at Oregon LSRCP facilities. Assistance was provided in data summarization and analysis for ODFW monthly and annual progress reports. Data used in scale pattern analysis to differentiate the scales of hatchery from naturally-produced spring chinook salmon collected on spawning grounds was summarized and provided to the ODFW scale reading laboratory in Corvallis. Details of data collection, summarization and analysis are not included in this report and are available in ODFW reports.

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