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Effects of Routine Handling and Tagging Procedures on Physiological Stress Responses in Juvenile Chinook Salmon

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Abstract.—Juvenile chinook salmon *Oncorhynchus tshawytscha* were subjected to handling and tagging protocols typical of normal hatchery operations and monitored for their physiological response to stress. Treatments included coded-wire-tagging, counting, ventral fin clipping, adipose fin clipping, and a procedure simulating a pond split. Treatment fish were also subjected to a standardized stress challenge (1 h confinement) to evaluate their ability to deal with disturbances subsequent to a handling or tagging procedure. Circulating levels of cortisol and glucose were used as indicators of stress. Each of the treatments elicited very similar responses among treatment groups. Cortisol increased from resting levels of about 20 ng/mL to about 90 ng/mL by 1 h poststress and returned to near resting levels by 8 h poststress. Glucose levels increased from 50 mg/dL to about 80 mg/dL by 1 h poststress and remained elevated for much of the experiment. The cortisol and glucose responses to the confinement stress did not differ over time or among treatments. However, the confinement stress results do suggest a small but significant cumulative response, indicating small residual effects of the original handling protocols. No deaths were noted among treatment groups.

The handling of fish is a necessary component of fish culture. For example, juvenile spring chinook salmon *Oncorhynchus tshawytscha* are subjected to a variety of handling protocols before release from hatcheries in order to implant or attach tags, otherwise mark fish, assess fish quality or developmental status, and adjust rearing densities (Wood 1968; Nielsen 1992). Such procedures are important elements in salmonid culture programs because they permit monitoring of migrations and the efficacy of rearing regimens. However, any physical handling or psychological disturbance is known to be stressful to fish (Don-

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aldson 1981; Schreck 1981; Wedemeyer and McLeay 1981). Decreased survival of fish can result when stress levels are high because stress can be immediately debilitating and may also increase the potential for vulnerability to subsequent challenges (Schreck 1981, 1982). The relative severity and duration of stress associated with the many different handling procedures used in fish culture are poorly understood. Thus, our objectives in this study were to examine stress in fish subjected to the handling protocols most typical of normal hatchery operations (such as crowding, counting, sorting, fin clipping, and tagging) and to attempt to identify procedures or combinations of procedures that were particularly stressful.

Methods

Treatment groups.—The experiment was carried out beginning in April 1994 at the Cowlitz Salmon Hatchery, Salkum, Washington, with spring chinook salmon from the 1993 hatchery brood. Six study groups of subyearling fish (5.54 ± 0.16 g) were created, with three replicates of each group. For each replicate, about 250 fish were distributed into 1-m-diameter, 260-L flow-through (7.5 L/min) circular tanks and allowed to acclimate for 2 weeks. Fish were fed Bio-Moist at 1.7% body weight/d during acclimation. Food was withheld for 24 h before the experiment, and fish were not fed during the experiment. Water temperature was approximately 8°C over the course of the experiment. Groups of fish were randomly assigned to the 18 tanks. Treatment 1 was a control (CONTROL), and fish were disturbed as little as possible. Treatment 2 consisted of fish captured in a dip net, anesthetized in tricaine methanesulphonate (MS-222, 50 mg/L), adipose-fin-clipped, allowed to recover, reanesthetized, and coded-wire-tagged (TAGGED). Treatment 3 contained fish subjected to a procedure for separating fish from one raceway into two or more raceways (the fish were crowded, seined, weighed in a screen bucket, transferred through a header box, and returned to their tank; SPLIT). Treatment 4 contained fish that were passed through a Bioscanner® fish-counting device (the fish were crowded, netted, transferred to a bucket, carried to the Bioscanner, counted, and returned to their tank; COUNTED). Treatments 5 and 6 consisted of fish that were ventral-fin-clipped and adipose-fin-clipped, respectively (the fish were netted, anesthetized, fin-clipped, and returned to their tanks; VENTCLIP, ADCLIP). The TAGGED, VENTCLIP, and ADCLIP treatments were conducted in a Washington Department of Fish and Wildlife (WDFW) coded-wire-tagging trailer with standard protocols (Schurman and Thompson 1990) and an experienced tagging crew. Duration of each treatment is provided in Table 1. For each treatment, the three replicates were carried out consecutively. The actual times at which the fish received the handling stresses were staggered so that subsequent samples could be obtained with precise timing after onset of the stress without conflicting with other sampling.

TABLE 1.—Descriptions and durations of each treatment to evaluate the effects of routine handling and tagging procedures on juvenile chinook salmon.

Treatment	Description	Duration (min)
1 CONTROL	No handling	
2 TAGGED	Captured, anesthetized, adipose-fin-clipped, allowed to recover, reanesthetized, coded-wire-tagged, and returned to tank	15
3 SPLIT	Crowded, captured, weighed, moved, returned to tank	5
4 COUNTED	Crowded, captured, moved, counted, returned to tank	10
5 VENTCLIP	Captured, moved, anesthetized, ventral-fin-clipped, returned to tank	10
6 ADCLIP	Captured, moved, anesthetized, adipose-fin-clipped, returned to tank	10

In addition to the monitoring of the direct response to the various handling protocols, we evaluated changes over time in the ability of the fish to deal with an additional disturbance imposed after the initial handling stress. For this portion of the experiment, fish from each replicate of each treatment were subjected to a standardized stress challenge. Ten fish were removed from each tank at 8, 24, and 168 h after the completion of the initial treatment, placed in a perforated, translucent, plastic 0.5-L container, and held submerged for 1 h in a separate stainless steel flow-through trough that was on the same water supply as the treatment tanks.

Sampling.—Physiological samples were taken beginning April 4, 1994. For the monitoring portion of the experiment, samples were taken by dipnetting six fish from their tanks into a bucket containing a lethal concentration of MS-222 (200 mg/L buffered with 500 mg $NaHCO_3$/L). Efforts were made to minimize inadvertent disturbance of fish remaining in the tank being sampled and in adjacent tanks. These efforts included allowing only one person near the tanks at any time, maintaining a low profile when approaching the tanks, and working quickly when dipnetting

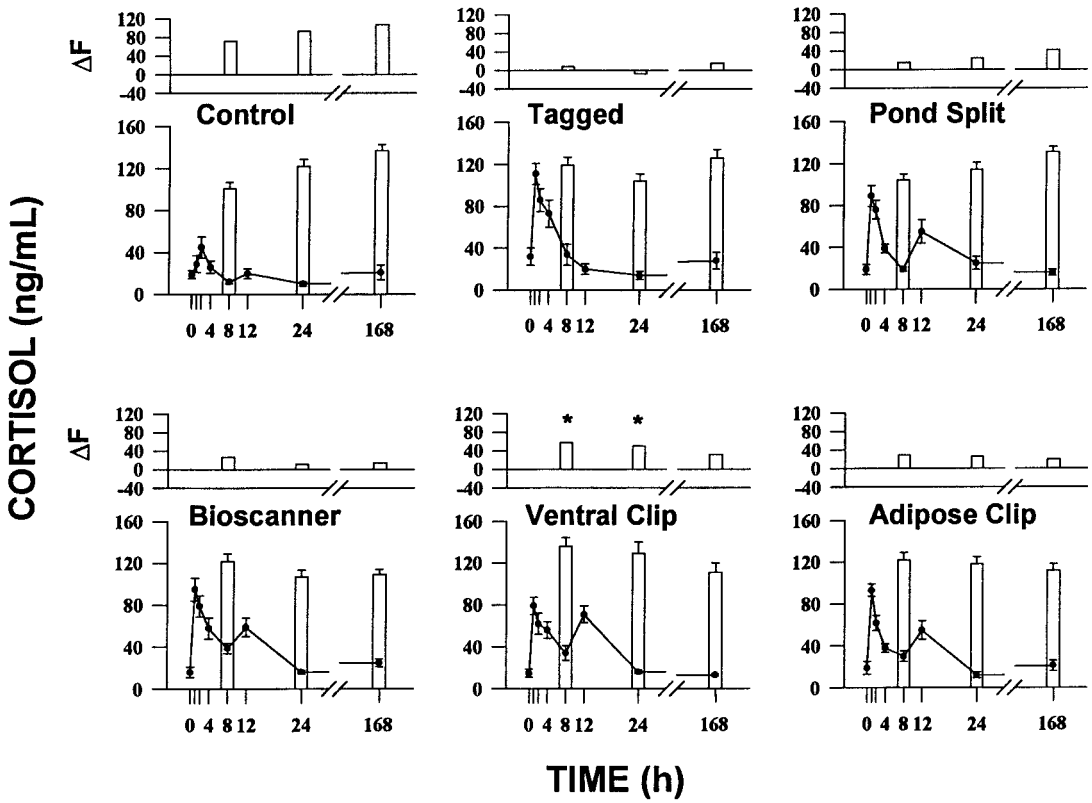

FIGURE 1.—Plasma cortisol concentration in fish subjected to various handling or marking stressors. The line in the lower graph of each pair provides the cortisol concentration (mean ± SE) after imposition of each handling stress. The bars in each lower graph show cortisol concentrations (mean ± SE) after the fish were subjected to a secondary stress challenge (confinement). The bars in each of the upper graphs show the difference between mean cortisol concentration 1 h after the secondary stress challenge and mean cortisol concentration 1 h after imposition of the original handling stress; an asterisk above the bar indicates the difference is significant ($P < 0.05$).

fish. Samples were taken immediately before treatment ($t = 0$ h) and at intervals of 1, 2, 4, 8, 12, 24, and 168 h posttreatment. For all fish, weight and fork length were measured, and blood was collected from the severed caudal peduncle in ammonium-heparinized capillary tubes. Blood-filled capillary tubes were held on ice until they could be centrifuged and plasma could be obtained. The plasma was immediately frozen. Plasma cortisol levels were established by radioimmunoassay (Redding and Schreck 1983; Foster and Dunn 1974), and glucose concentrations were determined by colorometric assay (Wedemeyer and Yasuake 1977). For the stress challenge portion of the experiment, the fish were removed from the containers after 1 h of confinement, and plasma samples were obtained as before.

Data analysis.—Cortisol and glucose levels were log-transformed to reduce both heterogeneity among variances and departure of sample distributions from normality. For each treatment group, a two-way analysis of variance (ANOVA) was performed on cortisol and glucose data to test for equality of means among replicates over time. Replicates did not differ significantly, and thus the data were combined across replicates. One-way ANOVA was performed on pooled cortisol and glucose data to test for equality of means among treatments at the individual time points and within treatments over time. When appropriate, multiple comparisons were performed with the Bonferroni multiple-comparison procedure. A significance level of 0.05 was used throughout.

Results and Discussion

Direct Response to Handling

Cortisol.—All of the handling protocols elicited a cortisol response comparable to the general re-

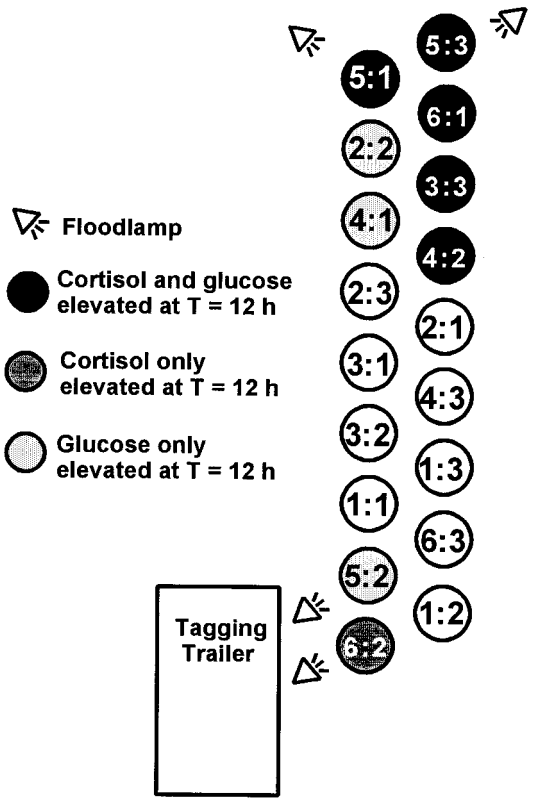

FIGURE 2.—Distribution of experimental tanks showing position of trailer and lighting. The shading indicates that at the time posttreatment (T) of 12 h the fish in those tanks had elevated cortisol or glucose levels relative to other replicates of the same treatment. Numbers in tanks indicate treatment group and replicate; treatment group codes are as in Table 1.

sponse to an acute stress (Figure 1). Initial cortisol levels (t = 0 h) were uniformly low for all six treatment groups, indicating that the animals were not stressed before the onset of the experiment. For all treatment groups except the CONTROL fish, circulating cortisol levels increased relative to control levels to a maximum at t = 1 h, but the peak cortisol response did not differ significantly among the treatment groups in which the fish were handled. At t = 2 h, cortisol levels did not differ among any of the groups. This is probably more a reflection of the slight increase in cortisol in the control fish rather than an indication that the handled fish had recovered to resting levels. In fact, at t = 4 and 8 h, cortisol levels in the control fish decreased to resting levels, and cortisol levels in all other treatment groups were again significantly greater than in control fish. The elevated cortisol level in the control fish at t = 2 h probably reflects the unavoidable minor disturbance of sequential sampling at t = 0 and 1 h. More problematic is an explanation for the tendency of the cortisol levels to be elevated in some treatment groups at t = 12 h. Although cortisol levels among replicates did not differ significantly within a treatment at any time, some replicates of the SPLIT, COUNTED, VENTCLIP, and ADCLIP treatment groups had cortisol levels that were significantly elevated over those of CONTROL and TAGGED fish at t = 12 h. Other workers have shown secondary rises in cortisol 6–24 h poststress that were apparently not the result of secondary disturbances (Barton et al. 1987; Mesa and Schreck 1989; Schreck et al. 1989), but inspection of a map of the layout of the tanks (Figure 2) reveals that the replicates with the highest cortisol levels at t = 12 h tended to be clustered together. Therefore, the elevation in cortisol at t = 12 h suggests an uncontrolled disturbance of fish occurring in some of the tanks after the 8-h sample was taken. We speculate that after dark, floodlamps near the ends of the tank field cast shadows on the end tanks when workers were sampling nearby. However, cortisol levels returned to resting or near resting levels in fish sampled at t = 24 and 168 h. At 24 h, the cortisol levels of the SPLIT treatment fish were slightly, but significantly, elevated over cortisol levels in CONTROL, TAGGED, and ADCLIP groups.

Glucose.—As with the cortisol data, the glucose response to the handling closely followed the general response expected from animals subjected to an acute disturbance (Figure 3). Glucose levels were low in all groups at t = 0 h and in the control fish throughout the experiment. Glucose levels in handled fish increased by 1 h posthandling and were generally sustained at high levels over 24 h. In all treatment groups, including controls, the glucose titer at t = 168 h showed a tendency to decrease. This probably reflects the fact that the fish were not fed over the course of the experiment. Other work has shown that the poststress hyperglycemic response is lower in fasted fish (Barton et al. 1988). Glucose levels at t = 12 h in the COUNTED and VENTCLIP groups were elevated. Again, this probably reflects the same disturbance suggested by examination of the cortisol data because both cortisol and glucose levels tended to be elevated in the same cluster of tanks.

Stress Challenge

Cortisol.—The cortisol response to the confinement challenge did not differ between confined treatment fish and confined control fish over time

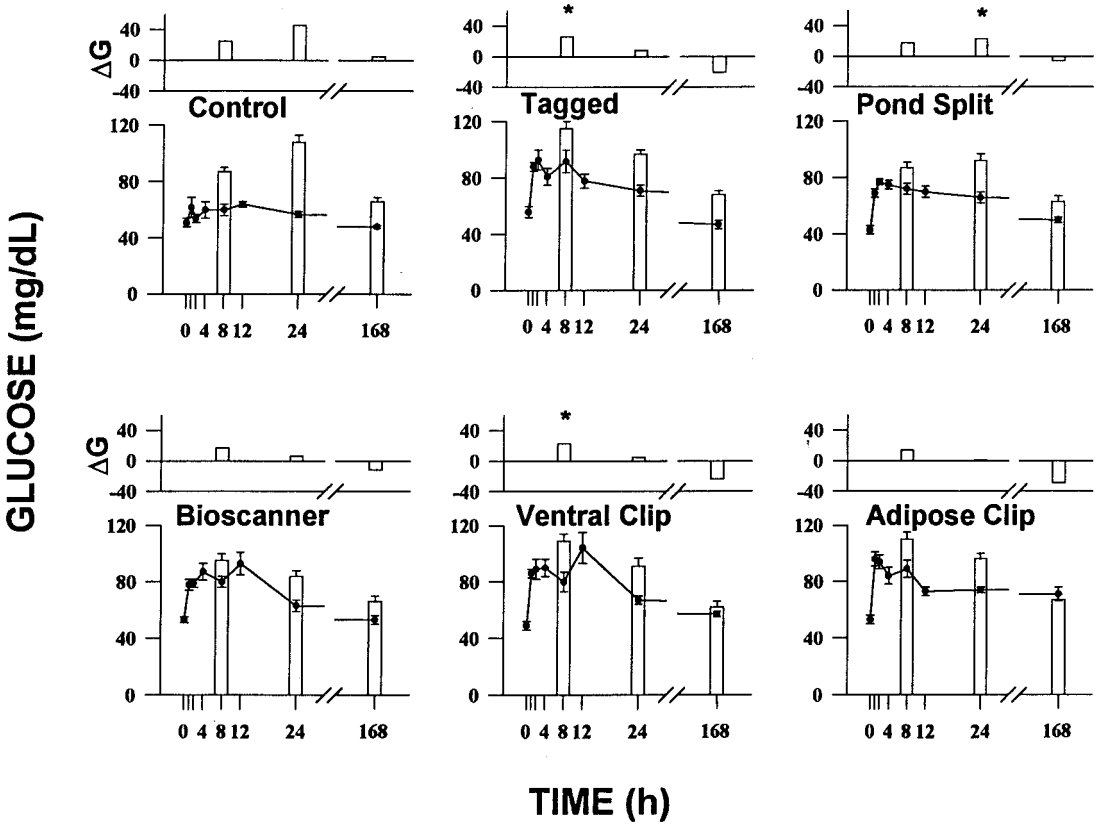

FIGURE 3.—Plasma glucose concentration in fish subjected to various handling or marking stressors. The line in the lower graph of each pair provides the glucose concentration (mean ± SE) after imposition of each handling stress. The bars in each lower graph show glucose concentrations (mean ± SE) after the fish were subjected to a secondary stress challenge. The bars in each of the upper graphs show the difference between mean glucose concentration 1 h after the secondary stress challenge and mean glucose concentration 1 h after imposition of the original handling stress; an asterisk above the bar indicates the difference is significant ($P < 0.05$).

or among treatments (ANOVA, $P > 0.05$; Figure 1, lower bar graphs). Cortisol levels in treatment fish subjected to the confinement stress were also compared with levels expressed in treatment fish 1 h after imposition of the initial treatments. Other work has shown that imposition of an additional stress on fish already responding to an initial disturbance can yield a cumulative physiological effect (Barton et al. 1986). In that work, cortisol and glucose levels in juvenile chinook salmon increased in a stepwise fashion in response to multiple acute handling stresses. We reasoned that a large positive difference between cortisol levels after a single and a subsequent second disturbance would indicate a large residual effect of the first disturbance. A small difference would suggest a small residual effect of the first disturbance, while a large negative difference would indicate that the effect of the first disturbance was so severe that the animals were exhausted and incapable of mounting an appropriate physiological response to an environmental disturbance. We noted a tendency for small positive differences in cortisol levels in each of the groups of fish receiving handling. That difference was statistically significant in one of the treatment groups (VENTCLIP, $P < 0.05$; Bonferroni multiple-comparison test) for two of the three times a secondary challenge was imposed (Figure 1, upper graphs). Taking the cortisol data alone, it appears that in these fish, the ability to mount a response to a second challenge is not compromised after 8 h of recovery but that even after an 8 h recovery, small residual effects of the first challenge were apparent.

Glucose.—The glucose response to the standardized stress challenge varied both among treat-

ments and over time (Figure 3). Glucose levels poststress in samples at $t = 8$ h were significantly elevated over control levels in the fish that received both the adipose clip (ADCLIP) and coded wire tags (TAGGED, $P < 0.05$; Bonferroni multiple-comparison test). Other treatment groups sampled at $t = 8$ h had glucose levels identical to those of control fish. Glucose levels in the samples taken at $t = 24$ h and at $t = 168$ h did not differ among control or treatment groups. It appears that, for glucose, a cumulative stress response was elicited in the TAGGED fish at $t = 8$ h but that sufficient recovery had occurred by $t = 24$ h that a cumulative effect was avoided. There was a strong tendency for glucose levels to be lowest after 168 h both before and after the 1-h confinement. Again, this probably reflects the fact that the fish were not fed over the course of the experiment. As with cortisol, we compared the difference between glucose levels 1 h after the initial treatment and 1 h after the confinement stress (Figure 3, upper graphs). This comparison was performed with the secondary stress challenges at 8 and 24 h only because the 168 h stress challenge results were apparently compromised by the fact that the fish had been fasted for a week. We noted significant positive differences in the challenged fish at 8 h in the TAGGED and VENTCLIP treatment groups and at 24 h in the SPLIT treatment group.

Conclusions

In this experiment, we have shown that individual handling procedures integral to many hatchery programs are indeed stressful and that each procedure has about the same physiological effect. However, the fish recover quickly, at least as measured by circulating cortisol levels. The glucose results indicate that the metabolic consequences of the handling procedures are more protracted than the initial (cortisol) response and that the fish exhibited a relatively greater response to the most protracted treatment (TAGGED), but only over the short term. For both cortisol and glucose, the ability to mount a physiological response to a second challenge was not compromised by any of the handling procedures, but relatively small residual effects were apparent. None of the handling procedures were associated with outright deaths. Thus, these results are of benefit to fishery managers, hatchery managers, and fish culturists because they indicate that carefully conducted handling and tagging operations need not compromise the well-being of the fish. However, a recovery period of at least 24 h, and perhaps longer, is warranted, especially if the animals are to be released into an environment more challenging than a hatchery raceway, such as a natural watercourse.

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