EVALUATION OF SHORT TERM SALTWATER IMMERSION TO ALLEVIATE HANDLING STRESS IN STEELHEAD TROUT

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Abstract

We evaluated the use of a short term saltwater immersion (10 minutes in isotonic saltwater) to alleviate handling stress in juvenile steelhead. Fish were assigned to either a control (freshwater recovery) or treatment group (saltwater immersion followed by freshwater recovery) and were blood sampled at five time periods (0, 1, 4, 6, and 24 hours) during recovery from a standardized stressor to determine plasma concentrations of the primary stress hormone cortisol and changes in plasma osmolality related to exposure to saltwater. Significant increases in plasma cortisol and osmolality indicative of the primary and secondary stress response were noted in fish in both the control and treatment groups at the 0 and 1 hour sampling periods. However, saltwater immersion had no effect on either the magnitude or duration of the stress response. Based on these findings, the use of a short term salt bath provides no benefit for mitigation of the stress response due to handling associated with tagging operations.

Introduction

When confronted with an external stress, an organism experiences a series of biochemical changes targeted at allowing the individual to maintain homeostasis and survive (Barton and Iwama 1991; Barton 2002). In fish, these biochemical changes include the release of catecholamines (adrenaline/noradrenaline) from the chromaffin tissue that induces the release of cortisol (colloquially termed the 'stress hormone') which, among many physiological changes, promotes the mobilization of energy reserves and diversion of blood flow to locomotary muscles and sensory organs (Barton and Iwama 1991; Morgan and Iwama 1997; Barton 2002). Over short time periods similar to predation events (termed acute stress), these changes provide the individual with the necessary energy and blood flow to avoid the stressor and survive (Barton and Iwama 1991; Barton 2002). However, if the stressor persists over extended time periods or is repeated multiple times (termed chronic stress), the organism can experience many negative consequences due to the fact that cortisol is generally seen as incompatible with daily life functions (Barton and Iwama 1991; Barton 2002). Chronic stress has been shown to cause decreases in immune function (and increased susceptibility to disease; Fries 1986; Barton and Iwama 1991), decreases in reproductive function (Campbell et al. 1992, 1994; Contreras-Sanchez et al. 1998), and increases in mortality. In a typical hatchery setting, juvenile Pacific salmon (*Oncorhynchus* spp.) encounter multiple stress events associated with rearing such as the air exposure, handling, and injury induced during implantation of coded wire tags (CWT) and passive integrated transponders (PIT), and the stress associated with these events has been implicated in causing juvenile mortality. As such, hatchery managers have long sought methods to mitigate the effects of these stress events and prevent associated delayed mortality.

A technique that has been proposed to alleviate stress in salmon production facilities is immersing fish in a saltwater bath following the PIT tagging event. Immersing fish in salt water has been theorized to prevent loss of blood ions due to acute stress events (Wedemeyer 1972; Carmichael et al. 1984). While originally counterintuitive as changes in salinity can induce a stress response in fishes (Fiol and Kültz 2007), studies of the effects of saltwater immersion to aid in recovery from handling stress have noted that immersion in isotonic saline water after a stress event can aid in decreasing recovery time (Barton and Peter 1992; Barton and Zitzow 1995; Reubush and Heath 1997). However, conflicting results have emerged as to whether immediate transfer to a salt bath after an acute stress event can wholly attenuate the corticosteroid stress response. Some studies have noted increases in plasma cortisol continue to occur in fish treated with salt (Harrell 1992; Barton and Zitzow 1995), while other studies have shown that the cortisol response is muted when fish are recovered in salt baths (Mazik et al. 1991; Carneiro and Urbanati 2001). Of the previously mentioned studies, two (Barton and Zitzow 1995; Reubush and Heath 1997) focused solely on placing fish in slightly saline water during extended recovery periods (between 1 and 48 hours), and both of these studies noted that the cortisol stress response was lessened and fish recovered from stress faster in saline recovery tanks. However, as recovering the number of fish commonly handled during mass marking in a production facility in saltwater for 24 hours is likely impossible, Abernathy Fish Technology Center (AFTC) proposed to determine if saltwater immersion for a short time period (10 minutes) immediately following an acute stressor is an effective method of ameliorating handling stress associated with PIT tagging of juvenile salmon in hatchery settings.

Materials and Methods

Juvenile steelhead were sampled on November 17th and 18th, 2009, concurrent with hatchery PIT tagging. Untagged fish (N = 60) were randomly selected for inclusion in the study. After overnight acclimation, all fish in each treatment group were subjected to standard acute stressor of air exposure for 120 seconds. Following stress, salt treated fish were placed in a 1.0% saline solution for a 10 minute immersion being moved to a recovery tank (~90 L aluminum hatchery incubation tank) supplied with a constant supply of freshwater. Conversely, control fish were transferred directly to recovery tanks following acute stress. A subset of fish (N = 6) was blood sampled via caudal venipuncture at five time periods (0, 1, 4, 6, and 24 hours) following stress and treatment by being rapidly euthanized with a lethal dose of anesthesia to minimize stress effects due to handling and blood sampling. Following sampling, blood was centrifuged at 10,000x gravity and plasma vials were stored at -80°C until further analysis.

Plasma samples were analyzed for concentrations of circulating cortisol, indicative of the primary stress response, using a commercially available ELISA kit (Cat. #901-071, Assay Designs, Ann Arbor, MI) following protocols included with the kit. Plasma was also analyzed for plasma osmolality (mOsm/Kg H_2O) using an automatic osmometer (Osmette II Model 5005, Precision Systems Inc., Natick, MA). To determine if there were differences for each response variable (plasma cortisol and osmolality) between treatments, between time periods, and if there was an interaction between these factors, a fully crossed two way analysis of variance (ANOVA) with Tukey's post hoc tests was used (Zar 1999). All analyses were performed in the statistical package JMP v7.0 (SAS Institute, Cary, North Carolina) and the level of significance for all tests (α) was assessed at 0.05 (Zar 1999). All values presented represent mean ± S.D. unless otherwise noted.

Results

Plasma levels of cortisol declined over time reflecting recovery from the handling stress event in fish from both treatments (Table 1, Figure 1). However, there was no difference between treatments in plasma cortisol content at any of the time periods (Figure 1), indicating that saltwater immersion did not have any effect on either dampening the stress response or shortening the recovery period. During the first two time periods (0 and 1 hour after stress), fish subjected to saltwater immersion had higher levels of circulating cortisol than fish that were recovered in freshwater indicating that the saltwater immersion potentially served as a second stressor (Table 1, Figure 1). Additionally, plasma osmolality followed a pattern similar to that of plasma cortisol as fish from both treatment groups recovered from handling stress (Table 1, Figure 2). Again, no significant treatment effect was noted (Table 1, Figure 2).

Discussion

During an acute stress event such as handling or air exposure, the gills of a fish become more perfused with blood allowing for greater oxygen uptake for the metabolic demands of coping with the stressor, and, subsequently, ions in the blood stream are inadvertently lost to the environment and later must be replaced (Barton and Iwama 1991; Morgan and Iwama 1997; Barton 2002). Given this, placing fish in a saltwater bath of a similar concentration to the blood stream (isotonic) has been theorized to prevent the loss of blood ions by preventing the passive movement of ions across the gills (Wedemeyer 1972; Carmichael et al. 1984). This has been used to beneficial effect during transport when fish can be kept in an isotonic saline for the duration of the recovery period (Barton and Peter 1992; Barton and Zitzow 1995; Reubush and Heath 1997). While the short duration of saltwater immersion used in the present study would have temporarily stopped ion loss immediately following the stressor, fish would have continued to lose ions when moved from the saltwater bath to freshwater recovery tanks. In effect, the saltwater immersion was too short of a duration and would need to take place on the same time scale as the full recovery process (greater than four hours in this study). Additionally, fish placed in the salt water bath had higher cortisol levels during the recovery indicating that salt water immersion may have had a negative effect by increasing the stress response in this group. Finally, there are many other physiological components of the stress response (i.e., hormone changes, glucose liberation, increased cardiac activity, behavioral changes, etc. [Barton 2002]) that are unrelated to ion loss and would not be affected by saltwater immersion.

From a practical standpoint, the use of a long duration (over four hours) saltwater immersion to prevent ion loss at a production facility would be most likely unfeasible. Particular care would be required to ensure that stocking densities within the saltwater bath are not high enough to induce chronic stress in the fish and prevent recovery from handling. Additionally, if the salt bath deviated from an isotonic concentration, it would either have no benefit (if too low) or could impart an additional stress (if too high) (Fiol and Kültz 2007).

Based on the data from this study, the use of a short term salt bath provides no benefit for mitigation of the stress response due to handling associated with tagging operations. On the contrary, exposure to a salt bath increased levels of the primary stress hormone cortisol, potentially indicating that the salt bath caused an additional stressor and may have impeded recovery from handling stress. While not explicitly tested in this study, subjecting fish to multiple acute stressors in a short duration of time similar to that of handling stress followed by osmotic shock (rapid changes in salt concentration in the blood stream) can lead to suppression of the immune response, increases in prevalence and intensity of disease, and long term mortality. For these reasons, the use of a short term salt bath after handling should be avoided.

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Tables

Table 1: Two-way ANOVA results for physiological recovery from an acute stress event in juvenile steelhead from Hagerman NFH. Bold and italic font indicates statistically significant differences at $\alpha = 0.05$.

Physiological variable	Source	d.f.	F value	P value
Cortisol (ng/mL)	Treatment	1	1.4	0.2
	Time Period	***4***	***16.7***	***<0.001***
	Treatment * Time Period	***4***	***2.8***	***0.04***
Osmolality (mOsm/Kg H_2O)	Treatment	1	0.9	0.3
	Time Period	***4***	***11.9***	***<0.001***
	Treatment *Time Period	4	0.8	0.6

Figures

Figure 1: Recovery pattern of plasma cortisol (ng/mL) following exposure to an acute stress in juvenile steelhead at Hagerman NFH. Grey bars represent fish treated by a saltwater immersion (SW) following stress, while black bars represent fish recovered entirely in fresh water (FW). Numbers indicate statistically significant differences in cortisol levels between time periods. There was no treatment effect.

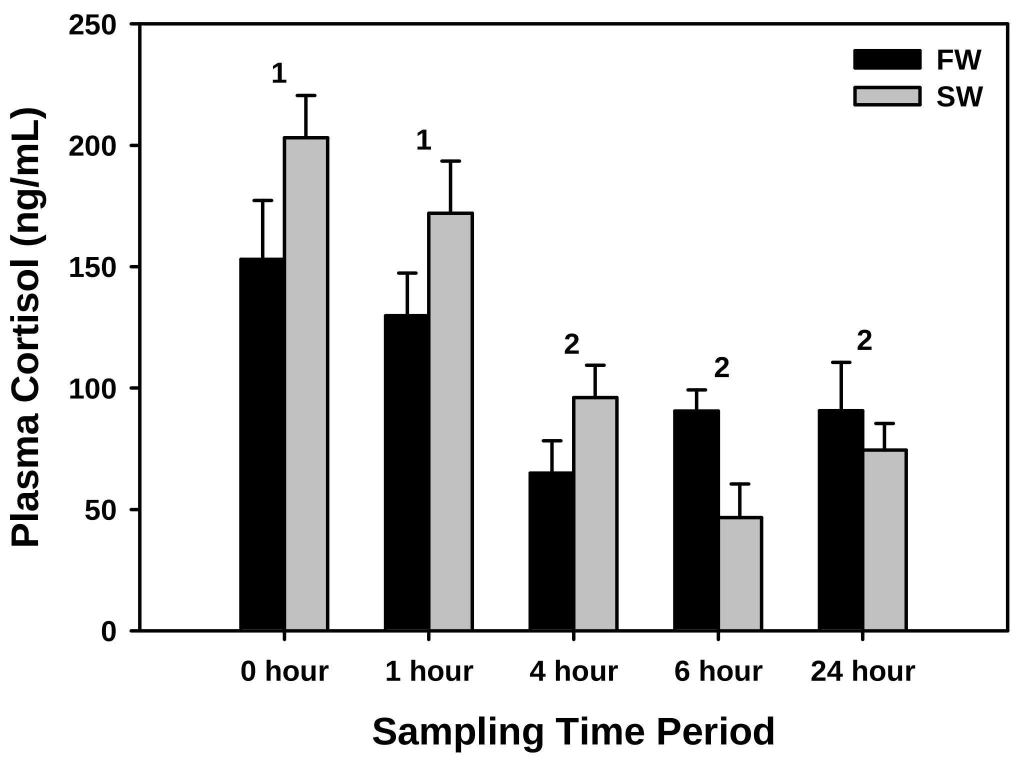

Figure 2: Recovery pattern of plasma osmolality (mOsm/Kg H_2O) following exposure to an acute stress in juvenile steelhead at Hagerman NFH. Grey bars represent fish treated by a saltwater immersion (SW) following stress, while black bars represent fish recovered entirely in fresh water (FW). Numbers indicate statistically significant differences in cortisol levels between time periods. There was no treatment effect.