SUB-LETHAL EFFECTS OF FLUORESCEIN DYE ON SMOLTIFICATION OF STEELHEAD (*ONCORHYNCHUS MYKISS*) FINGERLINGS

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Introduction:

Fluorescein (3',6'-dihydrospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one) sodium (fluorescein) dye is a common compound used in tracing flow rates of natural waters as it has been approved for this use by the Environmental Protection Agency. Fluorescein is a relatively nontoxic hydroxyxanthene dye that produces intense green fluorescence at low concentrations, thereby making it ideal for groundwater tracing applications as trace amounts can be detected far from the initial injection site. It has been shown to be non-toxic to fish at levels of 0.1mg/L (Schnick *et al.* 1989; Noga and Udomkusonsri 2002) and in steelhead (*Oncorhynchus mykiss*) at levels up to 10.0 mg/L (MacPhee and Ruelle 1969).

While it has been shown to be non-toxic to fish at these levels, nothing is known about the sub-lethal effects of fluorescein on the physiology of fish, especially during sensitive life history stages. In preparation for outmigration, juvenile salmonids undergo the process of smoltification, a series of changes to body morphology, coloration, behavior, and physiology that allow for survival in saltwater (Folmar and Dickhoff 1980; McCormick and Saunders 1987; Hoar 1989). Principal in the process of smoltification is the remodeling of the osmoregulatory system in preparation for the hyperosmotic environment of the ocean. This includes an increased proliferation of gill chloride cells and the well documented upregulation of gill Na^+,K^+ -ATPase (hereafter 'NKA') activity, an enzyme required to maintain osmotic balance by aiding in the extrusion of NaCl across the gills (Hoar 1989; Evans *et al.* 2005). Smoltification and preparation for saltwater entry is the result of a cascade of hormones, and disruption of the biochemical process at any point of this cascade would lead to a lack of upregulation of NKA activity and the inability of the fish to migrate to the ocean (Folmar and Dickhoff 1980; McCormick and Saunders 1987; Hoar 1989).

Currently there is interest in evaluating the Thousand Springs aquifer that is the source for rearing water at the Hagerman National Fish Hatchery (NFH) in Hagerman, ID, using fluorescein as the tracer dye. At present, Hagerman NFH raises an Endangered Species Act (ESA) listed stock of steelhead and has two ESA listed species of snails in its watershed. There is some concern as to what effect fluorescein might have on the process of smoltification in the protected steelhead. Abernathy Fish Technology Center (AFTC) was contracted to perform a study to determine if exposure to fluorescein dye has any sub-lethal effect on smoltification in steelhead.

Materials and Methods:

To determine the effects of fluorescein immersion on smoltification in steelhead, a study was designed whereby fish were placed in a bath of fluorescein mixed in freshwater for a short time period and then biopsied at two time periods prior to release from the facility. On March 15, 2010, 30 fish from a designated population of steelhead were sampled by gill clip, and gill clips were preserved in SEI buffer at -80°C for later lab analysis following the methods of McCormick (1993). These samples served as controls forming a NKA baseline for the study. On the same day, three groups of 150 fish were bath treated for one hour with 0.1, 1.0, and 10.0 mg/L fluorescein dye, and a separate group of 150 fish was held as non-treated controls. Bath treatment water was aerated so as not to impart any incidental stress due to declines in water quality. After treatment, three groups of 50 fish from each of the four treatments were moved to separate holding tanks. At one hour post treatment, 25 fish from each holding tank were sampled by gill clip following the above mentioned procedure. The remaining 25 fish per holding tank were maintained following standard hatchery feeding protocols until they were sampled by gill

clip on April 20, 2010, to coincide with release timing from the hatchery. All gill clips were assayed for NKA levels at the AFTC following the method developed in McCormick (1993).

Differences of NKA activity in fish among the treatment groups at the 1 hour time period, pre-release time period, and the untreated controls were compared by using a two way analysis of variance (ANOVA) with time period and treatment groups as main factors, and subsequent pairwise comparisons (Tukey's *post hoc* tests) where appropriate. NKA activity was $\log_{10}$ transformed to better meet the assumption of univariate normality (Zar 1999). All analyses were performed in the statistical package JMP v. 7.0 (SAS Institute, Cary, North Carolina, USA) and significance was assessed at $\alpha = 0.05$ unless otherwise noted. Additionally, all values are presented in this study as means ± S.D. unless otherwise noted.

Results

NKA activity of steelhead was affected by fluorescein immersion an hour after exposure ($F_{3, 193} = 8.10$, $P < 0.001$; Figure 1). Specifically, when compared to untreated controls, fish subjected to 0.1mg/L and 10mg/L both showed lower NKA activity although fish subjected to 1.0mg/L did not. However, there were no differences between treatment groups one month after exposure ($F_{3, 193} = 0.64$, $P = 0.59$; Figure 2), indicating that any effects of fluorescein exposure were transitory.

Discussion

In the short term, exposure to fluorescein dye did have a sublethal effect on NKA activity in juvenile steelhead relative to untreated controls, though there was no linear relationship between fluorescein exposure concentration and reductions in NKA activity. Currently, the

exact mechanism of action for this effect is not known. It is known that immersion in high concentrations of fluorescein can induce mortality in fish. Marking (1969) noted that mortality occurred at fluorescein concentrations of 1,372 parts per million [p.p.m.] after a 96 hour exposure in rainbow trout, and Pouliquen *et al.* (1995) noted an LD50 for fluorescein of ~1,000 p.p.m. in turbot after 96 hours. These concentrations required to induce mortality in a group of fish are many orders magnitude higher than those that caused a sub-lethal effect in this study (10mg/L = 10 p.p.m.) or that are typically used in dye tracing of waterways (typically less than 1 part per billion). Pouliquen *et al.* (1995) did note that toxicity affected the central nervous system, though there is no readily apparent link between this finding and any affect on NKA activity. One month after exposure to fluorescein, NKA activity was similar in all groups of study fish, indicating that any effects of fluorescein exposure dissipated across that time scale. As such, hatchery managers should be cognizant of the fact that fluorescein exposure may have short term effects on organismal physiology and behavior, but these effects are quite transient and highly dependent on fluorescein concentration. Future research should focus on the time scale over which fish recover NKA activity following fluorescein exposure to determine the duration of the effect. Information of this nature would serve to determine if there are certain time periods (i.e., hours or days prior to release) in which exposure to the chemical should be avoided. Nevertheless, given our results, it is likely that exposure of juvenile steelhead to water containing fluorescein used in mapping aquifers is not a major concern for hatchery personnel if hatchery release dates occur at least one months after the dye is used.

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Figures

Figure 1. Gill Na^+,K^+ -ATPase activity in steelhead one hour after exposure to varying concentrations of fluorescein dye (0.1, 1.0, and 10.0 mg/L) compared to untreated control fish. Dissimilar letter groups indicate statistically significant differences (α=0.05) between the dye concentrations.

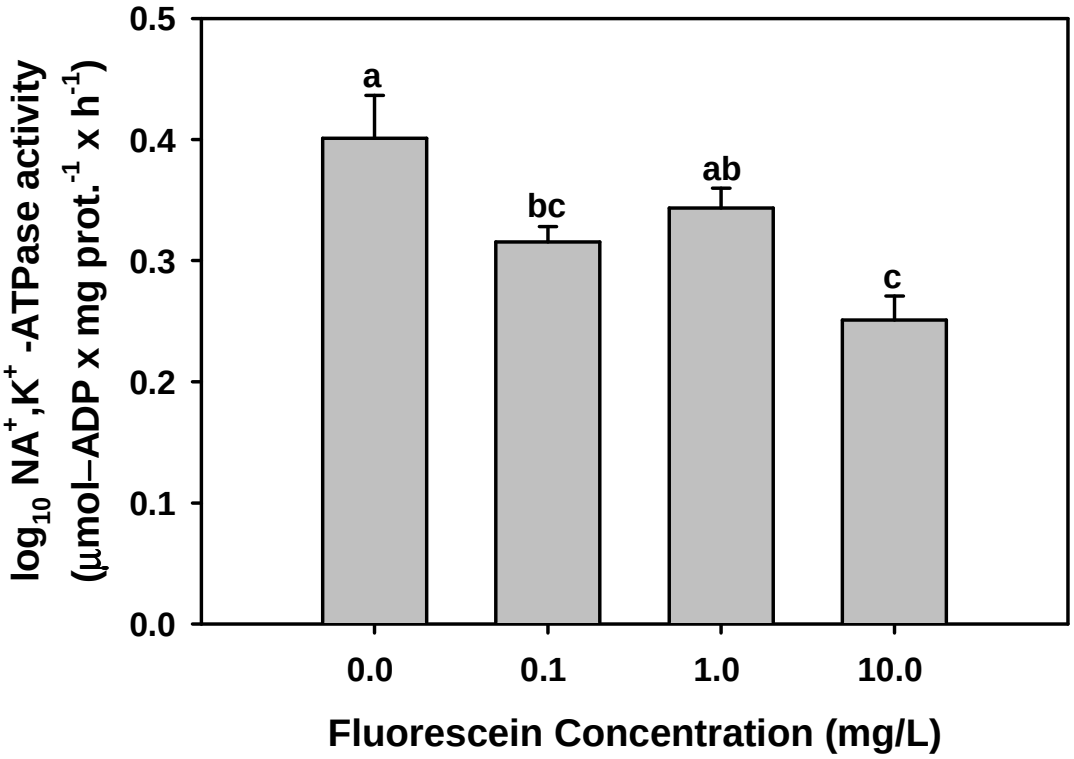

Figure 2. Gill Na^+,K^+ -ATPase activity in steelhead one month after exposure to varying concentrations of fluorescein dye (0.1, 1.0, and 10.0 mg/L) compared to untreated control fish. No statistical differences were observed among the fluorescein dye concentrations.