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Salinity effects on plasma ion levels, cortisol, and osmolality in Chinook salmon following lethal sampling

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ABSTRACT

Studies on hydromineral balance in fishes frequently employ measurements of electrolytes following euthanasia. We tested the effects of fresh- or salt-water euthanasia baths of tricaine mesylate (MS-222) on plasma magnesium (Mg^{2+}) and sodium (Na^{+}) ions, cortisol and osmolality in fish exposed to saltwater challenges, and the ion and steroid hormone fluctuations over time following euthanasia in juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*). Salinity of the euthanasia bath affected plasma Mg^{2+} and Na^{+} concentrations as well as osmolality, with higher concentrations in fish euthanized in saltwater. Time spent in the bath positively affected plasma Mg^{2+} and osmolality, negatively affected cortisol, and had no effect on Na^{+} concentrations. The difference of temporal trends in plasma Mg^{2+} and Na^{+} suggests that Mg^{2+} may be more sensitive to physiological changes and responds more rapidly than Na^{+}. When electrolytes and cortisol are measured as endpoints after euthanasia, care needs to be taken relative to time after death and the salinity of the euthanasia bath.

1. Introduction

The study of hydromineral balance of fishes frequently necessitates the measurement of plasma electrolytes (Clarke and Blackburn, 1977; Congleton, 2006). Changes in blood chemistry such as spikes in glucose or cortisol, which can cause ion movement, occur in as little as 2 min following a stressor (Svobodová et al., 1999). To avoid progressive changes in ion concentrations, fish are typically captured and immediately euthanized with lethal doses of anesthetic (Wedemeyer et al., 1990; Congleton, 2006). Unlike lower immobilizing doses of anesthetic, the lethal dose is believed to prevent a stress response that would cause a cascade of physiological changes (Wedemeyer, 1970; Strange and Schreck, 1978; Barton and Peter, 1982; Congleton, 2006). Fish from freshwater or saltwater holding salinities are euthanized by anesthetic overdose (Strange and Schreck, 1978; Ewing and Birks, 1982; Carter et al., 2011). Acute physiological changes can indicate passive water movement or active ion regulation; these processes can be affected by stress associated with experiments and sampling protocols.

Blood ion levels, measured following saltwater challenges are used as an indicator of the osmoregulatory ability of fish and as a predictor of successful survival and growth in a saline environment (Clarke and Blackburn, 1977; Folmar and Dickhoff, 1980; Shrimpton et al., 1994; Zydlewski et al., 2010; Aykanat et al., 2011). When sampling fish following saltwater challenges, anesthetics such as tricaine mesylate (MS-222) are used to reduce potential confounding handling stress (Strange and Schreck, 1978; Iversen et al., 2003) as well as for animal welfare concerns. Studies employing MS-222 typically examine changes in plasma sodium (Na^{+}) ions, but little is known about circulating levels of magnesium (Mg^{2+}) ions. Magnesium is particularly important because it reportedly increases with stress when other ions do not (Redding and Schreck, 1983; Staurnes et al., 1994; Arends et al., 1999; Raggs and Watts, 2015), and it is known to play a role in Atlantic salmon (*Salmo salar*) osmoregulation (El-Mowafi et al., 1997). However, the mechanism of Mg^{2+} regulation is not well understood (McDonald and Milligan, 1992; Prodocimo and Freire, 2001; Al-Jandal and Wilson, 2011).

Saltwater challenges were developed to identify salmon that were prepared for seaward migration to accelerate or to correctly

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time entry of juvenile salmon into seawater net pens (Clarke and Blackburn, 1977). Clarke and Blackburn (1977) found that fully smolted salmon had plasma Na^+ concentrations less than 170 mM following a 24-hour exposure to saltwater after direct transfer from freshwater. Cortisol concentration positively correlates with smolting (Barton et al., 1985; Richman and Zaugg, 1987; Madsen, 1990), but can also be induced by stress. As such, the goal of our study was to assess the effects of salinity and time on plasma ion and cortisol concentrations following saltwater challenges in juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*).

Our study had two objectives. First, we determined whether blood plasma Mg^{2+} and Na^+ ion concentrations and cortisol change when fish from a saltwater challenge are euthanized in a MS-222 euthanasia bath of a different salinity than the challenge tank. We predicted that the salinity of the anesthetic would affect osmoregulation, causing lower plasma Mg^{2+} and Na^+ concentrations in fish euthanized in freshwater compared to those euthanized in saltwater. We also predicted that plasma cortisol levels would be greater in treatments where fish were subjected to salinity changes and thus an osmoregulatory stress in addition to the confinement stress that is inherent to such tests. Second, we determined the magnitude of ion and cortisol changes over time following euthanasia. We predicted that over time there would be passive diffusion of water across the gill following death, when osmoregulation ceased, and this diffusion would alter plasma ion and cortisol concentrations, with decreases in freshwater and increases in saltwater.

While of interest from a biological perspective, our aim was also to help provide an understanding of possible sampling methodology. Because of constraints inherent in sampling under many field situations, it is often not safe or feasible to obtain blood samples immediately. Based on our experience we selected 30 min as an extreme time during which fish might sit in anesthetic before sampling. Also because of practical constraints in the field, we were interested in testing whether or not salinity of the anesthetic might have an effect.

2. Material and methods

2.1. Animals

We reared juvenile spring Chinook salmon originating from Oregon's McKenzie Fish Hatchery 2013 brood year at the Fish Performance and Genetics Laboratory (FPGL; Oregon State University, Corvallis, Oregon) under temperature and feeding regimes to achieve body size characteristics similar to those of sub-yearling or fall-migrating smolts. This included flow-through 11–12 °C, pathogen-free well water, exposure to natural daylight, low stocking density, and a low-lipid diet (fat content = 11.5% dry weight; manufactured and provided by W. Sealy, Bozeman Fish Technology Center of the US Fish and Wildlife Service). We fed fish this diet to emulate growth characteristics more like their wild counterparts compared to typical hatchery growth rates. We did not feed fish on the day they entered the 24-hour saltwater challenge. The fish ranged between 33 and 102 g in body weight and 109 and 216 mm in fork length at the time of the experiment. We conducted the 24-hour saltwater challenge in December 2014 when, based on our experience with these fish, they would presumably be undergoing or have completed smoltification.

2.2. Saltwater challenge and euthanasia

We used eight static 85 L opaque tanks (4 saltwater and 4 freshwater) with 20 fish each for a 24-hour saltwater challenge. We filled each tank with water from the stock tank from which the fish were reared. In half of the tanks, we added 3058 g of salt (Oceanic Natural Sea Salt Mix, Franklin, WI, USA) to produce a salinity of 34 ppt (comparable to seawater), once dissolved. We placed fish into each challenge tank at 1-hour intervals to allow sufficient time for sampling at the end of the 24 hour challenge test. Each tank had an opaque cover to minimize outside light from entering, with aeration from compressed air through diffusing stones to maintain dissolved oxygen levels at atmospheric saturation. Tanks were immersed in a water bath to maintain stable temperature (11.3–11.6 °C). Following the 24-hour test exposure, we placed 10 fish from each tank in a freshwater euthanasia bath of 200 mgL^{-1} MS-222 buffered to pH 7.0 with 500 mgL^{-1} sodium bicarbonate ($NaHCO_3$), and placed the other 10 fish in a saltwater euthanasia bath with the same concentration of MS-222. We repeated this for each tank following its 24-hour exposure with a new euthanasia bath prepared for each tank. In total, we had four final treatments, each in quadruplicate: freshwater challenge to freshwater anesthetic, saltwater challenge to freshwater anesthetic, saltwater challenge to saltwater anesthetic, and freshwater challenge to saltwater anesthetic. All fish lost equilibrium in less than 20 s (stage 4 anesthesia) after being placed in the anesthetic and were unresponsive to external stimuli in less than 90 s (stage 5 anesthesia, as defined by Summerfelt and Smith, 1990). From each euthanasia bath, we sampled 5 fish immediately (1.5–2 min after being placed in the bath) and sampled the other 5 fish after they had remained in the euthanasia bath for 30 min. For each sampling, bleeding of all the fish took less than 5 ½ min. Due to mechanical failure, we omitted one of the saltwater challenge tanks from the study.

2.3. Sampling and plasma analyses

We collected blood from each fish via caudal peduncle transection with ammonium heparinized blood collecting tubes (Natelson tubes, 250 μl), prior to collecting data on individual wet mass (g), fork length (mm), and sex. We separated the plasma from blood using centrifugation (Beckman Coulter Microfuge 16 Centrifuge) and froze samples at −20 °C until analysis. We measured plasma Na^+ and Mg^{2+} ion concentrations in an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100). Prior to measurement, we diluted plasma with deionized water by 1:1000 for Na^+ and by 1:200 for Mg^{2+}. We measured total cortisol using the radioimmunoassay procedure described by Redding et al. (1984), employing an antibody obtained from Fitzgerald (lot # P7071075), and validated by us for cortisol. Finally, we measured osmolality on duplicate 10 μl samples using vapor pressure osmometers (Wescor vapor 5100B and 5520, Wescor, Inc., Logan, Utah, USA).

3. Statistics

Analyses of plasma Na^+, Mg^{2+}, cortisol, and osmolality concentrations indicated the presence of a tank-level effect, requiring the use of a linear mixed model to evaluate the differences among treatments. We conducted statistical analyses using the lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2014) packages in program R (R Core Team, 2014). We assessed goodness-of-fit for each model by plotting residuals vs. predicted values (Lindsay and Roeder, 1992) and examining normal Quantile–Quantile (Q–Q) plots (Ricci, 2005). These evaluations indicated that no transformations were needed for Na^+ or osmolality, while an inverse transformation of Mg^{2+}, and a square root transformation of cortisol data were needed to meet constancy of variance assumptions. We evaluated the effect of challenge salinity, euthanasia bath salinity, and time on Na^+, Mg^{2+}, cortisol, and osmolality using additive linear models controlling for tank effects with a random variable. The best approximating linear model was selected using Akaike's information criterion (AIC) (Burnham and Anderson, 2002). The significance of variables in the best approximating models was assessed using Satterthwaite's approximation for degrees of freedom (Gaylor and Hopper, 1969). We considered differences as significant using an α of 0.05.