

**IONOREGULATORY DEVELOPMENT IN EARLY LIFE STAGES OF  
RAINBOW TROUT AND THE EFFECT OF SILVER.**

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**Introduction**

Active Na<sup>+</sup> uptake from fresh water has been demonstrated to occur in salmonid eggs starting at the eyed stage (Rudy and Potts, 1969) presumably driven by an apical H<sup>+</sup>-ATPase in series with a basolateral Na<sup>+</sup>,K<sup>+</sup>-ATPase, as is thought to be the case in the fish gill (Lin and Randall, 1995). The first objective of this study was to investigate the pattern of ion regulatory development from fertilization to pre-swim-up, based upon immunolocalization of the Na<sup>+</sup>,K<sup>+</sup>-ATPase and V-type H<sup>+</sup>-ATPase in the gills, skin and yolk sac epithelium of rainbow trout coupled with measurements of whole organism unidirectional Na<sup>+</sup> uptake and Na<sup>+</sup>,K<sup>+</sup>ATPase activity levels.

Silver acts as a Na<sup>+</sup> analogue and exerts its toxic effect by impairing branchial Na<sup>+</sup>,K<sup>+</sup>-ATPase in juvenile and adult fish, leading to loss of ion regulatory control and death (Wood et al., 1996). Very little is known of the effects of silver exposure on ion regulation in developing fish embryos and larvae which

are often the most sensitive to toxicants. Thus, the second objective of this study was to investigate the effects of chronic silver exposure on ion regulatory development in rainbow trout.

### **Materials and Methods**

Freshly fertilized rainbow trout (*Oncorhynchus mykiss*) eggs were obtained from Rainbow Springs trout farm (Thamesford, Ontario) and maintained in darkened chambers in flowing, dechlorinated Hamilton tap water at a constant temperature of 12 °C. Within 3 h following fertilization, up to 1 week post-hatch, eggs were continuously exposed to sublethal levels of silver (as AgNO<sub>3</sub>) at 0, 0.1 and 1.0 µg/l total silver in a flow-through set up. Every 5 days, unidirectional Na<sup>+</sup> influx was measured (using <sup>22</sup>Na) and eggs or larvae were collected for measurement of whole organism Na<sup>+</sup>, Cl<sup>-</sup>, Ag<sup>+</sup>, cortisol and ammonia levels as well as Na<sup>+</sup>,K<sup>+</sup> ATP-ase activity determinations. In a separate series, eggs and larvae that had not been exposed to silver were collected every 5 days, fixed in Bouins fixative and sectioned for immunolocalization of the Na<sup>+</sup>,K<sup>+</sup>-ATPase and V-type H<sup>+</sup>-ATPase in the gills, skin and yolk sac epithelium. Na<sup>+</sup>,K<sup>+</sup>-ATPase and V-type H<sup>+</sup>-ATPase were indirectly immunolocalized using the mouse monoclonal α 5 antibody and the rabbit polyclonal anti-peptide (A-subunit) antibody, respectively (Wilson et al. 2000).

### **Results and Discussion**

The ontogeny of ion regulation was investigated in rainbow trout from fertilization to swim-up, in the presence and absence of sublethal levels of silver, a Na<sup>+</sup> antagonist. Whole egg unidirectional Na<sup>+</sup> uptake increased dramatically from the “eyed stage” (10-15 nmol g h<sup>-1</sup>) through to post-hatch and swim-up (350-400 nmol g h<sup>-1</sup>) and this correlated well with the increase in whole egg Na<sup>+</sup>,K<sup>+</sup> ATPase activity levels. Na<sup>+</sup>,K<sup>+</sup>-ATPase and V-type H<sup>+</sup>-ATPase were immunohistochemically localized in the gills of pre-swim-up larvae not exposed to silver. While some labeling was also observed in the skin and yolk sac it was far less frequent indicating that the gills likely play the predominant role in driving active ion uptake at this stage of development. During exposure to sublethal levels of silver (0.1 and 1.0 µg/l total silver in hard water as AgNO<sub>3</sub>) from fertilization to post-hatch, there is a dose dependent acceleration in growth and ionoregulatory development as indicated by changes in unidirectional Na<sup>+</sup> uptake and Na<sup>+</sup>,K<sup>+</sup> ATPase activity levels (expressed per mg protein or per egg). Shortly following hatch, however, these sublethal levels of silver resulted in an

impairment of Na<sup>+</sup>,K<sup>+</sup> ATPase activity at which time no significant differences in Na<sup>+</sup> uptake were observed among treatments.

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