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Impacts of short-term acid and aluminum exposure on Atlantic salmon (*Salmo salar*) physiology: A direct comparison of parr and smolts

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Abstract

Episodic acidification resulting in increased acidity and inorganic aluminum (Al_i) is known to impact anadromous salmonids and has been identified as a possible cause of Atlantic salmon population decline. Sensitive life-stages such as smolts may be particularly vulnerable to impacts of short-term (days–week) acid/Al exposure, however the extent and mechanism(s) of this remain unknown. To determine if Atlantic salmon smolts are more sensitive than parr to short-term acid/Al, parr and smolts held in the same experimental tanks were exposed to control (pH 6.3–6.6, 11–37 $\mu g\,l^{-1}$ Al_i) and acid/Al (pH 5.0–5.4, 43–68 $\mu g\,l^{-1}$ Al_i) conditions in the lab, and impacts on ion regulation, stress response and gill Al accumulation were examined after 2 and 6 days. Parr and smolts were also held in cages for 2 and 6 days in a reference (Rock River, RR) and an acid/Al-impacted tributary (Ball Mountain Brook, BMB) of the West River in Southern Vermont. In the lab, losses in plasma Cl^- levels occurred in both control parr and smolts as compared to fish sampled prior to the start of the study, however smolts exposed to acid/Al experienced additional losses in plasma Cl^- levels (9–14 mM) after 2 and 6 days, and increases in plasma cortisol (4.3-fold) and glucose (2.9-fold) levels after 6 days, whereas these parameters were not significantly affected by acid/Al in parr. Gill Na^+,K^+-ATPase (NKA) activity was not affected by acid/Al in either life-stage. Both parr and smolts held at BMB (but not RR) exhibited declines in plasma Cl^-, and increases in plasma cortisol and glucose levels; these differences were significantly greater in smolts after 2 days but similar in parr and smolts after 6 days. Gill NKA activity was reduced 45–54% in both life-stages held at BMB for 6 days compared to reference fish at RR. In both studies, exposure to acid/Al resulted in gill Al accumulation in parr and smolts, with parr exhibiting two-fold greater gill Al than smolts after 6 days. Our results indicate that smolts are more sensitive than parr to short-term acid/Al. Increased sensitivity of smolts appears to be independent of a reduction in gill NKA activity and greater gill Al accumulation. Instead, increased sensitivity of smolts is likely a result of both the acquisition of seawater tolerance while still in freshwater and heightened stress responsiveness in preparation for seawater entry and residence.

Keywords: Acid rain; Aluminum; Atlantic salmon; Smolts; Ion regulation; Gill Na^+,K^+-ATPase activity

1. Introduction

Chronic (year-round) acidification and its associated aluminum (Al) toxicity is a known cause of Atlantic salmon population decline in Norway (Hesthagen, 1989) and Nova Scotia (Watt et al., 1983). Recent studies have suggested that episodic acidification (single or re-occurring episodes lasting several days) may also have effects on Atlantic salmon populations in regions of the northeastern United States including Maine, where several salmon rivers have been listed as endangered (Magee et al., 2001, 2003; National Academy of Science, 2004). As a result of their underlying geology, many rivers and streams in these regions have low concentrations of base cations (Ca^{2+}, Mg^{2+}) and consequent poor buffering capacity making them vulnerable to increases in acidity during episodic acidification events such as spring snowmelts and fall storms. During episodic acidification, Al is mobilized from the soil and enters the surrounding water leading to elevated Al concentrations. In addition, the solubility of Al increases as a direct result of decreased pH leading to the increased presence of inorganic Al (Al_i), the form of Al that is most toxic to fish (Gensemer and Playle, 1999).

The fish gill, a multifunctional organ involved in ion regulation and respiration, is the major site of acid/Al toxicity (Exley et al., 1991; Gensemer and Playle, 1999). During exposure to

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acid/Al, Al accumulates both on the surface and within the gill and is often associated with damage to the branchial epithelium (Youson and Neville, 1987; Lacroix et al., 1993; Wilkinson and Campbell, 1993; Teien et al., 2004). Consequently, acid/Al exposure results in the loss of ion regulatory ability due to an increase in branchial permeability and an inhibition of active ion uptake (Booth et al., 1988; McDonald et al., 1991). Increased permeability may be caused by the displacement of Ca^{2+} ions from anionic gill binding sites by Al, resulting in the weakening of intercellular tight junctions (Booth et al., 1988; Freda et al., 1991), whereas inhibition of ion uptake may result from damage to or alteration of gill chloride cells (Jagoe and Haines, 1997), and decreased gill Na^+,K^+-ATPase (NKA) activity (Staurnes et al., 1993, 1996; Kroglund and Staurnes, 1999; Magee et al., 2003).

Atlantic salmon are among the most sensitive of the salmonid species to acid/Al (Fivelstad and Leivestad, 1984; Rosseland and Skogheim, 1984). After several years of stream residence, Atlantic salmon enter the parr–smolt transformation, a developmental period necessary for seawater (SW) entry and residence (McCormick et al., 1998). This period is marked by the acquisition of SW tolerance (salt secretory capacity) resulting in part from an increase in the number and size of gill chloride cells and gill NKA activity (McCormick et al., 1998). Other physiological changes include silvering, darkening of fin margins, and increased growth and oxygen consumption (Hoar, 1988). Several studies have indicated that Altantic salmon smolts are the most sensitive of the salmon life-stages to ion regulatory disturbance resulting from acid/Al exposure (Rosseland and Skogheim, 1984; Leivestad et al., 1987; Staurnes et al., 1993; Rosseland et al., 2001). However, these studies have made life-stage comparisons during chronic exposures, under severe acid/Al conditions, or during different seasons. Thus, there is a need for direct life-stage comparisons of Atlantic salmon exposed to short-term and moderate acid/Al conditions. In addition, these studies have suggested that increased smolt sensitivity may be due to the acquisition of SW tolerance while still in freshwater, however the specific mechanism(s) underlying this remain unknown.

The present study was conducted to directly compare the impacts of short-term acid/Al on the ion regulatory ability and stress response of Atlantic salmon parr and smolts. We investigated impacts of acid/Al on plasma Cl^-, gill NKA activity, indicators of stress, plasma cortisol and glucose, and gill Al accumulation. Our objectives were to determine if smolts are more sensitive than parr to short-term exposure to moderate acid/Al, and to investigate the mechanism(s) of increased sensitivity. More specifically, we tested the hypotheses that decreased gill NKA activity and/or increased gill Al accumulation underlie increased smolt sensitivity.

2. Materials and methods

2.1. Fish rearing

Atlantic salmon (*Salmo salar*) were obtained from the Kensington National Fish Hatchery (Kensignton, CT), and held at the Conte Anadromous Fish Research Center (Turners Fall, MA). Prior to the initiation of studies, fish were held in fiberglass tanks receiving flow through ($4\,l\,min^{-1}$) Connecticut River water (Ca^{2+}, $9.0\,mg\,l^{-1}$; Mg^{2+}, $1.5\,mg\,l^{-1}$; Na^+, $6.8\,mg\,l^{-1}$; K^+, $1.10\,mg\,l^{-1}$, Cl^-, $11.0\,mg\,l^{-1}$), maintained under natural photoperiod conditions and ambient river temperatures, and fed to satiation twice daily with commercial feed (Zeigler Bros., Garners, PA).

2.2. Laboratory exposure

Laboratory exposures were conducted from May 12–18, 2005. Atlantic salmon parr (9.2–12.8 cm) and smolts (14.7–16.7 cm) were randomly assigned to two replicate tanks receiving control (pH 6.5, $0\,\mu g\,l^{-1}$ Al) or acid/Al (pH 5.2, $50\,\mu g\,l^{-1}$ Al) conditions. An acid only treatment was not included in this study, as it has been established that increases in inorganic Al occur together with decreased pH in rivers experiencing episodic acidification (Lacroix and Townsend, 1987). Each experimental tank contained 10 parr and 10 smolts. Food was withheld for 24 h prior to the initiation of the study, and fish were starved for the duration of the experiment. Parr and smolts were exposed to the two experimental water chemistries for 2 and 6 days, and five fish per tank were sampled at each time-point. Prior to the start of the experiment, eight parr and eight smolts were sampled directly from their rearing tanks as a reference group ($T = 0$). Experimental tanks (186 l) received artificial soft water prepared by mixing deionized water (Siemens, Lowell, MA) with ambient Connecticut River water (4:1), and target pH and Al concentrations were achieved in header tanks using 3 N HCl and an $AlCl_3 \cdot 6H_2O$ stock solution ($1000\,mg\,l^{-1}$ Al), respectively. Dilution of river water resulted in a reduction in ionic strength (including ambient Ca^{2+}, Na^+) similar to that which occurs following episodic rain events in low to moderately buffered streams (Lacroix and Townsend, 1987; Haines et al., 1990). These studies observed 2–5-fold decreases in ambient calcium concentrations shortly after periods of increased river discharge in Maine and Nova Scotia. Experimental water was mixed for >1 h before entering fish tanks to avoid unstable water conditions, and each tank received continuous flow of $14\,l\,h^{-1}$. Temperature was maintained at 10.3–12.4 °C using a re-circulating chiller system. Both header and experimental tanks were oxygenated continuously with airstones maintaining dissolved oxygen at $>10\,mg\,O_2\,l^{-1}$. pH measurements were made twice daily from water samples collected at the tank outlet using a bench top pH meter 145 (Corning, Medfield, MA) with a Ross Ultra pH probe (Thermo Orion, Beverly, MA). Water samples were also collected at the tank outlet twice daily in acid-washed 50 ml tubes for the measurement of Al, Ca^{2+} and Na^+.

2.3. Field exposure

Cage studies were conducted from May 17–23, 2005. Atlantic salmon parr (10.3–14.0 cm) and smolts (14.3–18.2 cm) were transported to two tributaries of the West River in Southern