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Recovery of Atlantic salmon smolts following aluminum exposure defined by changes in blood physiology and seawater tolerance

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ABSTRACT

Acidification is acknowledged as a cause for extinction or catch reductions in numerous rivers supporting Atlantic salmon (*Salmo salar* L.) populations in Norway. In freshwater, labile (cationic/inorganic) forms of Al (LAl) accumulate onto and in fish gills, where high concentrations can result in mortality due to respiratory and ionoregulatory dysfunction. At lower concentrations, Al may still have major population effects, mainly through the inhibition of gill Na^+,K^+-ATPase activity, reducing hypo-osmoregulatory capacity and thereby affecting marine survival. Following episodic exposure, normal smolt properties are expected to be restored. In 2006 and 2007 we exposed groups of 1100 to 1200 one-year old hatchery reared, Carlin tagged Atlantic salmon smolts of the Imsa strain (South-Western Norway) to moderately acidified water (pH 5.6–5.7; 10–15 μg L Al l^{-1}) for 2 to 14 days whereupon they were transferred to a water quality assumed to be satisfactory for smolt (good water; pH 6.8–7.2 and <6 μg L Al l^{-1}) for 2 to 14 days to monitor recovery from the prior exposure. Control fish had gill–Al concentrations in the range of 5 to 10 μg Al g^{-1} gill dry weight (dw) while Al-exposed fish had gill–Al concentrations exceeding 30 μg Al g^{-1} gill dw. Following transfer to good water, gill–Al did not return to control levels within a time span of 14 days. The physiological responses measured as blood ions (Na^+, Cl^-), blood acid-base balance (pH, pCO_2 and HCO_3^-), and blood glucose improved relative to the acid/aluminum (Al) exposed groups, but not to levels measured in the control fish. Mortality was elevated in all Al-exposed/recovery groups following transfer to seawater (34 ppt) and gill Na^+,K^+-ATPase was reduced. The results suggest that smolts had not fully recovered 14 days after a short-term acid/Al exposure, where recovery depends on what traits are used as indicators of healthy fish. An Al-exposure experienced by presmolt several weeks prior to the smolt spring migration can have negative population effects, both by reducing hypo-osmoregulatory capacity and by making the fish more vulnerable to secondary stressors in the marine environment.

1. Introduction

Anthropogenic acidification implies an increase in H^+ (reduced pH) and aluminum, where the prime toxicant is cationic species of aluminum (labile or inorganic Al (LAl or Ali)) (Gensemer and Playle, 1999, Rosseland and Staurnes, 1994). Sensitivity towards aluminum (Al) varies with life history stage, where sensitivity increases sharply towards the end of the parr-smolt transformation (smoltification) process (Monette and McCormick, 2008, Poléo and Muniz, 1993, Rosseland and Skogheim, 1984, Rosseland and Staurnes, 1994). During the smoltification process the individual becomes pre-adapted to the marine environment and imprinted on their natal stream (Hoar, 1988 McCormick et al., 1998). Smoltification is as such an endocrine driven metamorphism involving a number of physiological, morphological and behavioral changes, all acting to enhance survival when the fish moves from freshwater to seawater.

An important aspect of smoltification is the ability to hypo-osmoregulate. Seawater tolerance is brought about by changes in osmoregulatory capacity involving an upregulation of Na^+, K^+-ATPase (NKA), but also other hormones and enzymes e.g. carbonic anhydrase, cortisol and insulin-like growth factor (McCormick et al., 2007). Several of these are sensitive to Al (Kroglund et al., 2007, Kroglund and Staurnes, 1999, McCormick et al., 2009, Monette et al., 2008, Staurnes et al., 1993, Nilsen et al., 2010). Smolts entering the

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ocean with reduced NKA-activity have poorer hypo-osmoregulatory capacity that render the fish more susceptible to predation and diseases/parasites (Finstad et al., 2007, Handeland et al., 1996) resulting in reduced adult return rates (Kroglund et al., 2007, Kroglund and Finstad, 2003, Staurnes et al., 1996). In these studies, smolts exposed to Al for 3 days were as affected as smolt exposed for >30 days suggesting that the negative impact occurs within the first day(s) of exposure, where additional exposure duration period doesn't necessarily add to the stress response level.

There has been a large reduction of acid rain over Norway and Europe within the last 20 years (Skjelkvåle et al., 2001, Skjelkvåle et al., 2003), implying that measurements today show higher pH and lower concentrations of cationic Al than 10 to 20 years ago. While acidification in the past was often chronic, reduced acid rain has and will result in more episodic acidification events. This change is expected (Evans et al., 2008, Wright, 2008). It is further expected that many of the rivers currently affected by acidification will remain affected by seasonal episodes if acid deposition is not further reduced. Such episodes will often occur prior to or during the snowmelt in spring, at a time when the salmon are at their most sensitive life stage. As episodes by definition have a short duration they are often not recorded in national monitoring programs, causing a mismatch between smolt health and population status and recorded chemistry.

Smolt quality is affected even by episodes lasting only a few days (Kroglund et al., 2008, Magee et al., 2001, McCormick et al., 2009, Teien et al., 2004). Following an episode, water quality will gradually improve, taking from hours, days or weeks to return to normal levels, depending on intrinsic water basin properties. The timing of episodes will also vary with the timing of snowmelt, precipitation, contribution from seasalt rain events, etc. As water quality improves the pressure on the individual fish, and recovery of smolt characteristics, can be expected. When the fish is fully recovered, past exposures will by definition no longer have any negative effect at the individual or at the population level. The recovery takes from days to a week measured from the time the chemistry improved (Kroglund et al., 2001, Kroglund and Staurnes, 1999, Magee et al., 2003). However, recovery in an ecological setting relies also on which physiological traits or fish properties are used to define unaffected and recovered.

While mortality disappears quickly when water chemistry improves, it takes longer to restore full physiological health and seawater tolerance (Kroglund et al., 2007, Kroglund and Staurnes, 1999). It is likewise reasonable to assume that the recovery rate is related to which organs and physiological mechanisms are disturbed, how severe the damage is, and water chemistry and temperature experienced during the recovery period (Verbost et al., 1995). Due to increased sensitivity to a pressure during late smoltification, an episode occurring a few days prior to the smolt migration can have a major effect on smolt to adult salmon survival, whereas a similar episode 1 or 2 weeks earlier might have no appreciable effect at all. While there are numerous accounts of fish recovering from various stressors in the literature, demonstrating that recovery is possible, there are few studies dedicated towards defining recovery rates based on blood and gill physiology, including sensitivity to secondary stressor as an indicator of smolt quality.

Smolts exposed to suboptimal water quality (in freshwater) have in addition to impaired hypo-osmoregulatory capacity, an increased sensitivity towards salmon lice infestation (in seawater) (Finstad et al. 2007). Salmon lice infestation can therefore be used as an ecological valid "challenge test". Smolts that have recovered from an acidification episode should not be sensitized with respect to sensitivity towards a secondary stressor, such as salmon lice exposure (see Finstad et al., 2007, 2012).

The aim of this study was to study the recovery in smolts exposed to moderately acidified water (pH 5.6–5.7; 10–15 μg L Al l^{-1}) for 2 to 14 days whereupon they were transferred to a water quality assumed to be satisfactory for smolt (pH 6.8–7.2 and <6 μg L Al l^{-1}) for 2 to 14 days to monitor recovery from the prior exposure. The ability to recover was measured by various physiological parameters as plasma ions (Na^+, Cl^-), blood acid-base balance (pH, pCO_2 and HCO_3^-), and blood glucose, using gill–Al concentration as a measure of the dose. Results from this study are also discussed in relation to seawater tolerance. We here understand recovery from an exposure as a process where fish will regain physiological and behavioral characteristics of a normal fish. If physiological status after a period in water of a satisfactory quality still deviates from the unexposed control, recovery is interpreted as being incomplete. An incomplete recovery does not necessarily imply effects at the population level. Seawater and salmon lice challenge tests were included to address possible delayed effects and responses to incomplete recovery.

2. Materials and methods

2.1. Experimental setup

The experiments were carried out at the Norwegian Institute for Nature Research (NINA) salmon research station at Ims (SW-Norway), using 1-year-old, 1st generation hatchery reared pre-smolt originating from wild parents of the Imsa strain. All treatments were performed in 4 m^3 tanks. The same tanks were used both years. The eggs and subsequent juvenile fish were reared under hatchery conditions with a naturally simulated light regime. During this period the fish were fed ordinary commercial dry diet, according to temperature and fish size (Finstad et al., 2007, 2012). All fish were graded and Carlin tagged (Carlin, 1955) prior to transfer to the exposure tanks. Each tank was fed water (40 l min^{-1}) from Lake Imsa (Control; pH>6.5) for a minimum of 3 days prior to exposure start to allow recovery from handling stress. At the initiation of the exposure, the water source was changed to one of the exposure water qualities, and the flow reduced to 30 l min^{-1} (variation within and between tanks was less than 5%). The water current was maintained at 10–12 cm sec^{-1}, when measured 15–20 cm from the tank edge. The treatment tanks were stocked with approximately 1200 fish. The fish were 15.0 ± 0.9 cm, had a weight of 31.7 ± 5.7 g and had a condition (K) factor of 0.92 ± 0.06 in the spring of 2006. In 2007 the fish were larger and were 20.6 ± 1.6 cm, had a weight of 82.7 ± 19.9 g and a K-factor 0.92 ± 0.05. This difference in size can affect sensitivity to Al, where larger fish are most sensitive (Rosseland et al., 2001).

Fish groups were exposed to either GOOD or ACID water. The GOOD water from Ims contained calcium (Ca) concentration varying around 3 to 4 mg Ca l^{-1}, is slightly affected by organic carbon (TOC) being in the range of 2 to 4 mg C l^{-1}, and had an acid neutralizing capacity (ANC) >100 μeq l^{-1}. pH is >6.5 (Table 1). This water is in the text defined as GOOD. There are no known water chemistry related contaminants in GOOD.

The ACID groups in 2006 and 2007 were established by adding acid (HCl) and aluminum ($AlCl_3*6H_20$) to reduce pH and increase Al (ACID). All ions apart from Cl^- were identical across all water types. The acid-Al stock solution was acidified to pH (<2) to ensure that Al was present as $Al3^+$. To prepare Al-enriched water, the chemicals were continuously added into a header tank (size: 4 m^3) where water was mixed well and allowed to age for 100 min. From this tank water was fed to the various exposure tanks. Water age increased by an additional 100 min in the exposure tanks. The ACID water was thus aged for approx. 3 h and the Al^{3+} added was transformed into the various forms and species of Al normally encountered in natural water having a pH around 5.8 and a total organic content (TOC) of 2–5 mg C l^{-1}. This was confirmed by performing Al fractionations *in situ* at site (data not shown).

The exposure set up differed in 2006 from 2007 (Fig. 1). In 2006 all fish were exposed to acid water in a single tank. Groups of 400 fish were moved from ACID to GOOD after 2, 7 and 14 days (A2, A7 and A14) to initiate recovery. A2 was allowed to recover in GOOD for