Thy-SW

salt challenge;
10; Thy=plasma

ed to determine sig-
rent from each other.

to 26)
Total
1.76 ± 0.274^{a}
2.43 ± 0.51^{a}
4.04 ± 0.50^{b}

whereas 91% (9)
o. The total time
tions of the river
There was no cor-
any treatment.

smolts successfully left the upper estuary, sunset occurred 1-3 hrs after high tide, and these smolts migrated with the ebb tide. Those movements in which smolts were unsuccessful at leaving the upper estuary were random with respect to time of day and time of tide.

Smolt survival was lowest in the middle estuary (77%), where salinity and temperature fluctuated the most (0-32‰), and greatest in the lower estuary (100%), where salinity and temperature varied less than in other tidal sections (25-32‰). Survival was 96% and 93% in FW and upper estuarine sections respectively. There were no differences in survival between treatments.

4. Discussion

Although wild smolts had higher Na^+/K^+ ATPase activities than hatchery smolts, they were less able to osmoregulate in SW. Three wild smolts died or were moribund in SW, and those that survived had the lowest plasma Cl concentration in FW and the largest increase (70%) in SW. They also had the highest FW and lowest SW hematocrit, respectively. These conditions are characteristic of smolts with impaired osmoregulatory ability (Staurnes *et al.*, 1996). Thyroxine concentrations of smolts in this study were substantially lower than those of hatchery-reared smolts reported in McCormick and Bjornsson (1994). The reasons for this are unknown, but thyroxine can be reduced in wild smolts held in captivity for five days (S. McCormick, personal communication).

Mean residence time of wild smolts in FW was about four times that of ambient and acid smolts, even though these fish were actively migrating when captured. This may be related to differences between hatchery and wild fish in response to handling or surgical stress; however, 12 of the 26 wild smolts left the FW section during the first night after surgery. The majority of smolts in all treatments passed telemetry receivers during an ebb tide, indicating that they take advantage of the strong 'downstream' tidal flow (Moore *et al.*, 1998). Significantly more wild and acid-exposed smolts made large upstream movements from the middle to upper estuary than did ambient smolts. The lower part of the upper estuary section is where the smolts first encounter water of >8‰, and this is the zone of complex aluminum chemistry, which can be even more toxic than in freshwater (Rosseland *et al.*, 1992). If wild smolts were physiologically compromised, their movements upstream at this time may have been in search of a less physiologically stressful environment (lower salinity). Handeland *et al.*

(1996) found that predation rates were higher on smolts suffering from osmoregulatory stress after transfer to SW.

5. Conclusions

Wild smolts that had been exposed to acidic, aluminum-enriched river water were less able to osmoregulate in SW than hatchery smolts, and wild smolts spent more time in the river and made more repeat migrations than hatchery smolts. However, seaward migratory survival of all three treatments did not differ for the time we could track the fish.

Acknowledgements

We thank M. Tabone, C. Jarvis, N. Dube, and M. Martin for field assistance. Financial support was provided by the United States Geological Survey, Biological Resources Division, and National Marine Fisheries Service.

References

Batschelet, E.: 1981, Circular statistics in biology. *Eds.* Robin Sibson and Joel E. Cohen. Academic Press, New York.

Clarke, W.C.: 1982, *Aquaculture* **28**, 177.

Dickhoff, W.W., Folmar, L. C., and Gorbman, A.: 1978, *General and Comparative Endocrinology* **36**, 229.

Haines, T.A., Norton, S. A., Kahl, J. S., Fay, C. W., and Pauwels, S.J.: 1990, *Intensive studies of stream fish populations in Maine*, U.S. Environmental Protection Agency.

Handeland, S.O., Jarvi, T., Ferno, A., and Stefansson, S.O.: 1996, *Canadian Journal of Fisheries and Aquatic Sciences*, **53** 2673.

Hesthagen, T., and Hansen, L. P.: 1991, *Aquaculture and Fisheries Management* **22**, 85.

Lacroix, G.: 1989, *Water Air and Soil Pollution* **46**, 375.

Lacroix, G.L., and McCurdy, P.: 1996, *Journal of Fish Biology* **49**, 1086.

Mardia, K.V.: 1972, *Statistics of directional data*, Academic Press, London.

McCormick, S.D.: 1993, *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 656.

McCormick, S.D., and Bjornsson, B.: 1994, *Aquaculture* **121**, 235.

Moore, A., Ives, S., Mead, T. A., and Talks, L.: 1998, *Hydrobiologia* **371/372**, 295.

Rosseland, B.O., Blakar, I. A., Bulger, A., Kroglund, F., Kvellstad, A., Lydersen, E., Oughton, D. H., Salbu, B., Staurnes, M., and Vogt, R.: 1992, *Environmental Pollution* **78**, 3.

Specker, J.L., Whitesel, T. A., Parker, S. J., and Saunders, R. L.: 1989, *Aquaculture* **82**, 307.

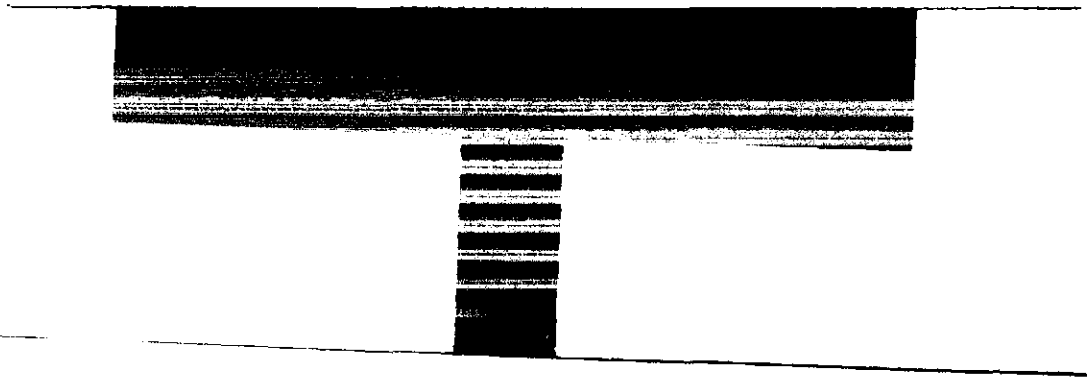
Staurnes, M., Hansen, L. P., Fugelli, K., and Haraldstad, O.: 1996, *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1695.

Thedinga, J.F., Murphy, M. L., Johnson, S. W., Lorenz, J. M., and Koski, K.V.: 1994, *North American Journal of Fisheries Management* **14**, 837.

USEPA: 1987, *Handbook of methods for acid deposition studies: laboratory analysis for surface water chemistry*, U. S. Environmental Protection Agency.

USFWS: 1999, *Report on the biological status of Atlantic salmon*, U. S. Fish and Wildlife Service.

White, G. 1998. *Program Mark.* http://www.cnr.colostate.edu/~gwhite/mark.



ELSEVIER

Aquatic Toxicology 86 (2008) 216–226

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

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

Michelle Y. Monette [a,b,*], Stephen D. McCormick [a,b]

[b] *USGS, Leetown Science Center, Conte Anadromous Fish Research Center, One Migratory Way, Turners Falls, MA 01376, USA*

Received 26 July 2007; received in revised form 29 October 2007; accepted 1 November 2007

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 popu-

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.

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

* Corresponding author at: Department of Cellular and Molecular Physiology, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA. Tel.: +1 203 785 7690.
E-mail address: michelle.monette@yale.edu (M.Y. Monette).

0166-445X/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2007.11.002

EXHIBIT
B

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 μg l^{-1} Al) or acid/Al (pH 5.2, 50 μg 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

Vermont. Prior to transport, nine parr and seven smolts were sampled directly from their rearing tanks as reference groups

eight smolts were placed into two tributaries of the West River; the Rock River (RR), a reference stream, and Ball Mountain Brook (BMB), an acid/Al impacted-stream. Cages were 76 cm × 46 cm × 31 cm and constructed of 3 cm wooden supports with 1 cm plastic mesh on the outside. Cages were placed behind large boulders or inside scour pools to ensure that they had adequate flow but were protected from both high flow and reduced water levels. During the time-course of the study temperature ranged from 8.7 to 11.0 °C in RR and 8.1 to 11.2 °C in BMB. Parr and smolts (four fish/cage) were sampled after 2 and 6 days. pH was measured directly in the stream after 0, 2, 3 and 6 days using a portable pH 105 meter (Corning, Medfield, MA) with a Ross Ultra probe (Thermo Orion, Beverly, MA, and water samples were taken at the same time as described above.

2.4. Sampling protocol and tissue collection

All fish were anesthetized with MS-222 (100 mg l^{-1}, pH

recorded to the nearest 0.1 cm. Blood was collected in heparinized 1 ml syringes from the caudal vessels and centrifuged at 3200 × *g* for 5 min. Plasma was then removed and stored at −80 °C. Gill biopsies (4–6 primary filaments) for the measurement of Al accumulation were taken as described by McCormick (1993), placed into acid-washed 1.5 ml centrifuge tubes, and stored at −80 °C. Gill biopsies were also taken for the measurement of NKA activity, placed into 100 μl SEI (250 mM sucrose, 10 mM Na_2EDTA and 50 mM imidazole, pH 7.3) and stored at −80 °C.

Water samples for Al analysis were taken and processed as described by Lacroix and Townsend (1987). Total Al (Al_{tot}) was analyzed from unfiltered water samples, whereas dissolved Al (Al_a) was analyzed from filtered (0.45 μm, nitrocellulose) water samples. Water samples were acidified (0.2%) with trace metal grade HNO_3 immediately upon collection

(HGA-800/AAnalyst 100, PerkinElmer, Wellesley, MA) atomic absorption spectrophotometry (GFAAS). Water samples were

every 10 samples with a reference standard. Acceptable recovery limits of reference standard were 90–110%, and when values were outside this range a re-slope function was applied. Inorganic Al was determined by the cation-exchange column method (Amberlite 120, prepared with Na^+) described by Driscoll (1984). Al present in the column-processed samples was called organically bound Al (Al_o). Al_i was then determined by calculating the difference between Al_a and Al_o. Ca^{2+} and Na^{2+} were measured by flame atomic absorption spectrophotometry (AAnalyst 100, PerkinElmer, Wellesley, MA).

2.6. Plasma anaylsis

chloridometer (Labconco, Kansas City, MO). Plasma cortisol was measured by enzyme immunoassay (EIA) as outlined by Carey and McCormick (1998). Plasma glucose was measured by enzymatic coupling with hexokinase and glucose 6-phosphate dehydrogenase (Stein, 1963).

2.7. Gill aluminum analysis

Al accumulation in a gill tissue biopsy was analyzed by the method outlined in Teien et al. (2006). Gill biopsies were thawed, dried at 60 °C for 24 h, and weighed to the nearest 0.0001 mg using a Series 30 microbalance (Cahn Instruments, Cerritos, CA). Gill biopsies were then digested by adding 98 μl of 100% trace metal grade HNO_3 and 2 μl of H_2O_2 to biopsy tubes, and heating at 100 °C until completely evaporated (~3 h). The same amounts of HNO_3 and H_2O_2 were again added to biopsy tubes and heated with tube caps on at 60 °C for 1 h. Samples were diluted (9:1) by the addition of 900 μl of ultrapure water, and Al concentration was analyzed by GFAAS as described above. A

tracting the Al present in digestion blanks. Gill Al was expressed as μg Al g^{-1} gill dry weight.

2.8. Gill NKA activity

Gill NKA activity was measured following the method described by McCormick (1993). Gill biopsies were thawed immediately prior to assay, and 25 μl of 0.5% SEID (0.1 g sodium deoxycholate in 20 ml SEI) added to the microcentrifuge tube with tissue and homogenized for 10–15 s using a Kontes pellet pestle motor. The homogenate was then centrifuged at

activity and total protein (BCA protein assay, Pierce, Rockford, IL). This kinetic assay was run at 25 °C for 10 min in a temperature-controlled plate reader (Thermomax, Molecular Devices, Menlo Park, CA) and read at a wavelength of 340 nm. Gill NKA activity was calculated as the difference in the production of ADP in the absence and presence of 0.5 mM ouabain, and expressed as μmol ADP mg protein^{-1} h^{-1}.

2.9. Statistics

For each physiological parameter, potential tank/cage effects were tested by nesting replicate tanks and cages within treatment and stream, respectively. Fish from replicate tanks/cages were pooled only if there was no significant tank/cage effect ($P > 0.05$). A one-way ANOVA on ranks was used to test differences between parr and smolts sampled at the start of each study ($T = 0$). For the laboratory study, a three-way ANOVA on ranks was used to determine the effect of treatment (control, acid/Al), exposure time (2 and 6 days), and life-stage (parr, smolt) on physiology. For the field study, a three-way ANOVA on ranks was used to determine the effect of stream (RR, BMB), exposure

time (2 and 6 days), and life-stage (parr, smolt) on physiology. For both studies, a one-way ANOVA on ranks was used to test differences between fish sampled at the start of each study ($T=0$) and control and RR fish sampled after 2 and 6 days. In all cases, when significant effects were observed ($P<0.05$), pairwise comparisons were made using Duncan's post hoc test. All statistical analyses were performed using Statistica 7.0 (Statsoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Laboratory exposure

Over the course of the study, pH ranged from 6.29 to 6.56 and 4.99 to 5.42 in control and treatment tanks, respectively (Table 1). Mean Al_{tot} concentrations were 33 ± 5 and $72 \pm 3\ \mu g\ l^{-1}$ and mean Al_i concentrations were 20 ± 5 and $53 \pm 4\ \mu g\ l^{-1}$ in control and treatment tanks, respectively (Table 1). Ca^{2+} and Na^+ concentrations ranged from 0.9 to 1.8 and 1.7 to 2.8 mg l^{-1}, respectively, and were similar in all tanks (Table 1).

Plasma Cl^- levels of parr and smolts sampled prior to the start of the study ($T=0$) were 136 ± 2.1 and 137 ± 1.7 mM, respectively (Fig. 1A). Plasma Cl^- levels of control parr and smolts were significantly lower (9–13 mM) than $T=0$ fish after both time-points ($P<0.01$, one-way ANOVA; Fig. 1A). Plasma Cl^- levels of parr were not affected by acid/Al, whereas plasma Cl^- levels of treated smolts were significantly lower (9–14 mM) than control smolts after 2 and 6 days, indicating disturbance of ion regulatory ability in this group (Fig. 1A). Plasma Cl^- levels of treated smolts were significantly lower (13 mM) than treated parr after 2 days (Fig. 1A).

Gill NKA activity of $T=0$ parr and smolts was 3.1 ± 0.2 and 7.2 ± 0.6 μmol ADP mg protein $^{-1}$ h^{-1}, respectively (Fig. 1B). Gill NKA activity of control parr did not differ from $T=0$ parr after either time-point ($P>0.65$; one-way ANOVA), whereas gill NKA activity of control smolts was significantly lower (34%) than $T=0$ smolts after 6 days (Fig. 1B). Gill NKA activity of both control parr and smolts did not differ from acid/Al treated fish throughout the study (Fig. 1B). Gill NKA activity of smolts was significantly greater (38%-2.3-fold) than parr in all groups (Fig. 1B). An elevation in gill NKA activity is typically used as an indicator of smolt development in Atlantic salmon (McCormick, 1993), therefore the observed life-stage differences in NKA activity confirm the status of parr and smolts used in the laboratory.

Plasma cortisol levels of $T=0$ parr and smolts were 1.1 ± 0.3 and 26 ± 8.0 ng ml^{-1}, respectively (Fig. 2A). Plasma cortisol levels of control parr were significantly greater (5–14-fold) than $T=0$ parr after both time-points ($P<0.01$, one-way ANOVA), whereas plasma cortisol levels of control smolts did not differ from $T=0$ smolts after either time-point ($P>0.20$, one-way ANOVA; Fig. 2A). Plasma cortisol levels of parr were not affected by acid/Al, whereas plasma cortisol levels of treated smolts were significantly greater (4.3-fold) than control smolts after 6 days (Fig. 2A). Plasma cortisol levels of both control and treated smolts were significantly greater (9–12-fold) than parr after 2 days, but were not significantly elevated in either group after 6 days (Fig. 2A).

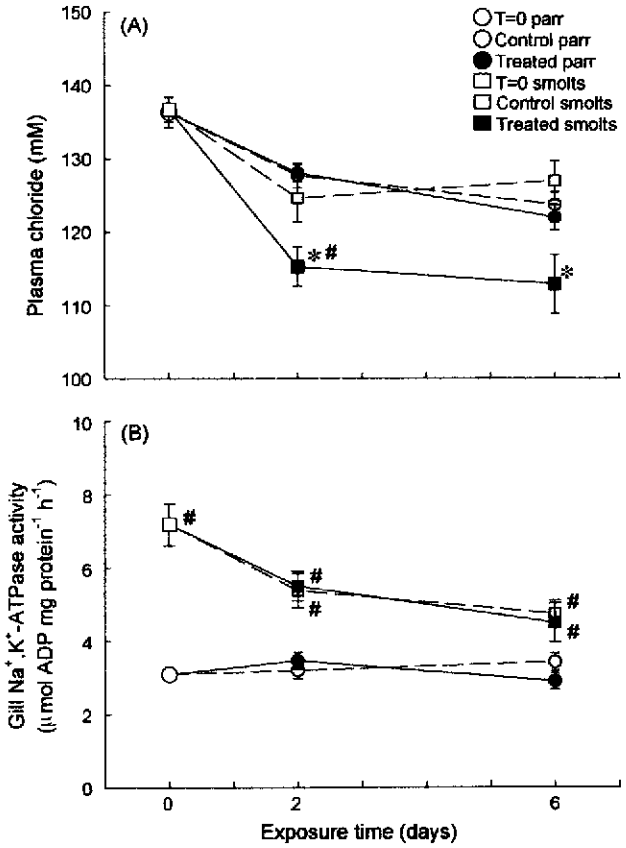


Fig. 1. Impacts of short-term laboratory exposure to acid/Al on the ion regulatory ability of Atlantic salmon parr and smolts. Plasma Cl^- (A) and gill NKA activity (B) levels of control and treated parr and smolts after 2 and 6 days. Values are mean ± S.E. ($n=7$–10). An * indicates a significant difference between control and treatment within exposure time and life-stage (Duncan's; $P<0.05$). An # indicates a significant difference between parr and smolt within a treatment and an exposure time (Duncan's; $P<0.05$). Values at day 0 represent parr and smolts sampled prior to the start of the study ($T=0$). Three-way ANOVA for plasma Cl^- levels determined significant effects of treatment ($P=0.004$) and life-stage ($P=0.01$), and significant treatment/life-stage ($P=0.02$) and timing/life-stage ($P=0.04$) interactions. Three-way ANOVA for gill NKA activity determined a significant life-stage effect ($P<0.001$).

Plasma glucose levels of $T=0$ parr and smolts were 3.5 ± 0.3 and 5.3 ± 0.8 mM, respectively (Fig. 2B). Plasma glucose levels of both control parr and smolts did not differ from $T=0$ fish throughout the study ($P>0.10$, one-way ANOVA; Fig. 2B). Plasma glucose levels of parr were not affected by acid/Al, whereas plasma glucose levels of treated smolts were significantly greater (2.9-fold) than control smolts after 6 days (Fig. 2B). Plasma glucose levels of treated smolts were also greater than control smolts after 2 days, but this difference was not statistically significant ($P=0.11$, Duncan's post-hoc test; Fig. 2B). Plasma glucose levels of smolts were significantly greater (51%-4.1-fold) than parr in all groups throughout the study (Fig. 2B).

Gill Al levels were $13 \pm 3\ \mu g\ g^{-1}$ for both $T=0$ parr and smolts (Fig. 3). Gill Al levels of all control fish remained between