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Exposure to moderate acid water and aluminum reduces Atlantic salmon post-smolt survival

F. Kroglund [a,*], B. Finstad [b], S.O. Stefansson [c], T.O. Nilsen [c], T. Kristensen [d], B.O. Rosseland [e], H.C. Teien [e], B. Salbu [e]

[a] Norwegian Institute for Water Research (NIVA), Televeien 3, N-4879 Grimstad, Norway
[b] Norwegian Institute for Nature Research (NINA), Tungasletta 2, N-7485 Trondheim, Norway
[c] Department of Biology, University of Bergen, N-5020 Bergen, Norway
[d] Norwegian Institute for Water Research (NIVA), Brekkevn. 19, N-0411 Oslo, Norway
[e] The Norwegian University of Life Sciences, N-1432 Ås, Norway

Abstract

Acidification is acknowledged as the cause for extinction or catch reductions in numerous Atlantic salmon (*Salmo salar* L.) populations in Norway. In freshwater, labile (cationic/inorganic) forms of Al (LAl) accumulate in fish gills, where high concentrations result in mortality due to respiratory and ionoregulatory dysfunction. At lower concentrations, Al may still have population effects by inhibiting gill Na^+,K^+-ATPase activity, thereby reducing hypoosmoregulatory capacity and marine survival. Over the years 1999 to 2003 we exposed groups of 1150 to 1200 one-year old hatchery reared, Carlin tagged Atlantic salmon smolts of the Imsa strain (South-Western Norway) to moderately acidified water (pH 5.8; 5–15 μg LAl L^{-1}) from 3 (short term exposure) to 60 (long term exposure) days. Fish exposed to Lake Imsa water (pH>6.5 and <5 μg LAl L^{-1}) acted as controls. 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 20 μg Al g^{-1} gill dw prior to seawater release. The physiological responses measured as plasma Cl^- and glucose were related to the LAl concentration in water and to the accumulation of Al onto the gills. Gill Na^+,K^+-ATPase activity was depressed in all groups having >25 μg Al g^{-1} gill dw. Following exposure, the smolts were released into River Imsa to monitor downstream migration and ocean return rates. Acid exposed smolts migrated out of the river together with controls. Adult return rates were reduced by 20 to 50% in all Al-exposed groups relative to the control groups, although marine growth was unaffected. The results suggest that even moderately and/or episodically acidified rivers containing 5–15 μg LAl L^{-1} can cause substantial reductions in returns of Atlantic salmon.

Keywords: Acid rain; Aluminum, Atlantic salmon; *Salmo salar*; Smoltification, Accumulation, Gill, Physiological stress responses, Marine survival

1. Introduction

It is commonly acknowledged that anthropogenic acidification has affected Atlantic salmon (*Salmo salar*) populations on both sides of the Atlantic Ocean (Haines and Akielaszek, 1984; Hesthagen and Hansen, 1991; Watt et al., 2000; NMFS, 2004). Acid water has led to the extinction or severe population reductions in 30 rivers in Southern Norway (Sandøy and Langåker, 2001) and is regarded as the most likely cause for, or as a contributor to, population reductions in several other rivers (Kroglund et al., 2002).

* Corresponding author. Tel.: +47 90 68 92 54.
E-mail address: frode.kroglund@niva.no (F. Kroglund).

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Acidification involves a reduction in water-pH, but water toxicity is primarily due to the concurrent increase in the concentration of labile (cationic/inorganic) species of aluminum (LAl). While low pH will aggravate the effects of Al, increased concentrations of calcium (Ca), increased ionic strength, and the presence of various organic and inorganic ligands can act to reduce toxicity (see reviews in: Exley et al., 1991; Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). The ecological effect of aluminum depends not only on exposure intensity, but also on the life stages exposed, exposure duration, and the possibility for post-exposure recovery (Kroglund and Staurnes, 1999; Kroglund et al., 2001). The smolt stage represents the most vulnerable life stage of Atlantic salmon (Rosseland and Staurnes, 1994; Rosseland et al., 2001). This life stage is in the wild only present in spring, a time period when water quality can change rapidly due to snow melt and/or to changes related to acid rain fall and to sea salt episodes (Teien et al., 2004, 2005).

While severe toxicity causing mortality is associated with ionoregulatory and respiratory failure (Exley et al., 1991; Rosseland and Staurnes, 1994, Gensemer and Playle, 1999), the ecological effect of sublethal exposures is more uncertain as the individual can recover following an episode. During the exposure phase, a sublethal water quality will initiate a long array of stress responses ranging from elevated cortisol to reduced growth rate and affect the ability to regulate plasma electrolytes (Rosseland and Staurnes, 1994; Wendelaar Bonga, 1997). Poor growth, reduced seawater tolerance and immunosuppression can all reduce the probability for subsequent post-smolt survival. While severe acidification will act to reduce smolt production in freshwater (Hesthagen and Hansen, 1991; Lacroix, 1989), moderate levels of acidification can still have population effects by affecting physiological traits important for freshwater growth and post-smolt survival (Kroglund and Finstad, 2003; Magee et al., 2001, 2003; Staurnes et al., 1995, 1996). The relationship between physiological responses and population effects is however more poorly documented.

We aimed to assess acidification effects on marine survival of post-smolts exposed to acid and aluminum of varying intensity and duration. Acidification exposed smolts were released into River Imsa early in May 1999, 2000, 2002 and 2003. The experiments simulate episodic to moderate chronic acidified rivers, where the fish could delay migration and recover within the good water of River Imsa before moving into the estuaries and seawater environment.

2. Materials and methods

2.1. Water analysis

pH, temperature and oxygen were measured *in situ* several times weekly. pH and temperature were measured using a PHM80 Portable pH meter with a Radiometer pHC2005 electrode (Radiometer; Denmark). Oxygen was measured in the tank outlet water using an Oxyguard (OxyGuard International A/S; Denmark). Water samples were taken at regular intervals (>once a week) from Control and from the inlet water of the exposure tanks. Aluminum was fractionated with respect to charge and reactivity. Total-Al was analyzed on an inductively-coupled plasma atomic emission spectrometer ICP-AES (Perkin Elmer), total reactive Al (RAl) and total non-labile Al (NLAl) was analyzed by flow injection analyses (FIA) using the pyrocatechol-violet (PCV) method (Røgeberg and Henriksen, 1985). NLAl is the fraction of Al that passed through a cation-exchange column (Amberlite). The labile Al fraction (cationic or inorganic monomeric) forms of Al (LAl), was calculated as the difference between RAl and NLAl.

Total organic carbon (TOC) was determined after oxidation by peroxydisulfate and UV-irradiation. Major base cations (Ca, Mg, Na, K, Si) were analyzed on an ICP-OES (Thermo Jarrell Ash Polyscan), anions (SO_2, Cl) by ion chromatography (IC), and F using an ion selective electrode and NO_2^{3+} on a CFA, auto analyzer using standard colorimetric methods. CO_2 was analyzed according to Standard Methods (APHA; AWWA; WEF; 4500-CO_2, 4–12; 4–18).

Acid neutralizing capacity (ANC) is a measure of water sensitivity to acidification. The ANC-value is calculated as: $\sum$ base cations minus $\sum$ strong acid anions (Reuss and Johnson, 1985).

2.2. Fish analysis

The tanks were inspected daily for mortality. Fish sampled for evaluation of physiological status were killed by a blow to the head, after which fork length (1 mm) and weight (0.1 g) were measured. Blood samples were then collected from the caudal vessels using heparinized syringes and analyzed *on site* using an I-stat blood/gas analyzer (Abbott, Chicago, USA). The second gill arch on the right side of each fish was dissected out and stored frozen in pre-weighed, acid washed polyethylene vials before freeze-drying and acid digesting. The digested samples were analyzed with respect to Al and iron on an ICP-AES (Teien, 2005). The Al-concentration measured includes Al bound to mucus, onto cell surfaces and Al incorporated inside gill cells. The Al concentration is given as μg Al g^{-1} dry weight (dw). The second gill arch on the left hand side was dissected out and frozen in 2 ml eppendorf tubes in SEI buffer for measurements of gill Na^+,K^+-ATPase activity according to McCormick (1993). Briefly, gill tissue was homogenized in 150 μl SEID (SEI buffer containing 0.1% deoxycholic acid) and centrifuged at 5000 g for 60 s. Ten microliters of supernatant was added in duplicate wells of a 96-well microplate containing 200 μl assay medium, with

and without 0.5 mM ouabain, and read at 340 nM for 10 min at 25 °C. Na^+,K^+-ATPase activity was determined as the ouabain sensitive fraction of the enzymatic coupling of ATP dephosphorylation to NADH oxidation, expressed as μmol ADP mg prot.$^{-1}$ h^{-1}. Five fish were sampled from each tank at each sampling date in 1999, while two groups of 5 fish were sampled the subsequent years (Table 1).

2.3. Fish material and exposure conditions

The experiments were carried out at the salmon research station belonging to Norwegian Institute for Nature Research (NINA) at Imsa (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 tanks (4 m^3). Exposures using natural populations would have been preferable, but not possible from a practical point of view. We therefore had to accept a semi-natural experiment, where exposures were performed in tanks, but where marine survival was assessed on fish released into a river and would thereafter be exposed to natural environmental conditions.

The fish were reared in the same tanks each year under a natural light regime and fed ordinary commercial dry diet, according to temperature and fish size. 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 for some recovery from handling. At the initiation of the exposure, the water source was changed to one of the exposure water qualities, and the flow in all tanks reduced to 25 to 30 L min^{-1} (variation between tanks within year was less than 5%). The water current was maintained at 10–12 cm s^{-1}, when measured 15–20 cm from the tank edge. The treatment tanks were stocked with approximately 1200 fish (Table 2). At the time of tagging the fish were 16 to 19 cm and had a weight of 39 to 66 g.

2.4. Exposure groups and water qualities

The Control group was exposed to water from Lake Imsa. The three Al-exposure groups were exposed to varying levels of acidity and Al, using the episodically acid River Fossbekken as the main water source (Fig. 1). This water source was lethal when pH was low (<5.4). In 1999 to 2003, 20 to 50 volume-% water from Imsa was added to reduce Al-related toxicity (Table 1). Because the water quality of Fossbekken was thought to be "satisfactory" (pH > 6.0) for parts of 2002 and 2003, acid (as HCl) and aluminum (as $AlCl_3 \bullet 6H_2O$) was added to maintain a sublethal water quality (pH < 6.0). Mixing water with different pH-levels and Al-concentrations generates mixing zones where Al can be more toxic than predicted from pH alone for a time-limited period (Rosseland et al., 1992; Kroglund et al., 2001; Teien, 2005). To minimize the importance of unstable forms of Al, the mixtures of water from Lake Imsa and River Fossbekken and water added acid and aluminum were aged for 10 min before being led into the exposure tanks in 1999 to 2002 and for > 1 h in 2003 (Fig. 1). The exposure tanks added another 3 h of ageing diluting the presence of transient toxic forms of Al.

The treatments are named Control, Low-Al, High-Al and Episode. The "High-Al" and "Low-Al" treatments were long term exposures lasting > 45 days (Table 1). The two treatments differed with respect to pH and labile Al-concentration (Table 3), although Δ pH within the individual year often was less than 0.3 pH-units (Fig. 2). The Episode group (short term exposure) received the same water as the Control group up to the last 3 days of the exposure period; thereafter the tank received the same water as High-Al.

Table 2
Fish size (length, weight and condition factor) for all Carlin tagged fish within year

	Treatments	Fish tagged	*L*	*W*	C-factor
			cm	G	
1999	2	1200	16.1±2.7	38.8±12.2	0.898±0.064
2000	2	1200	18.4±1.8	60.0±18.4	0.964±0.079
2002	4	1150	19.1±1.5	65.5±16.2	0.936±0.058
2003	4	1150	16.3±1.7	44.7±14.5	0.999±0.053

The number of fish tagged treatment^{-1} and the number of treatment groups (Control + Al-groups) are indicated. Between group variation in size within an individual year was <5%. The condition factor is calculated as $C = 100W * L^{-3}$.

Table 1
Exposure period, water treatments and the mixing ratio of water from Lake Imsa to River Fossbekken over the period 1999 to 2003

Year	Exposure period	Ratio Imsa to Fossb.	HCl and Al addition	Sample frequency × sample size	Control Tot-Al	High-Al Al-increase	Low-Al Al-increase	Episode Al-increase
1999	9 Feb–4 May	0.2:0.8	No	7 × 5 fish	42	+66		
2000	23 March–8 May	0.3:0.7	No	5 × 10 fish	43	+62		
2002	21 March–6 May	0.3:0.7	Yes	7 × 10 fish	32	+61	+39	+61
2003	18 March–6 May	0.5:0.5	Yes	7 × 10 fish	64	+113	+114	+113

The number of times fish were sampled from each tank and sample size is presented. The average concentration of total-Al in the Control tanks and the increase in total-Al relative to the Control is indicated for each treatment and year. The episode treatment received Control water up to episode start, thereafter the same water as High-Al for the remaining 3 days of the exposure period.

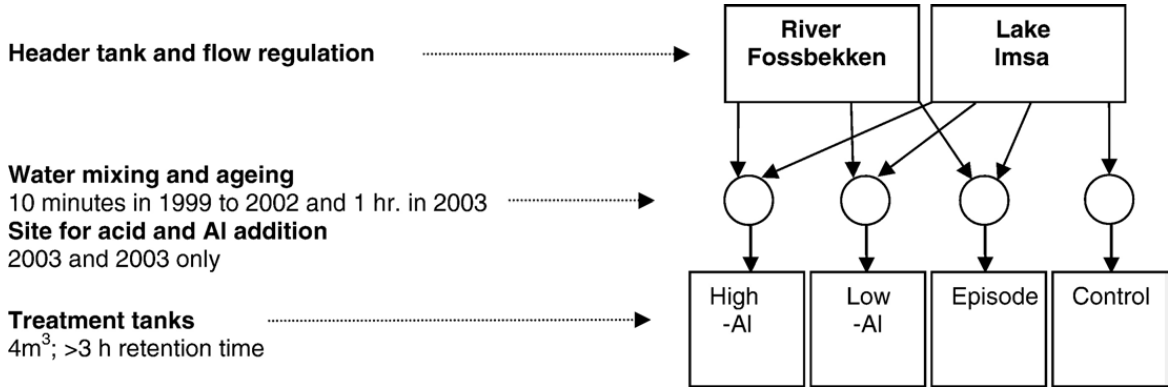

Fig. 1. Illustration of water flow, water mixing sites and site for chemical additions. Each treatment was performed in individual tanks. There were slight variations in the set up between years, indicated in Table 1.

The within year variation in temperature between treatments was small and generally <±0.2 °C. The between year variation was large in April (Fig. 2), where April was "cold" in 2003, and "warm" in 2002.

2.5. Post-exposure treatment

To obtain information of possible treatment effects on downstream migration rates and timing, 150 individuals from each treatment were released into River Imsa (pH>6.5) 800 m above an upstream/downstream Wolf trap (Wolf, 1951) in 1999, 2000 and 2003. To monitor marine survival and marine growth, the majority of the fish (n>900 treatment^{-1}) were released into River Imsa downstream from the Wolf trap or 150 m above the estuary. As the fish were released into freshwater (FW), movement from FW to seawater (SW) was not forced and the fish could migrate at will. The releases were timed to the natural migration of the wild fish. During the study years 25% of the wild smolts had migrated downstream between the dates of 29th April and 2nd May whereas 75% had migrated between the dates 12th and 14th May. Marine survival is evaluated with respect to adult returns to River Imsa and on the basis of total reported recaptures (River Imsa+ocean captures+captures in other rivers). All captures outside River Imsa depended on fishing activity. Marine growth and return dates were assessed from fish returning to River Imsa only.

2.6. Statistical analysis

All data presented are means±1 standard deviation (SD). Differences between treatment groups were tested using one-way ANOVA (StatGraphics plus). Significant ANOVAs were further analyzed using the non-parametric Kruskal–Wallis post-hoc test. Relationships between dose (LAl and gill-Al) and response (plasma Cl^-, glucose and Na^+,K^+-ATPase activity) were analyzed using regression analysis. If there were no differences in intercept and slopes between years, the data was pooled. Differences in marine survival were tested using Yates corrected chi-square test. A Pearson chi-square test was used for testing the probability of homogeneity in 2×2 contingency tables for different groups with respect to recaptures. Differences in downstream migration and adult return rates to River Imsa were tested using the Kruskal–Wallis rank test. This is a one-way analysis of variance by

Table 3
Average values for some water quality constituents in Control and Al-treatment waters during the exposure periods in 1999 to 2003

		Control				Low-Al		High-Al			
	Unit	1999	2000	2002	2003	2002	2003	1999	2000	2002	2003
TOC	mg L^{-1}	0.9 (0.2)	1.1 (0.1)	2.6 (0.1)	2.6 (0.1)	2.4 (0.2)	2.9 (0.2)	1.3 (0.2)	1.2 (0.2)	2.3 (0.3)	2.9 (0.2)
pH	H^+	6.9 (0.2)	6.9 (0.1)	7.0 (0.0)	7.1 (0.1)	6.6 (0.1)	6.0 (0.2)	5.7 (0.2)	5.8 (0.3)	6.0 (0.4)	5.8 (0.2)
Ca	mg L^{-1}	4.3 (0.1)	4.5 (0.1)	4.3 (0.1)	4.5 (0)	2.8 (0.2)	3.0 (0.1)	1.7 (0.1)	2.0 (0.3)	2.4 (0.4)	2.9 (0.1)
ANC	μeq L^{-1}	232 (96)	146 (7)	210 (28)	206 (12)	98 (19)	48 (7)	16 (9)	21 (21)	29 (28)	23 (6)
Tot-Al	μg L^{-1}	42 (18)	43 (9)	32 (9)	64 (5)	71 (26)	178 (36)	108 (15)	105 (29)	93 (35)	177 (37)
Ral	μg L^{-1}	16 (4)	15 (4)	16 (6)	16 (5)	30 (10)	80 (34)	43 (10)	54 (25)	46 (21)	79 (33)
LAl	μg L^{-1}	2 (1)	3 (2)	3 (2)	4 (2)	6 (2)	11 (4)	21 (15)	17 (13)	12 (9)	13 (4)

One standard deviation is given within brackets. TOC=total organic carbon, Ca=calcium, ANC=acid neutralizing capacity, Tot-Al=total aluminum (ICP), RAl=Al reactive to PCV, LAl=Al reactive to ion exchanger.

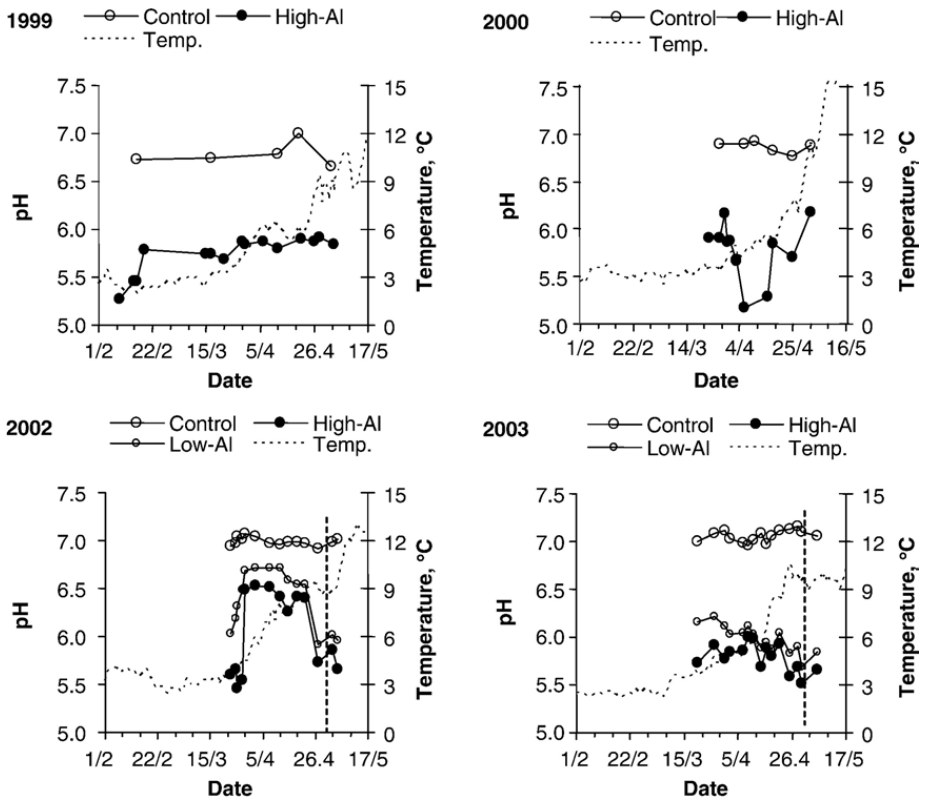

Fig. 2. Day to day variation (Feb. to May) in pH and temperature for each exposure year (1999 to 2003). Treatments can be identified by the legend. The episode group in 2002 and 2003 had pH-values close to the Control group prior to the start of the episode (indicated by vertical dashed line), then values close to High-Al for the remaining 3 days of the exposure period. Temperature in the treatment tanks was <±0.2 °C different from the temperature in the Control tank.

ranks, testing the null hypothesis that multiple independent samples come from the same population. Unlike standard ANOVA, it does not assume normality, and it can be used to test ordinal variables. All data are treated as being statistically significant when $P \leq 0.05$.

Specific growth rate (G) at sea was calculated from recaptured adults (Jonsson et al., 2003) as:

(ln adult body length (mm) − ln smolt body length (mm)/ number of growth seasons at sea).

3. Results

3.1. Water quality

The control water had an average pH > 6.8 and < 65 μg L^{-1} total Al in all years (Fig. 2; Table 3). Less than 10% of the total Al was recovered as labile Al (LAl). The ANC was > 150 μeq L^{-1}. In the acid treatments, average pH values ranged from 6.0 to 6.6 in the Low-Al exposure groups and from 5.7 to 6.0 in the

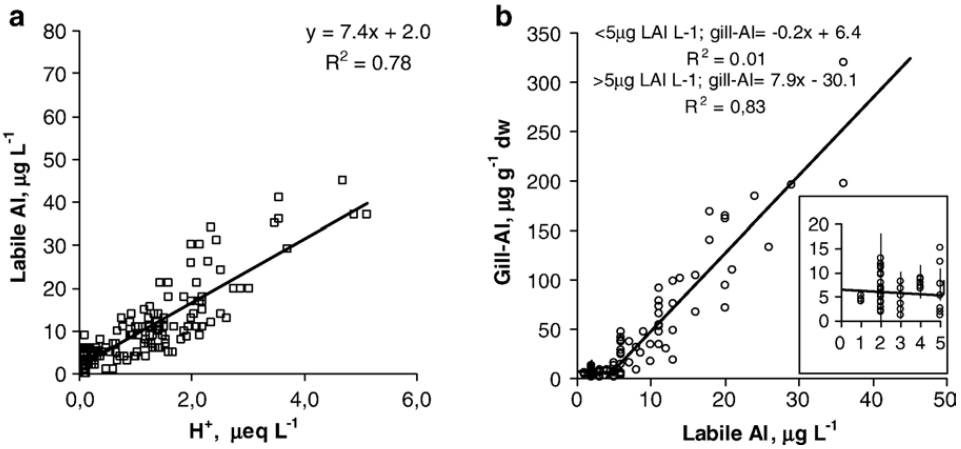

Fig. 3. a) Relationship between pH (as H^+) and LAl and b) LAl and gill-Al. The relationships are based on all samples from 1999 to 2003. There was no significant difference with respect to slope or intercept between years. The relationship between LAl and gill-Al for LAl concentrations < 5 μg L^{-1} is inserted to give a better resolution at low Al concentrations.

Table 4
Daily growth calculated as length (mm) or weight (g) change from tagging to the end of the exposure period (in freshwater) divided by the number of days the fish were exposed from March

	Length, mm/day				Weight g				Mortality number dead			
Year	1999	2000	2002	2003	1999	2000	2002	2003	1999	2000	2002	2003
Exp.days	84	46	44	49	84	46	44	49				
$n=$	>100	>60	>140	>120	>100	>60	>140	>120				
Control	0.10 (0.08)	0.13 (0.13)	0.35 (0.21)	0.12 (0.08)	0.07 (0.07)	0.04 (0.06)	0.36 (0.28)	0.03 (0.03)	6	10	8	23
High-Al	*0.04	*0.06	*0.18	*0.07	*−0.04	*−0.12	*0.15	*−0.06	33	28	54	130
	(0.11)	(0.11)	(0.24)	(0.05)	(0.09)	(0.06)	(0.31)	(0.06)				
Low-Al			*0.17	*0.08			*0.17	*−0.07	30	18	43	86
			(0.25)	(0.07)			(0.34)	(0.07)				
Episode			0.32 (0.18)	0.09 (0.09)			0.32 (0.26)	0.07 (0.06)			35	90

Standard deviation is given in brackets. Exposure year (1999–2003), exposure days and minimum sample size ($n=$) is indicated. Mortality is presented as the number of dead fish accumulated over the whole exposure period. The initial number of fish stocked into the individual tanks is given in Table 2. Changes that were significant from the Control are labeled with an asterisk (*).

High-Al exposures (Table 3). LAl ranged from 6 to 11 μg L^{-1} in Low-Al and from 12 to 21 μg L^{-1} in High-Al. The ANC level in Low-Al ranged from 48 to 98 μeq L^{-1} and from 16 to 29 μeq L^{-1} in High-Al. Calculated as H^+, pH and LAl were closely related (LAl=7.9*(H^+)−30); R^2=0.83; P<0,05) (Fig. 3a). There was also a close relationship between H^+ and ANC (ANC=−53.6 Ln(H^+)+49; R^2=0.83; P<0.05). Due to these relationships, water quality can be classified using pH, LAl and ANC. Due to the varying pH over time and the timing of and duration of periods with low pH, there were differences in water quality both within and between treatments and years (Fig. 2).

In all treatment waters TOC (total organic carbon) ranged from 0.9 to 3.0 mg C L^{-1}. Oxygen measured in the tank outlet water varied all years between 11 and 12 mg O_2 L^{-1} in early February to levels around 10 mg L^{-1} in May. CO_2 was measured in the inlet and outlet of the tank. The total CO_2 concentration increased in the tanks by 1 mg C L^{-1} from a background level of ~2 mg C L^{-1}.

3.2. Gill Aluminum and fish responses

Gill aluminum can be used as a measure of bioavailable Al. A bi-phasic relationship was observed, with a close (R^2=0.83) relationship between LAl and gill-Al when the LAl concentration exceeded 5 μg Al L^{-1} (Fig. 3). There were no differences in intercept and slope between years. For LAl-concentrations <5 μg Al L^{-1} there was no relationship.

Mortality was low in the Control tanks (<2%), slightly higher in the Al-exposure tanks (Table 4). Fish maintained under control conditions had plasma Cl^- concentrations >130 mM and glucose concentrations <9 mM in all years. Plasma Cl^- decreased and glucose increased with increasing gill-Al concentration (Fig. 4a,b). There was no difference in intercept and slope between years for plasma glucose. For plasma Cl^-, there was no difference in intercept between years, but the slope in 1999 and 2000 was significantly different from the slope in 2002 and 2003 (Table 5).

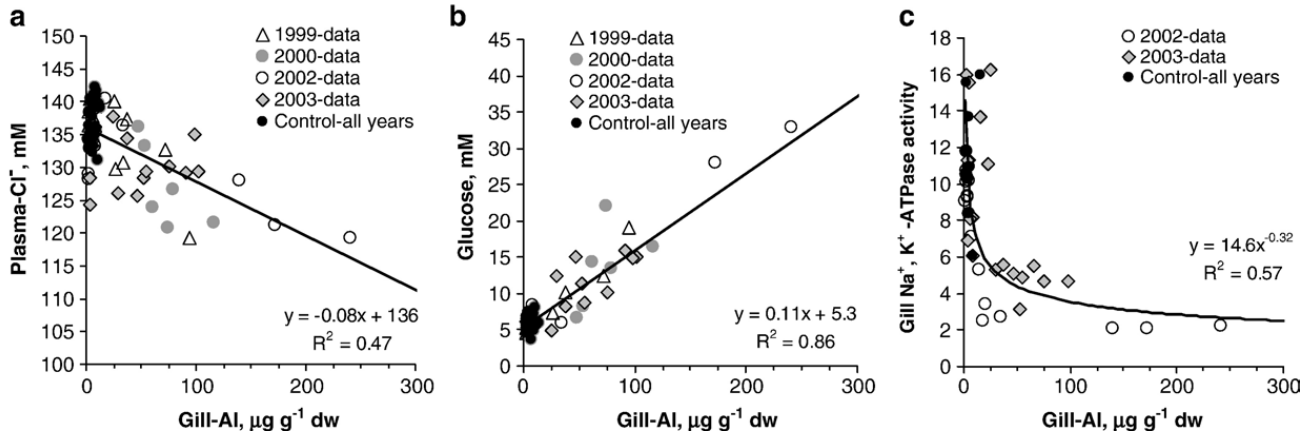

Fig. 4. Relationship between average gill-Al and average a) plasma Cl^-, b) glucose and c) gill Na^+,K^+-ATPase (μmol ADP mg $protein^{-1}$ h^{-1}) for all samples from all treatments all years. Sample year can be identified by the legend. Samples from the Control tanks are marked by heavy black dots. The regression lines are based on pooled data from all years. Year to year variation in intercept and slope is presented in Table 5.

Table 5
Within year relationship between gill-Al (μg Al g^{-1} dw) and plasma glucose, plasma Cl^- and gill Na^+,K^+-ATPase activity

Year	Glucose (mM)			Plasma Cl^- (mM)			Gill Na^+,K^+-ATPase (μmol ADP•mg protein^{-1}•h^{-1})		
	Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2
1999	4.8	0.13	0.96	140	−0.17[a]	0.67			
2000	5.3	0.12	0.67	141	−0.19[a]	0.75			
2002	5.9	0.12	0.97	135	−0.06[b]	0.65	14.5	−0.39	0.87
2003	5.4	0.11	0.75	135	−0.05[b]	0.15	17.1	−0.28	0.47
All years	5.3	0.11	0.86	136	−0.08	0.47	14.6	−0.32	0.57
P-value	0.733	0.525		0.139	0.002		0.722	0.011	

Statistical differences between years are indicated by (a) or (b), where different letters indicate differences that are significant ($P<0.05$). The relationship for data pooled over all years is included.

Gill Na^+,K^+-ATPase activity was only measured in 2002 and 2003. The activity in the Control treatments increased from normal parr levels in March to (<6 μmol ADP mg protein^{-1} h^{-1}) to normal smolt levels (>13 μmol ADP mg protein^{-1} h^{-1}) in May. In the Al-treatment groups, no such increase was observed. The activity remained at parr levels in all fish having gill-Al concentrations exceeding 25 μg Al g^{-1} dw ($R^2=0.59$) (Fig. 4c).

3.3. Freshwater growth

Growth was significantly reduced in Low- and High-Al relative to the Control. While the Control and Episode fish increased their length by an average of 6 mm and weight by 1 to 3 g over the whole exposure period, the two Al long term exposure groups increased their length by only 2 to 3 mm and had a weight loss most years (Table 4). The Episode group grew with a similar rate as the Control. The within year variations in growth cannot be related to temperature, nor to the feeding regime. There was also a between year variation in growth, where both the Control and High-Al groups grew significantly ($P<0.05$) better in 2002 than the other years.

3.4. Downstream migration

The fish were released earlier in 1999 than in 2000 and 2003 (4th, 8th, and 6th of May respectively). The majority of the fish released to monitor downstream migration were recaptured at the Wolf trap within days. From the Control groups, 96, 100 and 88% of the fish released ($n=150$) were recaptured at the trap in 1999, 2000 and 2003 respectively. From the High-Al group, the recaptures were 94, 97, 69% the three years. From the Episode and Low-Al groups in 2003, the recaptures were 89 and 86%. The migration rate was significantly different between Control and Al-exposure groups in 1999 (Chi-Square=34.7, $P<0.001$) and 2000 (Chi-Square=8.7, $P<0.001$), but not so in 2003 (Chi-Square=7.9, $P=0.095$). The time (hours) it took to reach the median number of migrants was 58.5 h for the Control and 84.5 h for High-Al exposed fish in 1999 (Table 6). Similar levels of migration were reached within 13.0 h for the Control and 10.0 h for High-Al in 2000. In 2003, the median time was 11.3 h for Control, 12.9 for Low-Al, 11.2 for High-Al and 10.3 for the Episode group. The time difference to a 50% recapture was in the order of hours between the Control groups and Al-exposure groups. The temperature during migration was around 9 °C in 1999 and around 14 °C in 2000 and 2003. All migrations occurred at night and between the hours of 23:00 and 04:00.

3.5. Recaptures

The majority of the fish (>80%) returned as 1-sea year fish. Sea-returns started early in September for the Control group and lasted until late November. The median date for 50% return to River Imsa was not significant different ($P>0.05$) between the treatment groups (Table 6). The size of returning fish ranged from 55 to 60 cm. Specific growth rate varied in the Control group from a low of 1.15 to a high of 1.26. There

Table 6
a) Median time (h) to 50% recapture of smolts released 800 m upstream the Wolf trap in River Imsa, b) median date for 50% adult returns and c) specific growth rate at sea (G)

Release year	1999	2000	2002	2003
A)	Hours to 50% smolt recaptures in trap			
Control	58.5	13.0	–	11.3
Low-Al	–	–	–	12.9
High-Al	84.5	10.0	–	11.2
Episode	–	–	–	10.3
P=	0.001	0.001		0.095
B)	**Date for 50% adult returns**			
Control	17. Oct.	15. Oct.	06. Oct.	18. Sep
Low-Al	–	–	01. Oct.	22. Sep
High-Al	19. Oct.	29. Oct.	01. Oct.	04. Sep
Episode	–	–	06. Oct.	17. Sep
P=	0.207	0.064	0.442	0.566
C)	**Specific growth rate at sea (G)**			
Control	1.23 (0.09)	1.20 (0.11)	1.15 (0.19)	1.26 (0.10)
Low-Al	–	–	1.15 (0.15)	1.22 (0.16)
High-Al	1.22 (0.10)	1.16 (0.15)	1.19 (0.14)	1.28 (0.14)
Episode	–	–	1.13 (0.09)	1.27 (0.12)
P=	0.7346	0.4470	0.7725	0.4535

Return date and specific growth rate is calculated on adults returns to River Imsa only.

Table 7
Number of fish released as smolts into River Imsa and subsequent adult recaptures in other rivers (River recap.), at sea (Sea recap.) and in the Wolf trap in River Imsa (Imsa recap.)

Release Year		Number released	Smolt size at release.	River Recap%.	Sea Recap. %	Imsa Recap. %	Sum recap.	Recapture rate %	Recaptures relative to Control %
1999	Control	934	16.9±1.5	12.7	11.8	75.5	110	11.8	100
	High-Al	973	*16.2±1.8	9.2	13.2	77.6	76	7.8	* 66 (0.004)
2000	Control	1057	18.9±1.8	5.4	29.3	65.2	92	8.7	100
	High-Al	1005	18.9±1.9	6.2	52.3	41.5	65	6.5	74 (0.056)
2002	Control	851	19.8±1.9	20.0	12.0	68.0	25	2.9	100
	Episode	951	19.2±1.2	12.5	20.8	66.7	24	2.5	74 (0.584)
	Low-Al	1103	19.2±1.2	21.2	21.2	57.6	33	3.0	87 (0.951)
	High-Al	1085	19.3±1.5	25.0	28.6	46.4	28	2.6	75 (0.627)
2003	Control	2975	16.2±1.9	15.4	15.4	69.2	65	2.2	100
	Episode	1197	16.6±1.5	11.8	17.6	70.6	17	1.4	66 (0.108)
	Low-Al	1028	16.3±2.3	23.1	15.4	61.5	13	1.3	57 (0.066)
	High-Al	1040	15.7±1.3	20.0	10.0	70.0	10	1.0	* 44 (0.012)

All numbers are in % of total recaptures (sum recap). Recapture rate (%) is the total recaptures relative to the number of smolts released. Percent (%) of Control recaptures is calculated from return rate of Al-exposure groups relative to Control returns (=100%). Significance levels (P=) are indicated within the brackets or tagged by (*). The smolt size at time of release is based on the actual measurements made during tagging, corrected with the average observed from tagging to release (Table 4).

was no difference ($P>0.05$) in growth between Control treatments and Al-exposure groups within years (Table 6).

The year-to-year variation in adult recaptures was large, ranging from a low of 2 to 5% return rates in 2002 and 2003 to a high return rate of 6 to 12% in 1999 and 2000. The Al-exposure groups had lower return rates than the Control groups in all years (Table 7). The adult returns ranged from 44 to 87% of the Control. The low returns to River Imsa from the High-Al group in the year 1999 and 2003 were significant. The difference was close to significant for High-Al in 2000 and Low-Al in 2003.

Averaged over the years, 69±4% of all adult recaptures from the Control group were made in River Imsa, compared to 69±3% for the Episode treatment, 60±3 for Low-Al treatment and 59±18 for the High-Al treatment. Remaining recaptures were made at sea or in other rivers. Although these differences are not significant, the results indicate a possible treatment effect on homing.

The variation in recaptures (all treatments) could be expressed as a simple relationship ($P<0.05$; $R^2=0.86$) to gill-Al (Fig. 5a). In the figure, returns are related to the gill-Al concentration the fish had upon termination of exposure. Other representations of dose, using time-averaged means for the whole exposure period or for the last few weeks prior to release, gave similar relationships but with a poorer R (0.6 to 0.7). The episode groups fitted into the same model as the smolts exposed to Al for 40 days or more. In addition to gill-Al, return rates were related to gill Na^+,K^+-ATPase activity (Fig. 5b). Returns were highest in groups having elevated Na^+, K^+-ATPase activity and low in groups having low activity.

4. Discussion

4.1. Dose–response relationships — a general overview

During our work on Atlantic salmon, it has become increasingly clear that the hypoosmoregulatory capacity

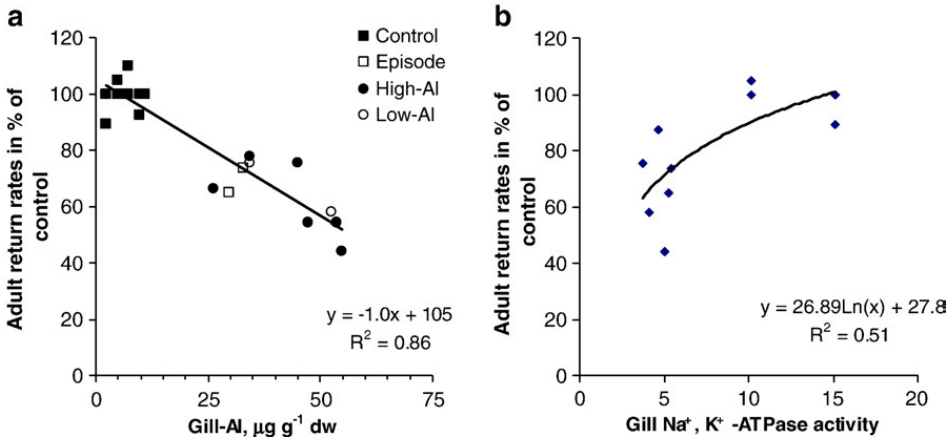

Fig. 5. a) Relationship between gill-Al measured at exposure termination and the adult return rate in% of Control. The four treatments are identified by the legend. b) Relationship between gill Na^+,K^+-ATPase (µmol ADP mg protein^{-1} h^{-1}) and adult return rate in % of Control.

of the smolt (as determined by a seawater challenge test) is extremely sensitive to Al, being affected at Al-concentrations that are clearly non-lethal in freshwater (Staurnes et al., 1995; Kroglund and Staurnes, 1999; Kroglund et al., 2001; Magee et al., 2001, 2003). The assumption in these works is that poor hypoosmoregulatory capacity will reduce seawater survival, thereby affecting adult return numbers. The link between gill Na^+,K^+-ATPase activity and hypoosmoregulatory capacity is well described (Staurnes et al., 1993a; Handeland et al., 1998; McCormick and Saunders, 1987; McCormick et al., 1998), but there are few works linking hypoosmoregulatory capacity to actual effects on seawater behavior and survival rates (Virtanen et al., 1991; Staurnes et al., 1993b) or Al to post-smolt survival (Staurnes et al., 1996; Kroglund and Finstad, 2003; Magee et al., 2003).

4.2. Water quality

The control water can be regarded as being unaffected by acidification based on the high pH and ANC, and the low Al-concentration. There was as expected a close relationship between pH (as H^+) and LAl (Fig. 3). The Al-treatment tanks were slightly acidic, having higher concentration of LAl and lower pH and ANC than the control tanks. While the exposures in 1999 and 2003 had more stable pH-levels, the exposures in 2000 and 2002 were more episodic. Episode timing, intensity and duration and post-exposure water quality varied as such within and between years (Fig. 2). LAl concentrations <10 μg Al L^{-1} were commonly calculated when pH$>$5.8. These LAl concentrations are highly uncertain (Teien, 2005). However, as LAl was highly related to gill-Al when the LAl concentrations >5 μg Al L^{-1}, we assume that the dose was low despite being uncertain. At LAl concentrations <5 μg Al L^{-1} the relationship was poor. It is likely that some of the LAl values calculated to be <5 μg Al L^{-1} in the Al-treatment tanks were actually higher based on gill-Al being >6 μg g^{-1} gill dw. Regardless of this uncertainty, the good relationship between LAl and gill-Al, suggest that the fish were exposed to low concentrations of LAl, where the dose increased from Control to High-Al.

4.3. Physiological responses — control fish

In the present experiment, the control groups had a physiological status that we regard as normal for smolts, with plasma Cl^- concentrations ranging from 132 to 143 mM and glucose from 4 to 8 mM. The fish underwent normal smoltification based on the increase in gill Na^+,K^+-ATPase activity (McCormick et al., 1998). The recapture rates were in accordance with rates observed for smolt releases at River Imsa (Jonsson et al., 2003). The control fish had gill-Al concentrations of 5.9 ± 3.3 μg Al g^{-1} gill dw ($n=250$ samples) averaged over all years. These are low levels, and levels that have not been associated with any reduction in seawater performance (Fivelstad et al., 2004; Kroglund and Finstad, 2003). Both water quality and the low gill-Al concentrations qualify the fish to be termed as acidification naïve at exposure start.

4.4. Physiological responses — exposed fish

LAl exercises its toxic properties by being bound to negative sites in mucus and onto the cell membrane of the gill (Handy and Eddy, 1989; Exley et al., 1991; Sparling and Lowe, 1996). After prolonged exposure time, Al is also found inside cells (Exley et al., 1991). It is reasonable to assume that the accumulation of Al at these different sites can result in similar response, but that there also can be site specific responses. E.g., accumulations that inhibit gill Na^+,K^+-ATPase activity need not be related to the same accumulation site as accumulations that affects blood electrolytes and respiration.

In water having elevated H^+ and Al concentrations, mortality is mainly related to H^+ when pH is close to 5 or lower and due to Al at higher pH values (Neville, 1985; Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). Atlantic salmon smolts exposed to pH values down to 5.4 without the presence of Al do not suffer any physiological impairment (Lacroix, 1989; Fivelstad et al., 2004). In our exposures, pH was higher than 5.4. The physiological responses were related to Al, but the variation in H^+ and the high concentration of Ca (>2 mg L^{-1}) in 2002 and 2003 could have acted to modify toxicity, e.g. be the cause for the significant difference in relationship between gill-Al and plasma Cl^- observed between years. As LAl is bound in a dose dependent manner to the gill, it is to be expected that there should be a close relationship between *in situ* fractionated Al, gill-Al and physiological response as observed in previous work from our group (Kroglund et al., 2001; Kroglund and Finstad, 2003; Kroglund and Rosseland, 2004; Teien et al., 2004). Similar cause–effect chains were observed in the data we report here, but due to the use of a different analytical protocol for Al, responses were observed at lower concentrations of cationic Al than previously reported. This does not affect the gill-Al/physiological relationship. The gill-Al concentration was at levels where the physiological responses in earlier work have ranged

from "no observed effect" to "moderate effect", and was only occasionally at levels (>250 μg Al g^{-1} dw) where some mortality can be expected provided exposure duration is long (>7 days). The physiological responses measured over the years 1999 to 2003 were all related to gill-Al, where elevated glucose (>9 mM) and reduced plasma Cl^- concentrations (<130 mM) was always observed in fish having >75 and >100 μg Al g^{-1} gill dw, respectively (Fig. 4a–c). The plasma Cl^- levels we measured were within levels that are not associated with any severe physiological impairment and mortality (Rosseland and Staurnes 1994). The exposures can as such be termed as sublethal. Gill Na^+,K^+-ATPase activity was low (<6 μmol ADP prot.$^{-1}$ h^{-1}) in all exposure groups having gill-Al >25 μg Al g^{-1} gill dw. This relationship suggests a "cut-off" response rather than a typical dose–response relationship. Similar reductions in gill Na^+,K^+-ATPase activity have previously been reported for Atlantic salmon following exposure to Al (Saunders et al., 1983; Farmer et al., 1989; Staurnes et al., 1993a; Staurnes et al., 1996; Kroglund and Staurnes, 1999; Magee et al., 2003).

There was some unexplained variation between LAl and gill-Al and between gill-Al and plasma Cl^-. Due to a time delay between the administration of a dose and the corresponding response; a poor relationship is to be expected at the start of an episode or following the termination of the episode. During periods when water quality is changing, water quality, accumulation or depuration of Al on the gill and the corresponding changes in physiological status will be mismatched to the changes in water chemistry. Tight relationships are therefore not to be expected. Furthermore, prolonged exposure can initiate various compensatory mechanisms resulting in a normalization of physiological properties (Wendelaar Bonga and Lock, 1992). Acclimation will as such affect the relationship between a dose and the corresponding response. Acclimation can be energetically costly and result in reduced growth or growth efficiency and thereby exercise population effects (Wilson et al., 1994).

4.5. Effects on growth

Increased plasma glucose is a known secondary stress response (Wedemeyer et al., 1980; Iwama et al., 1997; Wendelaar Bonga, 1997) The elevated glucose concentrations indicate that the fish are using energy to maintain homeostasis rather than growth. Growth reduction due to acid water is observed under experimental conditions, but the effects on wild populations are more variable (McKee et al., 1989; Buckler et al., 1995; Smith and Haines, 1995; Brodeur et al., 2001; Kroglund and Finstad, 2003). While sublethal water qualities can reduce growth, this effect can be counteracted by increased availability of food as fish density declines. The growth response we observed in the long term Al-treatments therefore need not be equally observable in a wild salmon population.

Reduced growth can also influence seawater survival as large smolts have higher survival, as they are less vulnerable to predation and have a larger body mass to counteract the initial disturbances to blood plasma electrolytes (Powell and McKeown, 1986; Salminen et al., 1995). Although we observed a growth reduction that was significant for High-Al, the absolute difference in length was only minor between the treatments. The weight loss we observed may be more important in determining later seawater survival.

4.6. Effects on migration

The treatments appeared to have no appreciable influence on the downstream movements, despite compromise of seawater survival. The time difference to reach a 50% median migration was significantly different between Control and Al-treated fish in 1999 and 2000, but not so in 2003. All groups migrated during the same time interval as the wild salmon in River Imsa. Based on this, and on the high gill Na^+,K^+-ATPase activity in control smolts, we assume that the fish migrated within the "smolt window". Fish migrating outside this time window are expected to have reduced seawater survival (McCormick et al., 1998). Migration rate was related to treatment in 1999 and 2000, but factors related to temperature, flow and degree of smoltification cannot be discounted. A time-delay to reach a 50% migration rate of 1 day is most likely not the cause for any major reduction in seawater survival.

The duration of the river period can be important with respect to the health status prior to ocean entry. The 5-day river period in 1999 and 2003 compared to the 1-day period in 2000 can imply that smolts in the first two years could have recovered partially or fully from the prior treatments. Salmon smolts exposed under sublethal conditions will regain full seawater tolerance over a time period of days to weeks, where the recovery rate depends on water quality (Kroglund and Staurnes, 1999; Kroglund et al., 2001; Magee et al., 2001). The possibility for a recovery period within River Imsa can as such have counteracted or acted to reduce the adverse effects Al has on seawater survival. The treatments could still have had an effect on estuarine movements

(Magee et al., 2003). Increased time within the estuary can lead to increased smolt mortality due to predation. It has been shown that predator avoidance in the estuary is reduced in physiologically stressed smolts (Järvi 1989; Handeland et al., 1996; McCormick et al., 1998).

4.7. Effects on marine survival

There was a close within year relationship ($R^2=0.90$) between the adult recaptures reported to ICES and our tank control recaptures (Norwegian Institute for Nature Research, Salmon tagging database). This suggests that our tank environment was not the cause for the year-to-year, nor between treatment variations in seawater survival. The year-to-year variation in adult recaptures was large, ranging from a low 2–5% return rate in 2002 and 2003 to a high return rate of 6–12% in 1999 and 2000. These values are all within the range published for Carlin tagged releases performed at Imsa (Jonsson et al., 2003). Most recaptures were made in River Imsa. This can be expected based on the Wolf trap capturing all returning fish, whereas recaptures in other rivers and at sea depend on fishing activity and fishermen's willingness to return tags (Jonsson et al., 2003). In this respect it is of interest that homing appears to be slightly poorer in the Al-treatment groups than in the Control. Adjusting the recaptures for capture efficiency at the Wolf trap suggests that a larger proportion of the Al-treatment fish were being caught outside their native river.

There was a close relationship between gill-Al and marine survival, (Fig. 5a). Exposure duration appears to have had no major influence on the relationship as both long- and short-term exposed groups fitted into the same model. Although the absolute returns are within levels previously reported from River Imsa (Jonsson et al., 2003), the returns of Al-treated fish were significantly lower than the Controls when the gill-Al concentrations at exposure termination were high. This strong ($R^2=0.86$) relationship suggests that marine survival was reduced as a direct response to Al being accumulated onto the gill, and most likely by inhibiting the activity of gill Na^+,K^+-ATPase. The Al-related effects on gill Na^+,K^+-ATPase activity and seawater survival were detected at far lower concentrations than the LAl effects on other responses such as mortality, reduced plasma Cl^- or elevated plasma glucose. Other release experiments have reported larger population effects than we observed. Salmon smolts released into rivers having high concentrations of LAl had close to "zero" returns as opposed to smolts released further into the estuary (Staurnes et al., 1996). The reduction in seawater returns were also linked to reduced gill Na^+, K^+-ATPase and the loss of hypoosmoregulatory capacity. The effects Al had on growth cannot be used to explain the reduced return rate observed in the Episode groups, indicating that size was not the main component causing treatment differences in response.

Variation in gill Na^+,K^+-ATPase activity cannot discriminate between groups having a 45 to 85% return rate compared to controls. This suggests that there is an additional component affecting seawater survival that we have not been able to identify. Moore (1994) showed that exposure of salmon parr to water of pH 5.5 significantly reduced the ability of the olfactory epithelium to detect relevant odours. Furthermore, the olfactory epithelium accumulates Al (Teien, 2005). Therefore, it is possible that our water qualities had an effect on the olfactory imprinting process during downstream migration, thus contributing to the observed differences in return rates. In addition, smolt size differences caused by Al effects on growth, year-to-year differences in predator densities, presence of parasites (sea lice) or other unknown factors will act in concert with the compromised seawater tolerance (Finstad et al., 2007-this volume). It is also likely that variation in Al-dose can result in various degrees of gill tissue changes (Mallat, 1985; Jagoe et al., 1987). It can be speculated that low gill Na^+,K^+-ATPase activity due to elevated tissue damage affecting Na^+,K^+-ATPase synthesis takes longer to recover than Al-induced effects on Na^+,K^+-ATPase activity alone.

It is known that predator avoidance in the estuarine environment is affected in physiologically stressed smolts (Järvi 1989; Handeland et al., 1996; McCormick et al., 1998), and that estuary movements are delayed (Magee et al., 2003). This is what we regard as the most likely cause for the reduced seawater survival. This argument is supported by the recaptured adults from the various Al-treatments having a marine growth that was equal to the Control. Assuming that the fish can restore full post-smolt properties while in seawater (as shown in Finstad et al., 2007-this volume), the physiological impairment will be transitional. During this recovery period, the smolt can still be more vulnerable to additional stressors, regardless of the stressors being predators or parasites.

4.8. Uncertainties — ecological relevance

In a normal river population, the smolt run lasts several weeks. During this period, water quality will vary depending on rain, snowmelt and temperature. Water quality will often improve during spring. This

change in water quality during migration implies that smolts leaving the river late in the season may experience a "better" water quality than fish leaving early. If this time-span is sufficient to permit recovery, the physiological status of late migrants can differ from the early migrants, having effects on the probability of marine survival. In our treatment, all fish from the same year-class were released at the same time, giving a picture of the response to a "single" dose. Gill-Al in the range of 25 to 60 μg Al g^{-1} gill dw suggested a reduction in adult returns in the order of 20 to 50%. This will not necessarily endanger the population, but will contribute to general declines in salmon stocks, similar to those observed over the last decade on both sides of the Atlantic (Parrish et al., 1998; Finstad and Jonsson, 2001). The number of pressures contributing to this decline is large. Here we add a delayed response component, where low concentrations of Al that have no appreciable effect on the fish while still in freshwater, affect salmon populations by reducing seawater survival.

Caution must be employed in transferring the absolute numbers we experienced in our treatments to actual population effects. Other rivers having different water chemical composition can result in different dose–response relationships. The experiments were performed at higher Ca-concentrations than normally encountered in acidified water in Norway. Base cations act to modify the toxicity of both Al and H^+, where the biological response is intensified at low base cation concentrations (Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). The results reported in the present study are as such likely to be minimum responses, where the responses could be more severe and damaging in the base-poor rivers on the western coast of Norway. The organic carbon content (TOC) was within the range normally encountered in rivers on the southern and western coast of Norway (2–4 mg C L^{-1}). Although not entirely typical for an acidified river, we do regard the treatments as representative for moderately acidified rivers. The cause–effect chain linking low levels of Al to reduced seawater survival is supported in this work.

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