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## Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon

Frode Kroglund<sup>a,\*</sup>, Bengt Finstad<sup>b</sup>

<sup>a</sup> Norwegian Institute for Water Research (NIVA), Televeien 3, N-4879 Grimstad, Norway

<sup>b</sup> Norwegian Institute for Nature Research (NINA), Tungasletta 2, N-7485 Trondheim, Norway

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### Abstract

Two strains of Atlantic salmon (*Salmo salar*) presmolt were exposed for 3 months to moderately acidic water (pH 5.8;  $6 \pm 2 \mu\text{g Al l}^{-1}$  inorganic monomeric aluminum-acid exposure group) or non-acid water (pH > 6.5–6.9;  $< 5 \mu\text{g Al l}^{-1}$ —Good/control group) at NINA Research Station, Imsa, Southern Norway. Exposure to low concentrations of Al raised the gill-aluminum (gill-Al) concentration by  $20\text{--}30 \mu\text{g Al g}^{-1}$  gill dry weight compared to control fish having  $< 10 \mu\text{g Al g}^{-1}$  gill dry weight. The fish responded to the Al loading with elevated blood glucose, but retained more normal hematocrit and plasma chloride levels. Fish exposed under acid conditions grew significantly poorer than the control fish. After 3 months of exposure, 150 Carlin-tagged smolts from both Imsa treatments were released into the non-acidic River Imsa, 800 m upstream from a trap that caught all migrating smolts. Acid-exposed fish migrated downstream slightly later than the controls. At the same time, Carlin-tagged fish from all four treatments (Acid and Good, both strains; approximately  $1000 \text{ fish group}^{-1}$ ) were released downstream of the trap located 150 m above the river mouth. Based on the number of adult recaptures (by May 2001), marine survival was 20–30% lower for the acid-exposed fish than for the controls. There were no differences in marine growth between the treatments. The results suggest that even very low concentrations of Al ( $6 \pm 2 \mu\text{g l}^{-1}$ ) can reduce seawater survival, thereby having effect on the population level. The physiological responses, reduced growth and reduced marine survival also suggest that the presmolt did not acclimate to the acid conditions.

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\* Corresponding author. Tel.: +47-37-29-50-55; fax: +47-37-04-45-13.

E-mail address: [frode.kroglund@niva.no](mailto:frode.kroglund@niva.no) (F. Kroglund).

## 1. Introduction

Acidification is one of several environmental factors affecting Atlantic salmon populations in Norway (NOU, 1999). Water toxicity is related to increased concentrations of H<sup>+</sup> (reduced pH) and inorganic monomeric aluminum (Ali) in freshwater. At lethal concentrations, H<sup>+</sup> acts primarily on the permeability of the cell membrane disrupting ionoregulation, whereas aluminum exerts its toxic properties by accumulation on and in the gill tissue, disrupting ionoregulation and impairing respiration. At lower concentrations, wide ranges of sublethal responses have been described (see, e.g. Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). While toxic water qualities will reduce smolt production in freshwater by increasing the density independent mortality, the ecological effects of sublethal water qualities are inevitably more uncertain as fish can acclimate to the water quality and will recover within days when removed from acid water (Rosseland and Staurnes, 1994; Kroglund et al., 2001b).

Atlantic salmon migrates from freshwater to seawater as smolts. Smolts are preadapted to full strength seawater and will leave freshwater within the short time-period called the “smolt-window” (Folmar and Dickhoff, 1980; McCormick et al., 1998), a time-period when the fish are both physiologically and ecologically prepared for the high-salinity environment. Seawater challenge tests have demonstrated that the blood salt regulation in seawater is affected at pH/Ali levels that do not cause major disruption of homeostasis in freshwater (Saunders et al., 1983; Staurnes et al., 1993a, 1995; Kroglund and Staurnes, 1999; Kroglund et al., 2001a). The hypothesis is that sublethally stressed smolts will have a reduced smolt-to-adult survival in the marine environment as a response to e.g. inhibited enzyme activities (important for maintaining physiological homeostasis in seawater), reduced growth and effects on migratory behavior and predator avoidance (see: Wedemeyer et al., 1980; Rosseland and Staurnes, 1994; Finstad and Jonsson, 2001). During long-term (months) exposure to sublethal water qualities, fish can become acclimated to these conditions, and normal physiological properties are restored. Acclimation will as such increase the tolerance to the toxicant (Mallat, 1985; Orr et al., 1986; Muller et al., 1991; Reid et al., 1991). If Atlantic salmon is “to be truly” acclimated to acidified water, the water quality should not interfere with smoltification or affect marine survival. If marine survival is reduced, then the acclimation has little ecological significance. In addition to acclimation, strain differences in tolerance to acid water have been described for several salmonid species (e.g. brook trout (*Salvelinus fontinalis*) Ingersoll et al., 1990; brown trout (*Salmo trutta*) Dalziel et al., 1995). If individual strains are genetically adapted to acidified water, water quality limits cannot be defined at the species level, and might have to be determined for the individual fish population or watershed.

In this study, we tested the effects long-term exposures to sublethal concentration of Ali have on physiological responses, acclimation, migratory behavior, hypoosmoregulatory capacity and marine survival in two strains of Atlantic salmon (*Salmo salar* L.). The two strains represent salmon populations that inhabit rivers with different levels of acidification.

## 2. Material and methods

### 2.1. Fish material

The experiments were carried out using 1-year-old, first-generation hatchery-reared Atlantic salmon presmoltos of the River Imsa and of the River Suldal strain. River Imsa has a pH >6.5 and an Al concentration <5 µg Al l<sup>-1</sup>, and is not regarded as being affected by acidification (Brodeur et al., 2001). River Suldalslägen is regarded as being moderately acidified (Blakar, 1995). pH is normally >6, but can fall to values <5.5 during episodes. At the same time Al increases to levels exceeding 15 µg Al l<sup>-1</sup>.

Both strains were reared under normal smolt production routines at NINA's Research Station, Ims, from eggs and sperm collected from adults caught in the two rivers. The two strains were kept in separate tanks, but under otherwise identical conditions. In March 1999, the presmoltos of the Imsa strain were 16.1 ± 1.6 cm, having a weight of 38.8 ± 12.2 g and a condition-factor of 0.90 ± 0.05. Presmoltos of the Suldal strain were slightly larger, having a length of 17.0 ± 1.5 cm, a weight of 48.2 ± 12.8 g and a condition-factor of 0.96 ± 0.06.

### 2.2. Exposure conditions

On February 9, 1999, two groups of 1250 individuals from each strain were sorted out of the two main rearing tanks (50 m<sup>3</sup>) and stocked into one of four 4 m<sup>3</sup> exposure tanks. Each tank received water (20 l min<sup>-1</sup>) from either River Imsa (Good; pH 6.5–6.9) or from the more acidic River Fossbekken (Fossbekken; pH 5.3–5.8). Due to increasing mortality in the two tanks receiving water from Fossbekken during the first exposure week, water toxicity had to be reduced. This was achieved by adding 30 vol.% water from Good into the flow from Fossbekken, starting on February 17. Addition of Good increased pH from 5.3–5.4 to 5.8 ± 2. This mixture is termed Acid. Tanks receiving water from River Imsa acted as controls.

The oxygen concentrations in the tank outlets varied between 11 and 12 mg O<sub>2</sub> l<sup>-1</sup> in early February and 9–10 mg l<sup>-1</sup> in May. The water current was maintained at 10–12 cm s<sup>-1</sup>, when measured 15–20 cm from the tank edge. Water retention time exceeded 3 h.

All fish were Carlin-tagged (Carlin, 1955) on March 9, when water temperature had increased above 3 °C. At the same time, all fish were length measured and weight was recorded on 25% of the fish. The Carlin tags permitted identification to treatment and strain, and allowed growth to be assessed on individual fish.

### 2.3. Analysis

Water samples were taken on a regular basis from Fossbekken, Acid and Good and analyzed for all major ions at the Norwegian Institute for Nature Research (Brodeur et al., 2001; Ytrestøy et al., 2001). The following Al fractions were analyzed; total or acid-reactive Al (TAI), total monomeric Al (TMAI) and organic monomeric Al (OMAI). Labile or inorganic monomeric species of Al (Ali) (representing the toxic form of Al) was determined as TMAI minus OMAI. The particulate/colloidal fraction (PCAI) was calcu-

lated as TAI minus TMAI. Aluminum was fractionated days after collection. Detection limit for Al is  $2 \mu\text{g Al l}^{-1}$ . Acid neutralizing capacity (ANC) is calculated as the sum of base cations minus anions (Henriksen et al., 1992).

The tanks were inspected for mortality (mort) daily. Fish sampled for physiological analyses were killed by a blow to the head. Blood samples were collected from the caudal vessels using heparinized syringes. Blood glucose (B-glu) was measured immediately on whole blood using Medisence Precision Q.I.D sensor. Hematocrit (Hct) was determined immediately on a Compur M 1100 microcentrifuge ( $1400 \times g$ ; 5 min). Blood plasma was fractionated on a Hettich EBA 85 blood centrifuge ( $3000 \times g$ ; 5 min). Plasma chloride (P-Cl) concentration was assayed on a Radiometer CMT 10 chloride titrator. The second gill arch on the right side of each fish was dissected out and frozen in pre-weighed, acid washed polyethylene vials for analysis of total gill-aluminum (gill-Al) content (Kroglund et al., 2001a,b). The results are presented as Al per gram dry weight (dw) gill. Physiological samples were taken on March 8 and 30, April 22 and May 4, representing 27, 49, 72 and 84 days of exposure. In addition, gill-Al was sampled after 7, 36 and 62 days. Prior to each sampling, 15 smolts from each tank were transferred to a 24-h challenge test performed in 30–32‰ saltwater at  $9\text{--}13^\circ\text{C}$  (Kroglund and Staurnes, 1999).

Mortality, blood physiology and gill-Al are used as indicators of fish response to water quality. We use 120–140 mM for plasma chloride, 35–45% for hematocrit and 3–6 mM for glucose as indicators of “no effect”. Physiological responses deviating from the predefined “no effect levels” or from the control, indicate a treatment response. Gill-Al concentrations higher than  $6 \pm 5 \mu\text{g Al g}^{-1}$  dw (background level in Good) are interpreted as Al accumulation on or in the gill tissue. Fish surviving the seawater challenge, having plasma chloride <160 mM, are regarded as smolts capable of normal osmoregulation in full strength seawater.

#### 2.4. Post-exposure treatment

On termination of exposure (May 4), subgroups of smolts were given different treatments. The majority of the fish (942 and 943 for Imsa Good and Acid, respectively, and 1118 and 1115 for Suldal Good and Acid, respectively) was mixed in a transport tank and released in River Imsa below an upstream/downstream migration trap located 150 m above the river mouth. The smolts were released to monitor marine survival and growth. Marine survival and growth was assessed from Carlin tags and catch data sent in to the authorities by fishermen.

Possible treatment effect on downstream migration rates was tested by releasing 150 individuals from both Imsa groups into River Imsa 800 m above the fish trap. Only the Imsa strain is permitted to be released above the trap to avoid the possibility of introducing non-native genetic material into the population. The two groups were transported to the release site in one transport tank and released at 15:30 h. The fish trap was inspected and emptied every hour between 21:00 and 04:00 from May 4 to May 10. In addition, the trap was emptied daily at 08:00 and 16:00 h. After May 10, the trap was only emptied at daytime. Blood samples were taken from smolts caught in the trap during the first five nights to assess the physiological status and seawater tolerance of downstream migrating smolts.

## 2.5. Statistical analysis

All data presented are means  $\pm$  1 standard deviation (S.D.). Differences between treatment groups (within and between strains) were tested using ANOVA one-way test. If the *F*-test in ANOVA suggested the presence of significant differences between means, these were identified using the Tukey post hoc test. Differences in marine survival were tested using Yates corrected chi-square. All data are treated as being statistically significant when  $p \leq 0.05$ .

## 3. Results

### 3.1. Water chemistry

Total organic carbon (TOC) was lower than 4 mg C l<sup>-1</sup> in both water sources. pH in Good was higher than 6.5 and ANC exceeded 90 µeq l<sup>-1</sup>. TMAI was lower than the detection limit of 2 µg Al l<sup>-1</sup> (Table 1), suggesting that Al was mainly present in particulate or colloidal form. In Fossbekken, pH varied between 5.3 and 5.8, with the lowest pH values occurring from February to mid-April. ANC was  $-35 \pm 10$  µeq l<sup>-1</sup>. TAl was  $136 \pm 22$  µg Al l<sup>-1</sup>. The concentration of Ali varied between 40 and 50 µg Al l<sup>-1</sup> up to mid-April, but dropped to concentrations around  $20 \pm 5$  µg Al l<sup>-1</sup> following the pH increase in April. The concentration of all major ions in Acid was directly related to the water qualities of Fossbekken and Good and to the mixing ratios (Table 1). Due to dilution with water from Good, TAl was reduced by 30% compared to Fossbekken. Ali was reduced to concentrations ranging from 15 to 30 µg Al l<sup>-1</sup>, with the lowest concentrations present from mid-April. The measured Ali concentration ( $6 \pm 2$  µg Al l<sup>-1</sup>) was lower than the estimated concentration.

There were no differences in water temperature between Good and Acid. Water temperature was lower than 3 °C until March 9 and increased gradually to 9 °C by May 4. From May 4 to May 17, temperature varied between 9 and 11 °C. Water discharge in River Imsa was low in May, varying between 1.0 and 1.5 m<sup>3</sup> s<sup>-1</sup> when the smolts were released.

### 3.2. Gill-Al

Gill-Al increased from a background level of  $8 \pm 1$  µg g<sup>-1</sup> for the Imsa strain and  $3 \pm 1$  µg g<sup>-1</sup> for the Suldal strain to concentrations higher than 200 µg g<sup>-1</sup> within the first 7 days of exposure (Fig. 1). Following dilution of water from Fossbekken with Good, gill-Al decreased and ranged from 30 to 50 µg g<sup>-1</sup> in March. On May 4, gill-Al was  $26 \pm 5$  µg g<sup>-1</sup> for Imsa and  $34 \pm 6$  µg g<sup>-1</sup> for the Suldal strain, representing an increase of 20 and 28 µg g<sup>-1</sup> dw, respectively, compared to fish exposed in Good. The gill-Al concentration of fish exposed to Good remained lower than 10 µg Al g<sup>-1</sup> (average value;  $6 \pm 5$  µg g<sup>-1</sup> dw) throughout the whole exposure period. A gill-Al concentration of 25 µg Al g<sup>-1</sup> or higher not only represents a response to being exposed in Acid but also a dose with respect to physiological responses.

Table 1

Average value  $\pm 1$  standard deviation (S.D.) of pH, TOC (total organic carbon), major cations (calcium, magnesium and sodium) and aluminum fraction composition in Good, Fossbekken and Acid from February 17 to May 4

	TOC (mg C l <sup>-1</sup> )	pH	Ca (mg l <sup>-1</sup> )	Mg (mg l <sup>-1</sup> )	Na (mg l <sup>-1</sup> )	ANC (μeq l <sup>-1</sup> )	TAl (μg l <sup>-1</sup> )	TMAI (μg l <sup>-1</sup> )	OMAI (μg l <sup>-1</sup> )	Ali (μg l <sup>-1</sup> )
Good	2.5 ± 0.4	6.8 ± 0.3	3.5 ± 0.1	1.4 ± 0.0	6.5 ± 0.1	97 ± 7	41 ± 19	<dl	<dl	<dl
Fossbekken	3.3 ± 0.4	5.5 ± 0.3	1.1 ± 0.1	0.6 ± 0.1	4.6 ± 0.3	-35 ± 10	136 ± 22	74 ± 15	34 ± 13	40 ± 15
Acid	3.1 ± 0.4	5.8 ± 0.3	1.7 ± 0.1	0.8 ± 0.1	5.1 ± 0.3	3 ± 5	108 ± 18	39 ± 6	31 ± 3	6 ± 2
Estimated	3.1		1.8	0.8	5.2	5	108	54	25	25

N=7. <dl; < detection limit (2 μg l<sup>-1</sup> for TAl, TMAI and OMAI; 5 μg l<sup>-1</sup> for Ali). The estimated concentration is calculated based on mixing ratio and measured concentration in Fossbekken and Good.

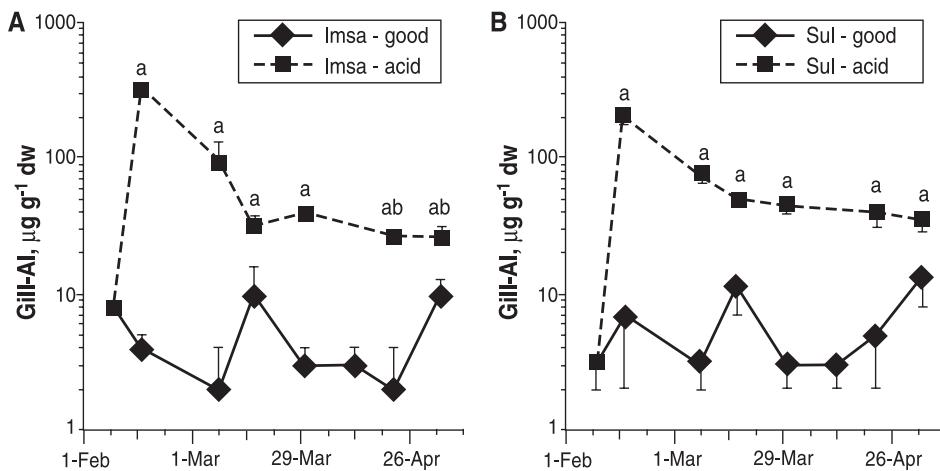


Fig. 1. Gill-Al ( $\mu\text{g Al g}^{-1}$  dw) concentration (average  $\pm 1$  S.D.) measured on fish from Imsa (A) or Suldal (B) exposed in either Good or Acid from February 9 to May 4. Significant differences (ANOVA;  $p < 0.05$ ) within strain, different treatments are denoted by “a”, while differences between strains, same treatment are denoted by “b”.

### 3.3. Fish responses

A few fish died, with mortality starting on February 13. Mortality ceased 4 days after water toxicity was reduced on February 17. Total mortality was 0.8% for the Imsa strain and 3.1% for the Suldal strain (Table 3).

Changes in individual growth were monitored by comparing individual length and weight measured during Carlin tagging with size measurements performed on the same fish late in April and in May (Table 2). Fish exposed in Good had a significant length gain ( $p < 0.05$ ) of 0.55 or 0.70 cm (Imsa and Suldal strain, respectively) compared to fish exposed in Acid. While fish in Good increased their weight by 2.7–3.2 g in average, fish

Table 2

Average value  $\pm 1$  standard deviation (S.D.) for fish lengths (cm) and weights (g) measured during Carlin tagging (March 9;  $n \approx 1200$  for each strain and treatment) and from fish sampled between April 22 to May 10 (number sampled is given as  $n$ )

	March 9		April 22–May 10			Change	
	Length (cm)	Weight (g)	<i>n</i>	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Imsa Good	$16.1 \pm 1.6$	$38.7 \pm 12.4$	167	$16.8 \pm 1.5$	$42.7 \pm 15.3$	$0.55 \pm 0.42$ a	$2.66 \pm 3.00$ a
Imsa Acid	$16.1 \pm 1.6$	$38.9 \pm 12.1$	157	$16.2 \pm 1.6$	$37.7 \pm 12.0$	$0.20 \pm 0.46$	$-1.77 \pm 3.82$
Suldal Good	$17.1 \pm 1.5$	$49.6 \pm 13.1$	66	$17.8 \pm 1.5$	$52.3 \pm 15.8$	$0.70 \pm 0.45$ a	$3.18 \pm 3.14$ a
Suldal Acid	$16.9 \pm 1.5$	$47.7 \pm 12.4$	51	$16.9 \pm 1.3$	$42.4 \pm 10.7$	$0.02 \pm 0.27$	$-4.16 \pm 2.88$

“Change” represents the average individual size change from March to April/May. Significant differences between treatments, same strain (ANOVA;  $p < 0.05$ ) are marked with an “a”. Fewer fish were measured from the Suldal group as these were not included in the downstream migration program.

exposed in Acid in average lost weight by 1.8–4.2 g. The weight loss was largest for the Suldal strain.

Fish exposed in Good had a physiological status well within the limits that we regard as indicating “no treatment effect” (Table 3). Seawater tolerance improved from March to May, when the majority of the fish had plasma chloride values lower than 160 mM. Fish exposed in Acid exceeded the “no effect limits”, having hematocrit >45% on March 8 and May 4, and glucose >6 mM on all but one sample date. The plasma chloride concentration was normally higher than the “no effect limit” of 120 mM, but was at the same time significantly ( $p < 0.05$ ) lower than the levels measured in Good (Table 3). Furthermore, fish exposed in Acid did not acquire normal seawater tolerance. By May 4, mortality in the seawater challenge test was high (40–50%) and surviving fish had plasma chloride concentrations higher than 180 mM.

### 3.4. Post-exposure; downstream migration

Regardless of treatment, more than 50% of all smolts had left the river within the first five nights after release, and more than 95% of the smolts had migrated within the first 2 weeks (Fig. 2A). Smolts reared in Good migrated prior to smolts reared in Acid. Maximum difference between the two treatments was recorded during the first four nights when >80% of the smolts from Good had migrated as compared to 50% of the smolts from Acid (Fig. 2A,B). Less than 1% of the outmigrating smolts was recaptured during daylight

Table 3

Accumulated mortality and physiological status (average  $\pm$  1 S.D.) of smolts from the Imsa and Suldal strains exposed to “Good” and “Acid”

Strain	Treatment	Date	Freshwater				Seawater		
			Mortality (%)	Hct (%)	B-glu (mM)	P-Cl (mM)	Mortality (%)	Hct (%)	P-Cl (mM)
Imsa	Good	8/3 0	40 ± 5	4.2 ± 0.4	135 ± 4	0			166 ± 7
		30/3 0	41 ± 3	4.7 ± 0.7	142 ± 1	0	31 ± 9 b	167 ± 14 b	
		22/4 0	37 ± 5	3.8 ± 0.5	137 ± 2	0	36 ± 3 a	160 ± 8 a	
		4/5 0	39 ± 3	5.2 ± 0.3	139 ± 2	0	39 ± 4 a	160 ± 9 a	
Imsa	Acid	8/3 0.8	51 ± 2 a	13.0 ± 1.8 ab	115 ± 4 ab	100	ns	ns	
		30/3 0.8	42 ± 2 b	11.1 ± 2.6 a	135 ± 4 a	0	33 ± 7	172 ± 17	
		22/4 0.8	38 ± 5	5.5 ± 0.5 ab	139 ± 2	0	29 ± 5	173 ± 16 b	
		4/5 0.8	50 ± 3 a	12.2 ± 3.4 a	127 ± 3 a	50	32 ± 3	191 ± 24	
Suldal	Good	8/3 0	40 ± 4	4.2 ± 0.6	139 ± 5	0			171 ± 12
		30/3 0	38 ± 3	5.3 ± 0.8	141 ± 2	0	40 ± 6 a	151 ± 5 a	
		22/4 0	38 ± 3	3.9 ± 0.5	139 ± 1	0	35 ± 4 a	155 ± 8 a	
		4/5 0	42 ± 3	5.0 ± 0.7	138 ± 4	0	38 ± 5 a	157 ± 6 a	
Suldal	Acid	8/3 3.1	48 ± 2 a	8.7 ± 0.4 a	131 ± 2 a	0			170 ± 8
		30/3 3.1	33 ± 3 a	10.1 ± 2.6 a	137 ± 3 a	0	27 ± 14	177 ± 11	
		22/4 3.1	38 ± 4	7.3 ± 0.8 a	136 ± 4	0	31 ± 4	189 ± 11	
		4/5 3.1	49 ± 6 a	12.1 ± 4.0 a	128 ± 3 a	40	29 ± 5	198 ± 9	

Significant differences (ANOVA;  $p < 0.05$ ) between treatment, same strain is denoted by an “a”. Significant differences between strains (ANOVA;  $p < 0.05$ ), receiving same treatment is denoted by “b”. No sampling due to mortality is marked as “ns”.

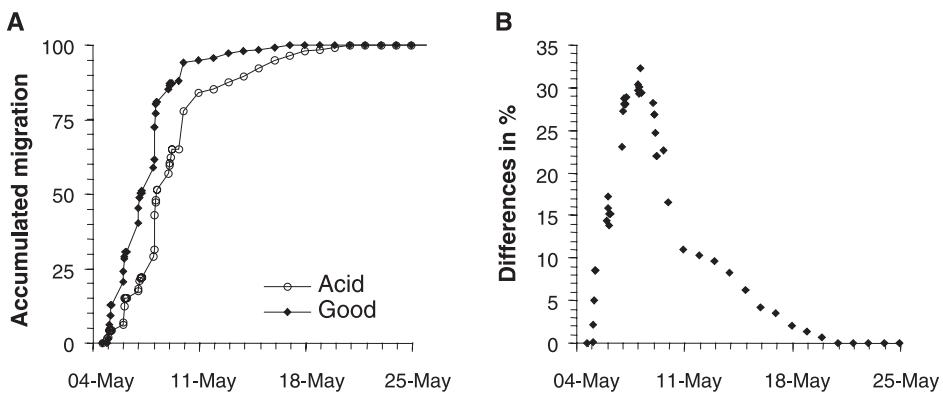


Fig. 2. (A) Accumulated downstream migration in percent ( $n = 150$ ) of smolts exposed in either Good or Acid. (B) Differences in percent number of smolts having migrated (Good–Acid).

(05:00–21:00 h) and more than 80% of all recaptures was done between the hours of 22:00 and 01:00.

The water quality in River Imsa and Good is close to identical as both originate in Lake Imsvatn. Transporting smolts to Imsa will constitute a handling stress, but for the Acid treatment group, transfer to a good water quality can also facilitate recovery. The transfer affected the physiological status of fish originating in Good (Table 4). Glucose values were elevated on smolts recaptured during the second night while the hematocrit values were elevated on smolts recaptured on the third and fourth night. Plasma chloride concentrations remained unchanged. Seawater tolerance was not affected, but a few individuals (<10%) had osmoregulatory problems. Fish originating in Acid had a slightly improved physiological status after four and five nights in Imsa, but did not establish seawater tolerance. Plasma chloride measured on surviving fish exceeded 180 mM at all times. The overall

Table 4  
Physiological status of downstream-migrated smolts released in River Imsa

Water quality	Time since release	Hours	Freshwater			Seawater				
			n	Hct (%)	B-glu (mM)	P-Cl (mM)	n	Mortality (%)	Hct (%)	P-Cl (mM)
Good	Background	0	6	39 ± 3	5.2 ± 0.3	139 ± 2	15	0	39 ± 4	160 ± 9
	2nd night	33–37	6	39 ± 2	7.3 ± 1.9 a	136 ± 3	18	0	40 ± 5	160 ± 4
	3rd night	57–61	6	47 ± 3 a	6.1 ± 0.5	139 ± 3	15	9	46 ± 5	162 ± 10
	4th night	81–85	6	53 ± 5 a	5.2 ± 1.0	131 ± 3	14	7	42 ± 4	162 ± 10
	5th night	105–109	6	45 ± 6	5.3 ± 0.9	135 ± 2	14	7	43 ± 4	142 ± 8 a
Acid	Background	0	6	50 ± 4	12.2 ± 3.4	127 ± 3	16	50	32 ± 3	191 ± 24
	2nd night	33–37	1	40	9.0	137	5	0	26 ± 6	190 ± 7
	3rd night	57–61	0	ns	ns	ns	15	53	43 ± 5	196 ± 10
	4th night	81–85	6	48 ± 5	6.1 ± 1.0 a	134 ± 2 a	10	60	38 ± 6	203 ± 13
	5th night	105–109	6	47 ± 5	7.5 ± 2.2 a	132 ± 4 a	15	13	34 ± 5	181 ± 12

Background levels represent physiological status prior to transfer. Results that are significantly different (ANOVA;  $p < 0.05$ ) from the background are denoted by an "a". No sampling due to too few migrating fish is marked as "ns".

Table 5

Length and weight (average  $\pm$  1 S.D.) of the released Carlin-tagged smolts, smolt size of the recaptured adults and length and weight of the recaptured adults

Strain	Whole material		Smolt size, recaptured adults			Adult size	
	<i>n</i>	Smolt length (cm)	<i>n</i>	Smolt length (cm)	Smolt weight (g)	Recapture length (cm)	Recapture weight (g)
Imsa Good	943	16.1 $\pm$ 1.6	101	16.4 $\pm$ 1.5	41.0 $\pm$ 12.5	55.5 $\pm$ 4.8	1465 $\pm$ 443
Imsa Acid	942	16.1 $\pm$ 1.6	73	16.1 $\pm$ 1.8	38.1 $\pm$ 10.9	54.3 $\pm$ 5.1	1410 $\pm$ 444
Suldal Good	1118	17.1 $\pm$ 1.5	39	17.0 $\pm$ 1.7	43.9 $\pm$ 12.9	64.6 $\pm$ 9.0	2480 $\pm$ 584
Suldal Acid	1115	16.9 $\pm$ 1.5	30	16.7 $\pm$ 1.5	43.2 $\pm$ 10.1	64.9 $\pm$ 3.6	2353 $\pm$ 471

results suggest a beginning recovery of fish from Acid, but recovery had not yet improved seawater tolerance. The smolts migrated downstream despite the physiological status being compromised.

### 3.5. Marine survival

The Imsa strain is a one-sea winter-salmon while the Suldal strain is a multi-sea winter-salmon. After 1 year at sea, 10.7% of the fish from Imsa—Good (*n* = 101) had been recaptured, compared to 7.7% from Imsa—Acid (*n* = 73), representing a significant ( $p = 0.05$ ) catch reduction of 28%. For the Suldal strain, 3.5% of the smolts exposed in Good (*n* = 39) was recaptured, compared to 2.7% of the fish from Acid (*n* = 30), representing a catch reduction of 23%. This difference was not significant (chi-square;  $p = 0.35$ ).

Upstream migration in River Imsa started for all treatments around August 20 and >50% had entered River Imsa by October 17 and >90% by November 2. Of the total adult recaptures, 77% and 75% for Imsa Good and Acid and 69% and 87% for the Suldal Good and Acid were caught in River Imsa, respectively. Smolt size did not appear to affect survival, as the average smolt size of the recaptured adults was similar to the average size the smolts had upon tagging (Table 5). There was no within-strain difference in marine growth (Table 5).

## 4. Discussion

### 4.1. Responses to treatment

The water chemical composition in Good is not associated with water quality properties suggesting acidification, while the composition of Fossbekken is typical for water qualities causing extinction in Atlantic salmon (Henriksen et al., 1992; Rosseland and Staurnes, 1994). Smolts reared in Good had a physiological status that was within the levels we regard as representative of smolts having a “normal physiological status”. The gill-Al concentration was lower than 10  $\mu\text{g Al g}^{-1}$  dw. The lack of any clear deviation from normality is consistent both with the water chemistry composition of River Imsa, but also suggests that the rearing regime did not impose any unwanted stress.

Fish exposed to water from Fossbekken from February 9 to February 17 accumulated gill-Al in excess of  $250 \mu\text{g Al g}^{-1}$  dw. During this period, the fish were exposed to water having an Ali concentration exceeding  $40 \mu\text{g Ali l}^{-1}$  and a pH of 5.3–5.4. This Al/H<sup>+</sup> dose, or the gill-Al accumulation, resulted in mortality, implying that the Fossbekken water quality reduced smolt survival early in February.

In Acid, the water from Fossbekken was added water from Good. This dilution reduced Ali by 30%, or to a concentration ranging from 30 to  $15 \mu\text{g Al l}^{-1}$ . The measured concentration was only  $6 \pm 2 \mu\text{g Al l}^{-1}$  ( $n=7$ ). The reason for this discrepancy is that when pH in acidic water is increased, Ali undergoes a polymerization and is transformed to OMAI and/or to PCAl. The transformation rate and reduction in Ali are dependent on both temperature (Lydersen et al., 1990) and pH (Kroglund et al., 2001a,b). The “true” Ali concentration experienced by the fish could therefore have been higher than the measured concentration of  $6 \pm 2 \mu\text{g Ali l}^{-1}$ , but had to be substantially lower than the concentrations measured in Fossbekken. Dilution, the pH increase and the long retention time in the exposure tanks (>200 min) would contribute to reducing Ali. A measured concentration of  $6 \mu\text{g Ali}$  is at the same time uncertain, being close to the detection limit ( $5 \mu\text{g Al l}^{-1}$ ).

From mid-March to May, the gill-Al concentration in fish exposed in Acid was only 20–40  $\mu\text{g g}^{-1}$  higher than the concentration measured in Good, with the highest differences between the two Suldal treatments. A low concentration of gill-Al was to be expected, provided the Ali concentration in Acid was low. Despite low concentrations of Ali, H<sup>+</sup> and gill-Al, smolts exposed in Acid had elevated blood glucose, slightly elevated hematocrit and lower plasma chloride concentrations than fish in Good. Likewise, growth was poor. The differences between treatments within strains were significant at most sampling points ( $p < 0.05$ ). Significant differences between the two treatments suggest the presence of one or more stressors related either to physio-chemical properties of the water and/or to stressors related to tank environment. Exposure tanks, water temperature, water flow and current and feeding regimes were all kept as similar in all tanks as possible. There were no indications of “stress” in Good. A difference related to the water chemistry is therefore the more probable factor, which can also explain the increase in gill-Al. While a pH of 5.8 and  $6 \pm 2 \mu\text{g Ali l}^{-1}$  are not normally associated with water qualities resulting in severe physiological responses (Rosseland and Staurnes, 1994; Gensemer and Playle, 1999), the results indicate that this was the case here. Physiological responses at similarly low concentrations of Ali have been observed in other experiments performed in low-TOC water (Staurnes et al., 1995; Kroglund and Staurnes, 1999; Kroglund et al., 2001a) as opposed to the limits suggested for TOC-rich rivers in Canada (Lacroix, 1989). Marine survival was not assessed in these studies.

#### 4.2. Seawater tolerance, downstream migration and seawater survival

Hypoosmotic regulation, based on seawater challenge tests, was compromised in the Acid groups, but developed normally in Good (ANOVA;  $p < 0.05$ ). Hypoosmotic capacity can be affected despite the physiological status of the fish appearing to be “normal” in freshwater (Saunders et al., 1983; Farmer et al., 1989; Staurnes et al., 1993a). Staurnes et al. (1995) and Kroglund and Staurnes (1999) reported that seawater tolerance was effected at Ali concentrations ranging from 7 to  $30 \mu\text{g Ali l}^{-1}$  within the pH range 5.7 to 6.1.

Smolt to adult survival is highest when the smolts leave the river and enter the ocean during the “smolt window” (Virtanen et al., 1991; Staurnes et al., 1993b). Based on 11 years of data, the date for 50% descent of wild smolts in River Imsa spans from May 7 to May 25 (Hvidsten et al., 1998). Of the smolts stocked into River Imsa on May 4, 50% of the smolts had migrated downstream by May 8, suggesting that the timing of the smolt migration was within normal time limits. The Acid group reached the 50% level 1 day delayed compared to fish from Good, but showed the same diurnal rhythm as smolts from Good. This time clustering suggests that the migration was active, and that the treatments had no or little effect on the migratory behavior, despite the smolts from Acid lacking hypoosmotic capacity. There were signs of recovery among fish from the Acid group in River Imsa by night five after release. Assuming identical migration pattern of smolts released above or below the trap, it is possible that smolts leaving the watershed from May 9 and onwards were more seawater tolerant than the smolts migrating the first five nights.

Fewer adult recaptures (23% and 27% reduction) were reported from the two Acid treatments than from the two groups raised in Good. The difference was significant ( $p=0.05$ ) for the Imsa strain but not significant ( $p=0.35$ ) for the Suldal strain due to few recaptures. We interpret both catch reductions as indicating a response to treatment, despite the lack of statistical significance for the Suldal strain. Reduced marine survival can be related to poor hypoosmotic capacity and growth differences between treatments, but probably not to the timing of seaward migration. We assume that the time delay in migration of a few days was not the cause for differences in marine survival. Fish exposed in Acid grew poorly compared to the control fish in freshwater. Differences in growth (smolt size) could therefore explain the differences in marine survival. However, the average smolt size of the recaptured adults was similar to the average size the fish had upon tagging. If survival was only related to differences in size, the recaptured adults should have belonged to the upper size mode. This was not the case, suggesting that differences in freshwater growth were not the main factor regulating marine survival. Marine growth, based on adult recaptures, was related to strain but not to treatment. We interpret the results as suggesting that low concentrations of Ali impair seawater tolerance. It is previously shown that predator avoidance is affected in physiologically stressed smolts (Järvi, 1990; McCormick et al., 1998; Finstad and Jonsson, 2001). Smolts exposed in Acid had impaired hypoosmotic capacity and the reduction in marine survival could be due to disturbed predator avoidance. Similar and greater reductions in marine survival are described in experiments where the smolts were exposed to higher concentrations of Ali, but for shorter (<48 h) periods of time (Staurnes et al., 1996; Finstad et al., 1999). A reduction in marine survival of 25% can have population effects by reducing the size of the spawning population.

#### 4.3. Acclimation and strain

Long-term exposure to sublethal water qualities has resulted in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*S. fontinalis*) becoming acclimated and thereby more tolerant to aluminum (Mallat, 1985; Orr et al., 1986; Mount et al., 1988; Muller et al., 1991; Reid et al., 1991). Acclimation with respect to acidification has been associated with changes in metal–gill surface interactions resulting in a reduction in gill-Al concentration

despite prolonged exposure (Mallat, 1985; Wood et al., 1988; Muller et al., 1991). The reduction in gill-Al concentration from February to April in the present experiment may have been related to alterations in gill tissue properties, but the effects on physiological status, growth and the lack of seawater tolerance do not suggest that the smolts were becoming acclimated to the ambient water quality. Saunders et al. (1983) did not find that pretreatment of Atlantic salmon smolts in acidic water increased tolerance, on the contrary, pretreated smolts were more sensitive suggesting sensitization.

The results do not indicate that there was any major strain variation in tolerance, irrespective of river of origin. Large strain-dependent variations in acid tolerance have been demonstrated for various freshwater fish species (e.g. brook trout; Ingersoll et al., 1990; brown trout; Dalziel et al., 1995), but were not detected in Atlantic salmon (Rosseland et al., 2001).

## 5. Conclusion

There was no evidence of any significant strain-related difference in tolerance to acid water. Presmolts/smolts of Atlantic salmon exposed in Acid water ( $\text{pH } 5.8; 6 \pm 2 \mu\text{g Al l}^{-1}$ ) for 3 months did not become acclimated to the ambient water quality. On the contrary, the exposure affected growth, physiological status with respect to blood glucose and impaired seawater tolerance. The treatment did not affect the timing of downstream migration, but reduced the adult returns by 20–30% depending on strain. Marine growth was not affected in the survivors. The results suggest that sublethal exposures (which only with difficulty can be interpreted as being harmful at the population level in freshwater) may affect marine survival and the size of the returning adult population, thereby having ecological implications.

Seawater tolerance should be included as a response measure when determining water quality criteria for anadromous species. Gill-Al is a valuable addition when monitoring effects of aluminum at concentrations close to or below the analytical detection limit.

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