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# Water quality limits for Atlantic salmon (*Salmo salar* L.) exposed to short term reductions in pH and increased aluminum simulating episodes

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## Abstract

Acidification has caused the loss or reduction of numerous Atlantic salmon (*Salmo salar* L.) populations on both sides of the North Atlantic. Acid deposition peaked in the 1980's and resulted in both chronically and episodically acidified rivers. At present, water quality is improving in all affected rivers due to reduced acid deposition. However, spring snow melt, heavy rainfall and sea salt episodes can still cause short term drops in pH and elevated concentrations of bioavailable aluminum. Technical malfunction in lime dozers will cause short termed episodic spates in the limed rivers. The current situation has prompted a need for dose-response relationships based on short term exposures of Atlantic salmon to assess the potential population effects of episodic acidification. Water quality guidelines for salmon have been lacking, despite a large number of experiments, all demonstrating dose-response relationships between water chemistry and fish health. We have summarized results from 347 short-term (<14 days) exposures of salmon parr and smolt performed between 1990 and 2003 in Norway. The experiments have been performed as bioassays, where fish have been exposed in tanks fed river water, in tanks where the river water quality has been manipulated (added H<sup>+</sup> and Al) and as Carlin-tagged smolt releases after preexposure to moderately acidic waters. The results from the various bioassays are compared to water quality limits proposed on basis of the relationship between water quality and population status/health in Norwegian rivers. The focus of this article is placed on chemical-biological interactions that can be drawn across experiments and exposure protocols. We propose dose-response relationships for acid neutralizing capacity (ANC), pH, cationic Al and gill accumulated Al, versus mortality in freshwater, effects on hypo-osmoregulatory capacity in seawater challenge tests and on smolt to adult survival in release experiments. The "no effect" dose depends on the life history stage tested and on the sensitivity of the biomarkers. Parr are more tolerant than smolt. Concentrations of Al that have no significant impact on freshwater life history stages can still have major population effects if they occur prior to smolt migration. While smolt can

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survive in freshwater for a prolonged period of time (>10 days) at an AI dose resulting in a gill AI concentration of up to  $300 \mu\text{g Alg}^{-1} \text{ dw}$ , a 3 day exposure resulting in a gill AI accumulation in the range of 25 to  $60 \mu\text{g Alg}^{-1} \text{ dw}$  reduces smolt to adult survival in a dose related manner by 20 to 50%. For smolt to adult survival, the biological significant response is delayed relative to the dose and occurs first after the fish enters the marine environment. In addition to exposure intensity and timing, exposure duration is important for the setting of critical limits.

## 1 Introduction

Acidification has affected the Atlantic salmon (*Salmo salar* L.) populations in >50 rivers in Norway (Hesthagen and Hansen, 1991; Kroglund et al., 2002; Sandøy and Langåker, 2001). Of these, salmon is classified as extinct in 18 rivers while the catches are reduced in the remaining. Acidification entails a pH reduction, and also a mobilization of aluminum (Al). Toxicity is normally attributed to Al, unless the water is very acid (Gensemer and Playle, 1999; Rosseland and Staurnes, 1994). Numerous other water quality constituents can also affect toxicity, including total organic carbon (TOC) and calcium (Ca). TOC binds metals rendering them unavailable for accumulation whereas Ca reduces the organism's sensitivity to metals (Gensemer and Playle, 1999; Rosseland and Staurnes, 1994).

In northern Europe, acid deposition peaked in the 1980's and resulted in chronic acid waters in many areas. Water quality is at present improving due to reduced sulfur deposition (Evans et al., 2001; Skjelkvåle et al., 2003). However, many salmon rivers are still severely affected by chronic acid water while others are more impacted during acid episodes. An acidification episode has a short duration where the pH depression most often is related to sulfate and possible nitrate pulses, snowmelt, heavy rainfall and sea salt deposition. During an episode, chemical elements such as  $\text{H}^+$ , Al, Ca and organic carbon will be continuously changing in response to dilution, mobilization and transformation processes (Evans et al., 2001; Henriksen et al., 1984; Hindar et

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al., 2004; Teien et al., 2004b, 2005a). While severe acidification (both chronic and episodic) can cause population extinction, the biological response to a more moderate episode depends not only on the increase in H<sup>+</sup> and Al concentrations, but also on exposure duration and timing and on changes in other water quality relevant elements.

5 The effects an episode will have on fish will as such depend on numerous simultaneous interacting elements including the prior exposure history of the fish. The ecological impacts of episodes are still poorly documented as fish kills and density reductions are rarely observed and documented. This is not necessarily due to kills being a rare event, and is just as likely due to kills being difficult to observe on juvenile life-stages.

10 Field bioassays have the capacity to document both mortality rates and sub-lethal responses in fish, and link these to the intensity and duration of an acid/Al episode (Barlaup and Åtland, 1996; Lacroix and Korman, 1996; Magee et al., 2003; Teien et al., 2004b, 2005a). The effects of an episode can be simulated in short-term experiments where the toxic components and the toxicity moderating variables can be controlled and varied (data included here). Population responses to prior exposures can be tested in exposure/release experiments (Staurnes et al., 1996; Kroglund and Finstad, 15 2003; Kroglund et al., 2007; Magee et al., 2003). The results can later be feed into water quality/biological response models (Korman et al., 1994; Monteith et al., 2005). To identify water quality limits with respect to acidification, it is necessary to identify 20 the critical biological properties that need protection and the physio-chemical factors that affect these. Water that does not inflict mortality is not synonymous to a “healthy” or satisfactory water quality. Although sub-lethal biological responses can be measured, the impacts these have on population status is still unclear. Although sub-lethal doses can affect health status and growth, the effects are time limited. Fish surviving 25 an acidification episode will enter a recovery phase which depends on the severity of the initial stress response, the water quality present following the episode, and temperature (Kroglund and Staurnes, 1999; Kroglund et al., 2001a; Lacroix and Korman, 1996; Magee et al., 2003). If fish are exposed to a new episode during the recovery phase, the biological response can be more severe than expected from chemistry

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alone, as fish health already is compromised (Henriksen et al., 1984). Furthermore, the ecologically relevant responses can be delayed relative to the timing of the dose (see below).

For salmon, the timing of an acid episode is important because the various life stages are not present at all times of the year and have differences in sensitivity where smolt are more sensitive than parr and fry (Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). Due to this variation in tolerance, an episode prior to or during the final smoltification stage (in spring) can be more detrimental to a salmon population than events of similar severity and duration occurring at another time of the year (Staurnes et al., 1995). The chemical/biological interactions are further modified by water temperature, as toxicity increases with temperature (Poléo and Muniz, 1993).

Al is toxic by acting on the gill altering gill tissue structure and function (see review in: Sparling and Lowe, 1996; Gensemer and Playle, 1999; Rosseland and Staurnes, 1994). The biological responses related to  $H^+$  can be similar to, but also different from responses related to Al (Gensemer and Playle, 1999; Rosseland and Staurnes 1994). Mortality in acid water is often related to ionoregulatory disturbance at low pH values, and to respiratory disturbances at high Al concentrations.  $H^+$  and Al act in concert at intermediate pH-levels. While  $H^+$  by itself has no effect on the population status of Atlantic salmon down to a pH of 5.4 (Fivelstad et al., 2004; Lacroix, 1989; Watt et al., 2000), this pH value is highly toxic when present together with cationic Al (Rosseland and Staurnes, 1993; Gensemer and Playle, 1999; Staurnes et al., 1995). Understanding how  $H^+$  and aluminum interacts with fish health is thus crucial to the interpretation of water quality. Acidification represents therefore a combined pressure where the ecological effect of the two main stressors needs to be evaluated separately, but also how they interact and magnify toxicity.

Current water quality guidelines are often related to the acid neutralizing capacity (ANC) of water, and as  $H^+$ , Al and ANC are interrelated, all can be used as indicators of water quality (Bulger et al., 1993; Kroglund et al., 2002; Lien et al., 1996). However, this statement is only valid when both  $H^+$  and Al is present and impact water quality:

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e.g., in water where low pH is due to organic acids, metal toxicity can be insignificant, falsifying pH-based limits generated from Al-enriched water. Not all forms of Al are toxic. Only cationic species of Al contained within the operational forms termed labile Al (LAl) or inorganic monomeric Al (Ali), are gill-reactive and hence affect fish health (Driscoll et al., 1980; Oughton et al., 1992; Teien et al., 2005b). Experiments show close relationships between cationic forms of Al and the Al concentrations accumulated on gills (Kroglund et al., 2001a, b; Teien et al., 2006b). Gill Al thus provides an independent measure of the Al-dose and is related to the concentrations of cationic Al. Not all Al-species contained within cationic Al are equally bioavailable as both DOC and silicate interferes with the relationship between Ali and gill Al, leading to variation in a dose response model (Teien et al., 2006a, b).

A no-observed-effect-concentration (NOEC) relates to “no” biological response and is defined as: the highest concentration of an effluent or toxicant that causes no observable adverse effects on the test organisms (EPA, 2000). The biological response used to identify NOEC for salmon has changed over the last decades; from focus on mortality and ionoregulatory responses in freshwater to an increased awareness that acidification also effects growth and behavior (Gensemer and Playle, 1999; Rosse-land and Staurnes, 1994). More recently, research has provided data demonstrating how pre-smolt and smolt exposed to Al in freshwater can produce a delayed response, leading to mortality and population effects after the fish left freshwater and entered sea-water (Kroglund and Finstad, 2003; Kroglund et al., 2007; Magee et al., 2001; Staurnes et al., 1996). While severe acidification affects population status by causing mortality in freshwater, moderate to low levels of acidification can have equal strong effects on population health by reducing smolt quality having an effect on post-smolt survival (Finstad et al., 2007; Kroglund and Finstad, 2003; Kroglund et al., 2007; Magee et al., 2001; Staurnes et al., 1996). Because smolt leave the river over a time span of several weeks and episodes can last days, parts of the smolt run can contain fish affected by Al, while other parts of the population leave the river at a time with satisfactory water quality or after they have recovered from the episode. In this respect, not only the timing of an

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episode is important, but also how well the fish can recover before they leave the river and enter seawater.

There is increasing evidence that with reduced acid deposition, water quality improves and that the intensity of episodes diminishes (Wright, 2007; Evans, 2007), though severe acidification episodes still occur during extreme weather events (Barlaup and Åtland, 1996; Hindar et al., 1994; Hindar et al., 2004; Teien et al., 2005a, 2006a). Fish can also experience higher concentrations of Al than predicted on basis of pH or ANC in rivers affected by acid Al-rich tributaries (Rosseland et al., 1992; Poléo et al., 1994; Kroglund et al., 2001a, b). Fish are affected by the general water quality, but it can still be the extremes that have the largest impact on population status. Episodes can in part explain the poor biological recovery recorded to date, despite an impressive long-term water chemical recovery (see discussion in: Monteith et al., 2005). The main objective behind the work presented here is to identify empirical relationships between water quality components and their possible effects on salmon populations. Focus is on exposure intensity, duration and timing. The results are interpreted as a simulation of an episode.

## 2 Material

The material consists mainly of fish exposure experiments carried out in Norway during the period 1990 to 2003. Only brief descriptions of methods are provided here and we refer back to the original articles for full descriptions (Rosseland, et al., 1992; Poléo et al., 1994; Kroglund and Staurnes, 1999; Kroglund et al., 2001a, b, c; Rosseland et al., 2001; Kroglund and Finstad, 2003; Teien et al., 2004a, 2006a; Kroglund et al., 2007). These experiments involve a total of 347 groups of salmon at various life stages exposed in tanks to water qualities ranging from satisfactory to lethal (Table 1). In this data compilation, focus is placed on conclusions that can be drawn across experiments through a large range of water chemical compositions and biological responses. The chemical limits derived from the exposure experiments are compared to limits sug-

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gested for Atlantic salmon based on population surveys (Kroglund et al., 2002).

We have included only experiments where we have access to the raw data to avoid uncertainties related to exposure environment; differences in analytical protocols and large differences in TOC concentrations. Experiments not included can and should be used for model validation. The material is divided into two main parts differing with respect to experimental approach. In the short-term experiments, fish were exposed up to 10 days, while in the sea-survival experiments, the exposures were sub-lethal and exposed fish were released to monitor effects on movements and survival in the marine environment.

## 2.1 Short-term exposures

### Fish material

The fish were offspring of wild parents reared at local hatcheries or wild smolt caught by electro-fishing. It is not possible to define the pre-exposure history of a wild salmon smolt as this life stage is not stationary and it is likely that these fish had experienced acidification episodes prior to our exposures. Fish originating from the hatchery in River Suldal (SW Norway) experienced acidification pulses prior to being used in the experiments, while fish from the other hatcheries were not pre-exposed to acidic waters. Comparative studies have not been able to detect ecological relevant strain differences in responses to water quality for salmon (Kroglund and Finstad, 2003; Rosseland et al., 2001) as opposed to the large strain related differences in sensitivity observed for brown trout (*Salmo trutta* L.) (Dalziel et al., 1995; Andrén et al., 2006). We therefore assume that strain differences are not the cause for response variations in our material.

Salmon migrate from the ocean to spawn in rivers. In the river, the fish develop from eggs to fry to parr and to smolt and this latter stage will migrate into the ocean in the spring (Mills, 1989). Here, data are presented for the life stages parr and smolt. The definition of a life stage is not straightforward, especially for smolt, as smoltification is a process preadapting the individual to the later marine life. For simplicity, fish >12 cm,

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showing loss of parr marks and coloration and that were exposed within the period March to May are regarded as being pre-smolt or smolt, while fish exposed prior to this time span are treated as parr. Fish size varied between life stages and experiments. Larger fish are more sensitive to Al while smaller fish are more sensitive to H<sup>+</sup> (Rosse-  
land et al., 2001). This size-dependent response and sensitivity to the stressors adds to variation in the models.

### Biological protocols

All experimental tanks were monitored for mortality at an interval of hours the first days, less frequent thereafter. Mortality is presented as accumulated mortality and time (h) to 1st fish died and in relationship to exposure duration and dose. Before sampling of gills and blood, the fish were killed by a blow to the head. The 2nd gill arch of the fish was cut out for gill Al determination and analyzed according to Teien et al. (2006b). Concentration of Al is reported as  $\mu\text{g Alg}^{-1}$  gill dry weight (dw). The bioconcentration factor (BCF) is estimated as the ratio between cationic Al in water and gill Al. Only data from the terminal samples are presented here. The material is as such valid with respect to biological response recorded within a 7 to 10 day exposure period.

### Exposure environment – freshwater, seawater challenge test and smolt to adult survival

All fish were exposed in 1–4 m<sup>3</sup> tanks, in 90 L black tanks or in cages placed in the rivers. Depending on the experiment, fish were exposed to natural acid and non-acid source water and water added Al<sup>+</sup> and H<sup>+</sup> to increase toxicity or limestone or sodium-silicate to reduce toxicity.

Seawater challenge tests were performed as a terminal test in all smolt studies. The test was run at salinities of 33–34 ppt, at temperatures of 6–11°C and for 24 h. Responses are here presented as mortality and as blood-plasma Cl<sup>-</sup> concentrations. The responses are presented as average group performance in seawater minus performance in freshwater (SW plasma Cl<sup>-</sup> – FW plasma Cl<sup>-</sup>). Plasma Cl<sup>-</sup> cannot be

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measured on dead fish; thus the average increase in plasma  $\text{Cl}^-$  in groups suffering from mortality is underestimated.

To address the relationship between seawater challenge tests and population responses, a seawater survival program was initiated in 1999. Over the years 1999, 2000, 2002 and 2003, groups of 1200 to 1500 pre-smolt originating from wild salmon broodstock of the lmsa strain were Carlin tagged (Carlin, 1955) prior to exposure to control water or to one of three acid doses where episodic high AI was administered only for the last 3 days prior to release and long term low-AI and long term high-AI lasted >30 days. Following treatment termination, the fish were transported to River lmsa (pH >6.5) and released 150 m above the river mouth. Timing of migration from freshwater to seawater was as such voluntary. Additional details are given by Kroglund et al. (2007).

## Chemical protocols

AI is present as unstable inorganic species following changes in pH; e.g. after liming. “Unstable” AI is here functionally defined as acid water containing elevated concentrations of AI that has been aged for <30 min after an increase in pH. Fish exposed to unstable forms of AI are excluded from the models as the aim was to make a “general” model relating stable water chemistry to biological responses.

pH was measured using two protocols. pH was either measured in field or at NIVA’s laboratory in Oslo after transport. Positively charged (cationic) AI was identified using two different protocols and is defined as AI retained in an Amberlite ion exchanger column; pyrechatecol-violet method (Røgeberg and Henriksen, 1985) termed LAI, and in situ modification of the Barnes-Driscoll method (Teien et al., 2004a) termed Ali. Both protocols are commonly used in Norway, the first in the national monitoring program, the latter in fish experiments dedicated to study AI-biological interactions and AI-species transformations and mechanisms. The two different protocols have different properties and do not estimate identical concentrations of cationic AI (Andrén, 2003). The in situ method produces better relationships between AI and a biological response

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as Al speciation can be changed during transport to the laboratory (Teien et al., 2004a). Aluminum fractionated after transport to a laboratory can therefore underestimate the “true” dose experienced by the fish (Kroglund et al., 2001ab; Poléo et al., 1994; Rosse-land et al., 1992; Teien et al., 2004a, 2005b, 2006ab). This effect is best detected using gill Al or in situ fractionation of Al. All major cations and anions were measured at laboratory (NIVA) using standard protocols.

### NOEC-limits

We use mortality as the unacceptable, non-recoverable biological response. Elevated mortality related to the exposure is understood as an effect of water quality. Water quality limits are based on various biomarkers and are graded as “no effect”, “low to high” and “high”. “No effect” implies that no or few exposure groups responded negatively to the treatment. “Low to high effect” defines the chemical range where all response levels can be present, whereas “high effect” defines water chemistry where all exposure groups responded strongly. Water quality limits are presented with respect to dose and exposure duration for the life stages parr and smolt.

Performance in seawater challenge tests were used as an indicator of possible effects of freshwater quality on hypo-osmoregulatory capacity of the fish. The test was only performed on groups where freshwater survival was high. Water qualities that cause high mortality in freshwater are therefore lacking in the material. The ecologically more relevant response, actual effects on post-smolt survival affecting adult return rates, was used to define limits with respect to the survival of smolts to the adult stage. Dose response relationships were tested using linear regressions.  $R^2$  values and equations are entered into the graphs whenever  $p < 0.001$ .

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### 3 Results

#### Relationship pH and cationic Al

The relationships we observed between pH, ANC and cationic Al are superimposed onto data from the 1000-lake survey in Norway (Henriksen et al., 1989). The relationship between our data and the national dataset was satisfactory for all elements (Fig. 1). Our data are thus representative of the chemical ranges present in Norway. There was a close relationship between cationic Al and H<sup>+</sup> in the water with pH <6.4 (Fig. 1a). More cationic Al was measured as Ali than as LAI for a given pH (P<0.001). This difference is probably purely analytical, but may also be due to different pH/Al relationships in the various water qualities used in these experiments, and/or due to pH being analyzed in the field when Ali was determined. The systematic difference supports an analytical interpretation. Regardless of protocol, all fish were exposed to a combination of H<sup>+</sup> and Al in these experiments. Due to the differences in relationships, guidelines for pH/Al must be related to the analytical protocols being used. The use of different protocols within acid rain research makes comparisons between studies more difficult (Hindar et al., 2000; Andrén, 2003). There were similar differences in the relationship between cationic Al and ANC and pH to ANC (Fig. 1b).

#### Cationic Al – gill Al concentrations

There was a highly significant relationship (p<0.0001) between cationic Al, ANC and gill Al (Figs. 2a, c). The relationship was not significantly influenced by Al protocol or life stage (p>0.1). The bio-concentration factor (BCF) varied within a range of 2 to 8, and increased with Al concentration with respect to LAI but not so for Ali (Fig. 2b). This demonstrates differences in the bio reactive properties for the two forms of cationic Al. The relative large variation in factor suggests that there are other environmental, biological and chemical factors that also influence Al accumulation. Gill Al concentrations in salmon from water having pH values >6.5 and in water outside regions affected by

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acid rain is usually  $<5 \mu\text{g Alg}^{-1} \text{ dw}$  and almost always  $<15 \mu\text{g Alg}^{-1} \text{ dw}$  provided there are no contaminants such as clay present. These values represent background levels (Kroglund et al., 2007).

The relationship between cationic Al and gill Al will be affected by: a) underestimation of LAI in mixing zones, b) analytical errors in Ali and LAI fractionation, c) gill Al was not in a steady state to cationic Al, d) background concentration of gill Al were elevated due to prior Al-exposures and e) contamination of sample. These factors contribute to uncertainty in the regression models. Most of the uncertainty is probably related to the determination of cationic Al. Furthermore, not all forms of Al species determined as cationic Al are bio-reactive and hence toxic. Measurement of cationic Al can overestimate toxicity in water rich in DOC, silicate (Si) and fluoride (F) (Peterson et al., 1989; Teien et al., 2005b, 2006a). Organically bound Al can have cationic properties with respect to an ion exchanger, but not to the gill. This has been tested using  $\text{Al}^{26}$  by Oughton et al. (1992) and in exposure experiments where fish were exposed to source water and water that was passed through filters with a  $10^{-3}$  nominal cut-off removing about 50% of DOC (Teien et al., 2005b). Regardless of these uncertainties, there were strong relationships between gill Al and biological responses (see later). The BCF can be used to generate a probability range for gill Al given a concentration of cationic Al.

During an episode there need not be any clear relationship between cationic Al and gill Al. While changes in water quality can occur over a time span of minutes, kinetic constraints will delay the corresponding change in gill Al. During exposure, gill Al increases fast and reaches a “steady” state to the ambient concentration of cationic Al within a time span of 1 day. Likewise, at the end of the episode, water quality can improve faster than gill Al depuration (Teien et al., 2005a, 2006b).

### 3.1 Biological responses-parr

There is only limited data on mortality in the parr studies. Reduced survival was recorded in only 9 of 101 exposure groups. All fish survived when  $\text{pH}>5.6$ , Ali

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<45  $\mu\text{gL}^{-1}$  and ANC >15  $\mu\text{eqL}^{-1}$  (Figs. 3a–c). When water quality deteriorated relative to these limits, mortality increased relatively steeply, but could also remain low at pH <5, Ali >90 and ANC <–10. Only toxic responses were never observed within the range in water qualities we exposed the fish to.

Parr tolerated gill AI concentrations above 500  $\mu\text{g Alg}^{-1}$  dw. The number of observations are insufficient for determination of NOEC, but 100% of the fish died when the gill AI concentration was 1000  $\mu\text{g Alg}^{-1}$  dw and there was zero mortality when gill AI <400  $\mu\text{g Alg}^{-1}$  dw. There was also a clear relationship between accumulated mortality and the time it took to kill the 1st fish. When accumulated mortality exceeded 25% over a 7 to 10 day exposure period, the first kills were observed within the first 24 h exposure separating acute from moderately lethal water qualities.

### 3.2 Biological responses-smolt

Mortality was frequently observed in the smolt studies (Figs. 4a–d). Reduced survival was recorded in 39 of 228 exposure groups. All fish survived when pH>5.8, LAI <20  $\mu\text{gL}^{-1}$  or Ali <40  $\mu\text{gL}^{-1}$  and ANC >15  $\mu\text{eqL}^{-1}$  (Figs. 4a–c). When water quality deteriorated relative to these limits, mortality increased relatively steeply, and was always high when pH <5.5, LAI >45  $\mu\text{gL}^{-1}$  or Ali >65  $\mu\text{gL}^{-1}$  and ANC <0  $\mu\text{eqL}^{-1}$ . Mortality started when gill AI exceeded 300  $\mu\text{g Alg}^{-1}$  dw and was thereafter correlated to dose. In water qualities that resulted in high mortality, the first kills were observed within the first 24h of exposure (Fig. 4e). At lower doses, the fish had to be exposed for 4 days or more to initiate mortality.

### 3.3 Seawater challenge tests

There was a relationship between impaired hypo-osmoregulatory capacity and mortality (Fig. 5). Mortality increased when plasma  $\text{Cl}^{-}$  in the seawater challenge test exceeded 160 mM or when the difference in plasma  $\text{Cl}^{-}$  in seawater to freshwater exceeded 45 mM (Figs. 5a, b). These levels differentiate between groups having in-

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creasing mortality from groups not suffering from mortality.

When pH in the pre-exposed freshwater  $>6.5$  and cationic Al  $<5 \mu\text{g Al L}^{-1}$ , we observed close to zero mortality in a subsequent seawater challenge test. At lower pH or higher Al concentrations, mortality could range from low to high irrespective of dose (Table 2, Figs. 6a, b). Mortality was however significantly ( $p < 0.0001$ ) related to gill-Al and in a clear dose-response manner (Fig. 6c).

The fish had reduced hypo-osmoregulatory capacity with decreasing freshwater pH and increasing cationic Al (Fig. 6d). The increase in plasma  $\text{Cl}^-$  from freshwater to seawater increased beyond 40 mM when cationic Al exceeded  $5 \mu\text{g Al L}^{-1}$  as LAI or  $10 \mu\text{g Al L}^{-1}$  as Ali (Fig. 6e) or when gill Al exceeded  $25 \mu\text{g Al g}^{-1}$  dw. Mortality in the tests was always high when cationic Al exceeded  $25 \mu\text{g Al L}^{-1}$  as LAI and when gill Al exceeded  $500 \mu\text{g Al g}^{-1}$  dw. Hypo-osmoregulatory regulation was always poor when pH  $< 6.0$ , cationic Al exceeded  $15 \mu\text{g Al L}^{-1}$  as LAI (or  $25 \mu\text{g Al L}^{-1}$  as LAI) and when gill Al exceeded  $100 \mu\text{g Al g}^{-1}$  dw.

The dose-response relationships with respect to the seawater challenge tests suggest cut-off limits rather than dose related responses with the exception of gill Al. The strong relationship to gill Al suggests that Al bound to the gill is a stronger indicator of the dose than the chemical indices.

### 3.4 Seawater survival

Large groups of Carlin-tagged smolt were exposed to control water (average pH  $>6.5$ ;  $<5 \mu\text{g Al L}^{-1}$ ) or to Al-containing water (pH range 5.7–6.6; LAI range 6–17  $\mu\text{g L}^{-1}$ ). At exposure termination, the fish had accumulated Al onto gills in a dose related manner (25 to 60  $\mu\text{g Al g}^{-1}$  dw), while the control fish had  $5.9 \pm 3.3 \mu\text{g Al g}^{-1}$  dw). Gill Al could account for  $>80\%$  of the variation in return rates (Kroglund et al., 2007). Hypo-osmoregulatory capacity was related to both ANC and cationic Al (measured as LAI) (Figs. 7a, b). Adult return rates was related to performance in the seawater challenge test, to cationic Al (as LAI) and to ANC. The number of exposure groups is insufficient to set definitive limits for ANC, but support previous conclusions that even low

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concentrations of Al have detrimental effects with respect to survival in seawater.

### 3.5 Water quality limits

In an experimental study, Atlantic salmon smolt exposed to pH 5.4–5.6 for 35 days, in waters having “no” gill-reactive Al. showed no negative effects monitored as post-smolt survival and growth following 100 days in seawater (Fivelstad et al., 2004). In Canada where high organic content bind most of the Al in water even at pH 5.4, “no effect” on smolt production is expected at pH levels above 5.4 (Lacroix, 1989; Watt et al., 2000). These observations suggest that the  $H^+$  concentration at pH of 5.4 has no adverse effect on smolt quality by itself. Leivestad et al. (1980) did not observe any reduction in plasma  $Cl^-$  above pH 4.5 in waters not containing LAI, suggesting  $H^+$  limits to be around pH 4.5 for freshwater life stages of Atlantic salmon. It is reasonable to assume that fish in our studies were mainly responding to Al as pH was generally  $>5.4$ . Even if pH had no direct toxic role, pH contributes to toxicity by mobilizing Al from the catchments and by transporting Al on its toxic form to the river. Within the river environment  $H^+$  interacts with Al speciation and hence toxicity and acts together with Al to give a combined stressor.

The response relationships presented here were mainly generated on fish that were not pre-exposed to acidic waters. Acclimation to acid water has been suggested as a mechanism to cope with impaired water quality to enhance survival (e.g. Allin and Wilson, 1999; Mueller et al., 1991). We did not observe acclimation in any of the long-term studies performed on Atlantic salmon smolt, but we did observe growth reduction and immunosuppression (Finstad et al., 2007; Kroglund and Finstad, 2003; Kroglund et al., 2007) but no signs of genetic adaptation to acid water (Rosseland et al., 2001). This lack of adaptation can be due to the extreme effects Al has on seawater survival (Staurnes et al., 1995) and the flooding of adapted genes by non-adapted genes from fish originating in non-acidic rivers (Rosseland et al., 2001).

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## Limits affecting freshwater survival (parr to smolt survival)

Parr exposed to pH values  $<5.6$  or cationic Al concentrations  $>45 \mu\text{g} \mu\text{gL}^{-1}$  suffered from increasing mortality (Table 3). Mortality was recorded within the first 24 h mainly when pH was  $<5.1$  and when cationic Al exceeded  $90 \mu\text{gL}^{-1}$ . At lower dose levels, mortality occurred first after several days. For smolt, mortality increased when pH was  $<5.8$  or when LAI exceeded  $20 \mu\text{gL}^{-1}$  or gill-Al exceeded  $300 \mu\text{gg}^{-1}$  dw (Table 3). Mortality could occur within 24 h when pH was  $<5.5$ , LAI  $>25 \mu\text{gL}^{-1}$  or gill-Al  $>750 \mu\text{gg}^{-1}$  dw (Fig. 7). At lower levels of the dose, the fish had to be exposed for days before mortality was observed. The differences in limits are to be expected on basis of the differences in sensitivity previously reported (Rosseland and Staurnes, 1993; Gensemer and Playle, 1999).

## Limits affecting survival in seawater (smolt to adult survival)

Performance in the seawater challenge tests were increasingly poor when LAI $>5$  or Ali $>10 \mu\text{gL}^{-1}$ . All smolt having gill Al  $>25 \mu\text{gg}^{-1}$  dw had poor hypo-osmoregulatory capacity (Table 4). Results from the sea-survival program indicate that smolt exposed to  $>5 < 10 \mu\text{gL}^{-1}$  had 25 to 50% reduction in survival, where the reduction was strongly related to dose. The return rates were reduced in all groups having gill Al concentrations  $>25 \mu\text{g} \text{Alg}^{-1} \text{dw}$  ( $p < 0.0001$ ). This strong relationship between Al and reduction in adult returns was independent of exposure duration as fish exposed for 3 days fitted into the same model as fish exposed for  $>30$  days.

The cause-effect mechanisms for this phenomenon are different from those associated with mortality in freshwater. Reduced seawater survival is most likely due to the direct effects Al has on the gill  $\text{Na}^+, \text{K}^+$ -ATPase activity (ability to maintain homeostasis in seawater), on mucus quality (charge and density), immunosuppression (increased sensitivity to diseases and parasites) and behavior effects related to loss of fright response and willingness to enter full strength seawater (Berntssen et al., 1993; Finstad et al., 2007; Kroglund and Staurnes, 1999; Kroglund et al., 2007; Magee et

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al., 2003; Staurnes et al., 1984; Staurnes et al., 1995, 1996; Rosseland et al., 1992). Of these, AI-induced effects on gill  $\text{Na}^+, \text{K}^+$ -ATPase activity has received most attention. The activity of this enzyme increases steeply during smoltification as a part of the pre-adaptation to tolerate full strength sea water (Wedemeyer et al., 1980). While  
5 compromised seawater tolerance has little effect on performance while the individual is in freshwater, this response is critical for post-smolt survival and can have the same effects on population health as heavy mortality in freshwater (Kroglund and Finstad, 2003; Kroglund et al., 2007). Smolt released into the acidified Rivers Mandalselva and Moisåna in the early 1980's gave zero returns, while fish released into the estuary had  
10 a low return rate (Hansen, 1987). Similar results were observed after releasing smolt into the acid river Lygna, while smolt released into the limed River Audna or into the estuary of the two rivers had recoveries (Staurnes et al., 1996). Improvements in liming strategy can increase salmon adult return rates (Alenäs et al., 1995; Larsen and Hesthagen, 1995; Hesthagen and Larsen, 2003).

As the timing of the smolt run varies across regions, care must be taken when interpreting the ecological effect of an episodic AI exposure to the actual timing of the smoltification period. The smolt run can last for weeks, where part of the smolt leaving the river can have reduced hypo-osmoregulatory capacity, while individuals migrating earlier or later have normal sea water performance. The ecological effect depends on  
20 the timing of the episode and how well fish recover after the episode.

#### 4 Discussion

*Both in situ* bioassays and field surveys suggest that acid episodes are harmful to fish (Barlaup and Åtland, 1996; Hindar et al., 2004; Magee et al., 2003; Teien et al., 2004b, 2005a). In short-term exposures, fish can be exposed to controlled levels of water  
25 quality constituents mimicking elements of a natural episode, but is at the same time a poor representation of the complex variation in water chemistry the wild and native fish populations are exposed to prior to, during and following an episode. Results

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from short-term exposures cannot therefore easily be extrapolated to effects at the population level (Lepori and Ormerod, 2005). For instance, fish constrained within tanks or cages during an episode are denied the possibility of behavioral avoidance that could enhance their survival in the wild. Atlantic salmon has poor developed avoidance behavior with respect to aluminum, compared to other fish species (Åtland and Barlaup, 1995; 1996). To substantiate the water quality limits suggested on basis of short-term exposures, these limits are compared to limits suggested for Atlantic salmon based on population surveys (Kroglund et al., 2002). The results used in this paper were generated by the use of acidification naïve fish, exposed for a short period (<10 days) under controlled conditions. The short exposure duration mimics, but is at the same time an oversimplification of an episode.

The response limits derived from the parr and smolt studies are not very different from limits suggested on basis of acidification-related effects on adult return rates reported from 73 rivers in Kroglund et al., (2002). Salmon was extinct from all rivers having an annual average pH <5.2 and >50 µg LAIL<sup>-1</sup>. This is a water quality that affects both parr and smolt survival in the bioassays. Within the pH-range of 5.2–5.7 and 20–50 µg AIL<sup>-1</sup> as LAI, salmon was extinct in some rivers while other rivers had reduced catches. This is a water quality that can cause some mortality in the bioassays, depending on i.e. the calcium level in the river, especially during episodic events. Mortality also will depend on exposure duration. All rivers within the pH range of 5.7–6.2 or a LAI concentration within the range of 5–20 µg AIL<sup>-1</sup> had reduced catches. This is a water quality where parr and smolt survival in freshwater is not affected in the bioassays, but the hypo-osmoregulatory capacity of the smolt is compromised. Rivers having pH >6.2, <3 µg AIL<sup>-1</sup> and ANC values >35 µeqL<sup>-1</sup> were all categorized as unaffected by acidification. Similar conclusions are drawn from the bioassays. The population status of salmon in the individual river will be controlled by chemical and biological factors and in-between year variations in critical exposures (timing, duration and “intensity” of episodes). Furthermore, it is well known that returning adult salmon, being accounted for in the catch statistics, can have an origin from neighboring rivers

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(strayers) within a region and that escaped salmon from fish farms can contribute to the annual catches as well. Salmon catches in some of the acid rivers can thus be due to smolt produced in other rivers.

The population responses reported in Kroglund et al. (2002) were all based on annual average water qualities. Fish are affected by the general water quality, but it is probably the extremes (taking exposure duration into account) that have the largest impact on population status. The relationship between episode intensity and annual average chemistry is therefore of interest (Wright et al., 2007). Smolt will migrate from the river and into the ocean during high discharge periods in spring, a time period when acidification episodes were common. Short-term episodes in spring (down to 3 days) can as such be one of the factors contributing to the present low and declined salmon populations in many rivers draining to the Atlantic Ocean (Kroglund et al., 2007).

The above limits suggest that population extinction occurs mainly in water qualities where the critical limit for all life history stages is exceeded, since parr (which we have included in our experiments) probably is the most resistant life history stage. Catch reductions, however, occur in rivers where water quality is predicted to affect the hypo-osmoregulatory capacity of the smolt. The low tolerance limits of smolts to AI suggest that more rivers than presently accepted being acidified can be affected by acidification. This can then have restricted the interpretation of cause and effect, and thus the use of chemicals (lime) as a mitigation method to improve fisheries. In those cases, additional data on gill AI would aid to the interpretation of ecological status.

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**Table 1.** Number of exposure groups, separated and sorted according to life stage and the analytical protocol for AI fractionation.

Life stage	Number of exposure groups		
	LAI-protocol	Ali-protocol	Total
Parr	34	67	101
Smolt	114	114	228
Post-smolt	18	0	18
Total	116	181	347

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**Table 2.** Percent of the seawater challenge tests (24 h, 33–34 ppt salinity, 6–11°C) resulting to from zero, >0–<50% and >50% mortality relative to the LAI concentration the fish experienced while in freshwater. N=179.

LAI $\mu\text{gL}^{-1}$	No mortality 0%	Moderate to high mortality >0–50%	High mortality >50%
< 5	100	0	0
5–10	6	54	40
10–15	38	40	22
15–20	11	65	24
>20	22	18	60

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**Table 3.** Dose levels that separate “no effect” levels from doses where responses can either range from “low to high” or are always “high” with respect to ANC ( $\mu\text{eqL}^{-1}$ ), pH ( $\text{H}^+$ ), cationic Al ( $\mu\text{gL}^{-1}$ ) and gill Al ( $\mu\text{gg}^{-1}$  dw) as the dose and mortality as the response. Limits are proposed for the life stages parr and smolt. Exposure duration was <10 days.

	Mortality-parr				Mortality-smolt				
	ANC	pH	Ali	Gill Al	ANC	pH	LAI	Ali	Gill Al
No effect	>15	>5.6	<45	<400	>15	>5.8	<20	<40	<300
Low-high	<15	<5.6	45–90	>1000	<15	5.5–5.8	20–40	40–65	300–450
High					<15	<5.5	>40	>65	>450

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**Table 4.** Dose levels that separate “no effect” levels from doses where responses can either range from “low to high” or are always “high” with respect to ANC ( $\mu\text{eqL}^{-1}$ ), pH ( $\text{H}^+$ ), cationic Al ( $\mu\text{gL}^{-1}$ ) and gill Al ( $\mu\text{gg}^{-1}$  dw) as the dose. All fish were exposed for 7 to 10 days. Responses are evaluated using plasma  $\text{Cl}^-$  (on smolt surviving a 24 h seawater challenge test) and on effects on adult return rates (smolt release experiments; 3 – >40 days exposure).

	Plasma $\text{Cl}^-$ (SW-FW)				Adult return rates			
	pH	LAI	Ali	Gill Al	ANC	pH	LAI	Gill Al
No effect	>6.5	<5	<10	<25	>50	<8	<25	
Low-high	6.5–6.0	5–15	10–25	25–100	<50	8–12	25–60	
High	<6.0	>15	>25	>100		>12	>60	

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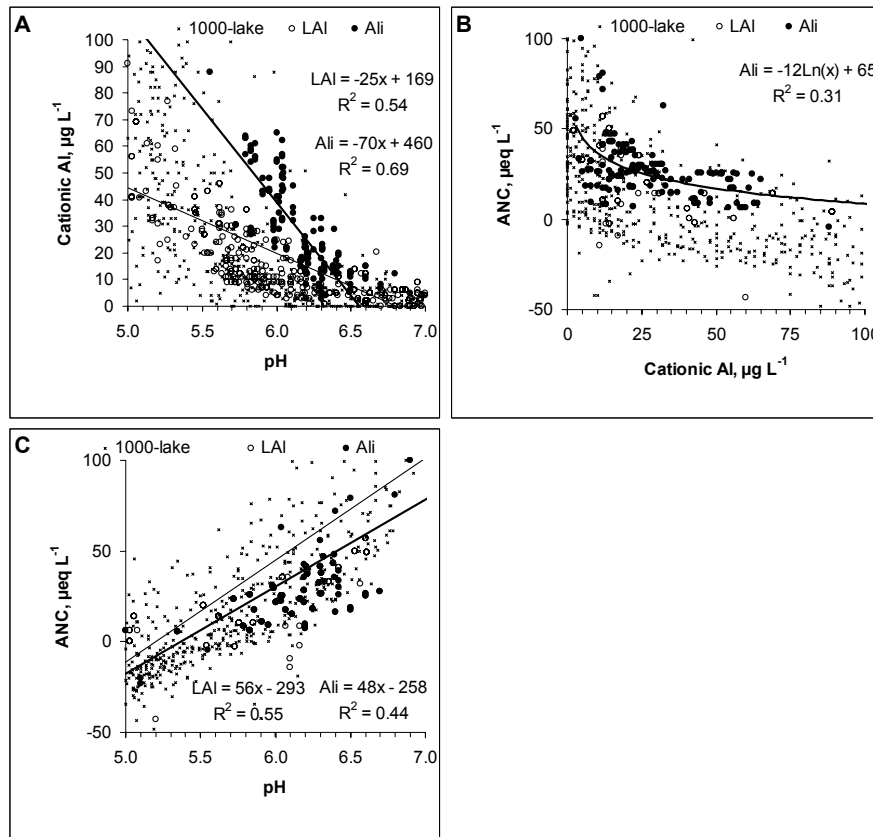
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**Fig. 1.** (a) Relationship between pH and cationic Al ( $\mu\text{g L}^{-1}$ ) measured in situ (Ali) or on samples after transportation to NIVA for Al fractionation (LAI). Figures (b), (c) relationship between cationic Al and ANC and pH to ANC. The relationships are superimposed on relationships from the Norwegian 1000-lake survey from 1986 (Henriksen et al., 1989; Rosseland and Henriksen, 1990; Bulger et al., 1993; Lien et al., 1996). Linear relationships are entered into the graphs whenever significant.

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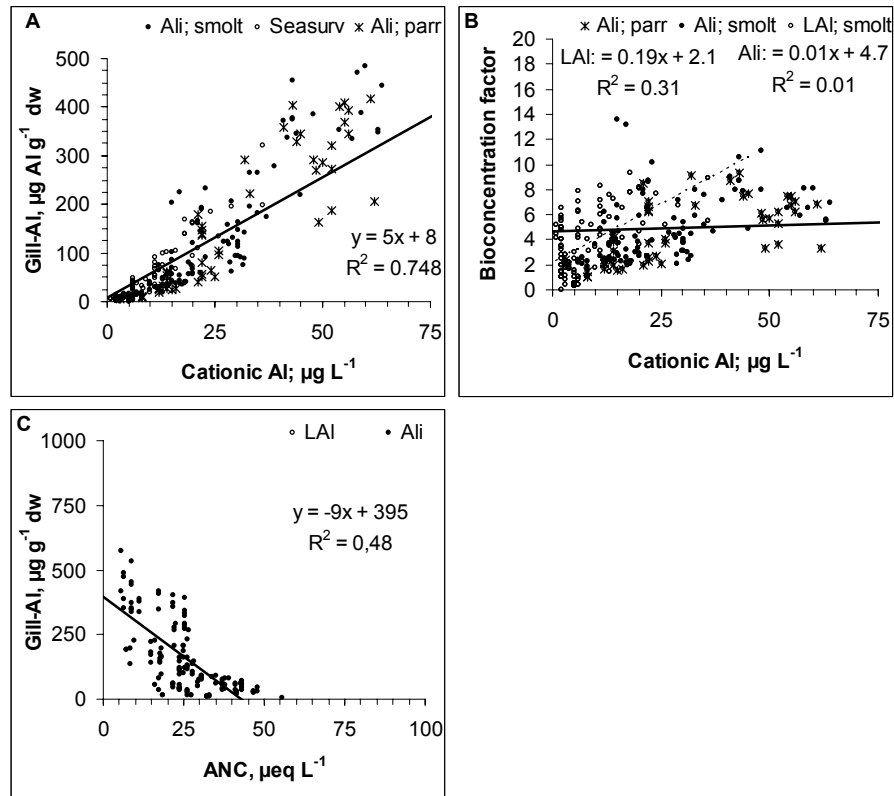
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**Fig. 2.** Relationship between cationic AI (LAI and Ali) and gill AI for parr and smolt. Figure (b): Relationship between cationic AI and bio-concentration factor. Figure (c): Relationship between ANC and gill AI. Linear relationships are entered into the graphs whenever significant.

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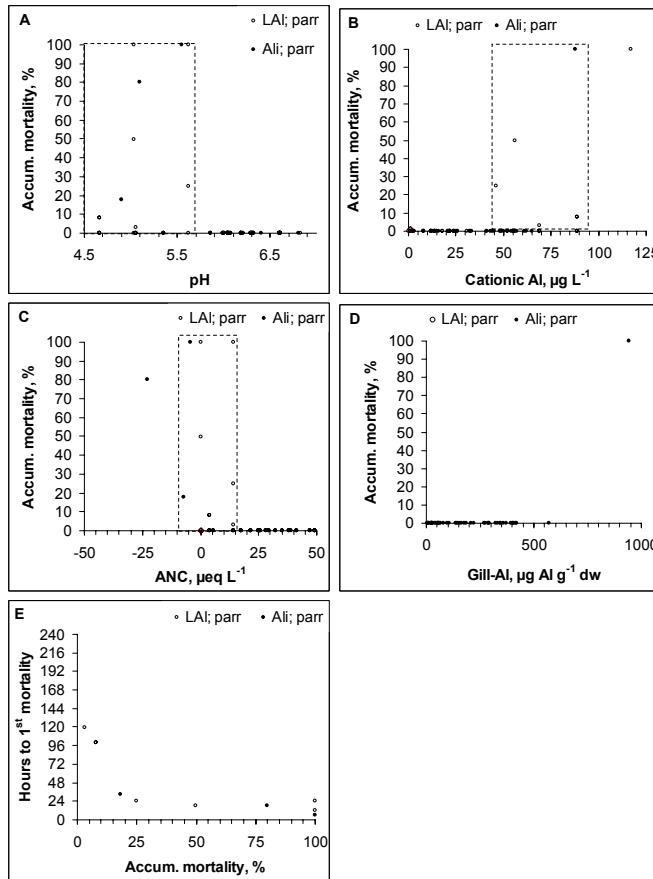
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**Fig. 3.** Relationship between (a): pH, (b): cationic Al, and (c): gill Al and accumulated mortality of parr. In Fig. (d): the relationship between accumulated mortality over a 10 day period is related to how long it took to kill the first fish. The dashed lines suggest dose levels separating “no effect”, “low to high” effect and always “high” effect.

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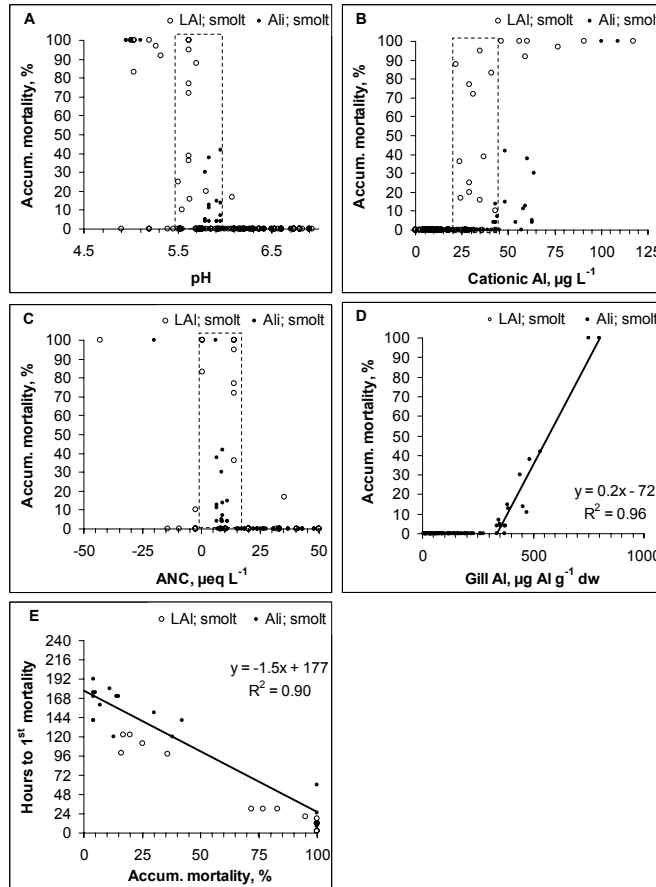
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**Fig. 4.** Relationship between (a): pH, (b): cationic Al, (c): ANC and (d): gill Al and accumulated mortality of smolt. In Fig. (e): the relationship between accumulated mortality over a 10 day period is related to how long it took to kill the first fish. Linear relationships are entered into the graphs whenever significant. The dashed lines suggest dose levels separating “no effect”, “low to high” effect and/or always “high” effect. 3351

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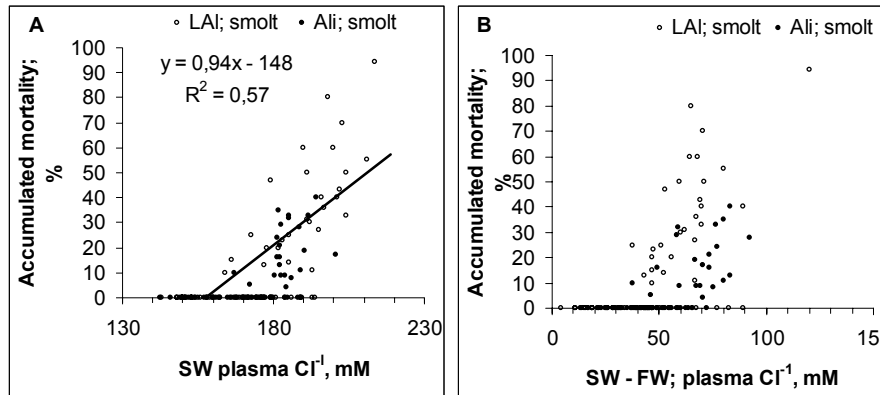
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**Fig. 5.** Relationship between (a) plasma Cl<sup>-</sup> or (b) increase in plasma Cl<sup>-</sup> relative to freshwater values and mortality in seawater challenge tests (n=178). Linear relationships are entered into the graphs whenever significant.

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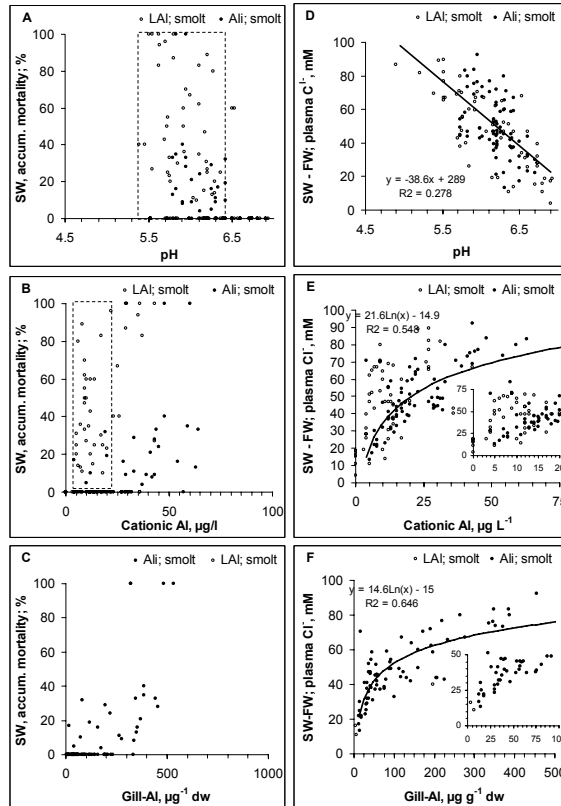
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**Fig. 6.** Mortality in a seawater challenge test with respect to (a) pH, (b) cationic Al (as LAI and Ali) and (c) gill Al concentrations measured in freshwater prior to the test. Figures (d)–(f) show the same data, but using the increase in plasma Cl<sup>-</sup> from freshwater to seawater as the biological response. To increase resolution, the relationship for low concentrations is inserted into Figs. (e) and (f). Linear relationships are entered into the graphs whenever significant. The dashed lines suggest dose levels separating “no effect”, “low to high” effect and/or always “high” effect.

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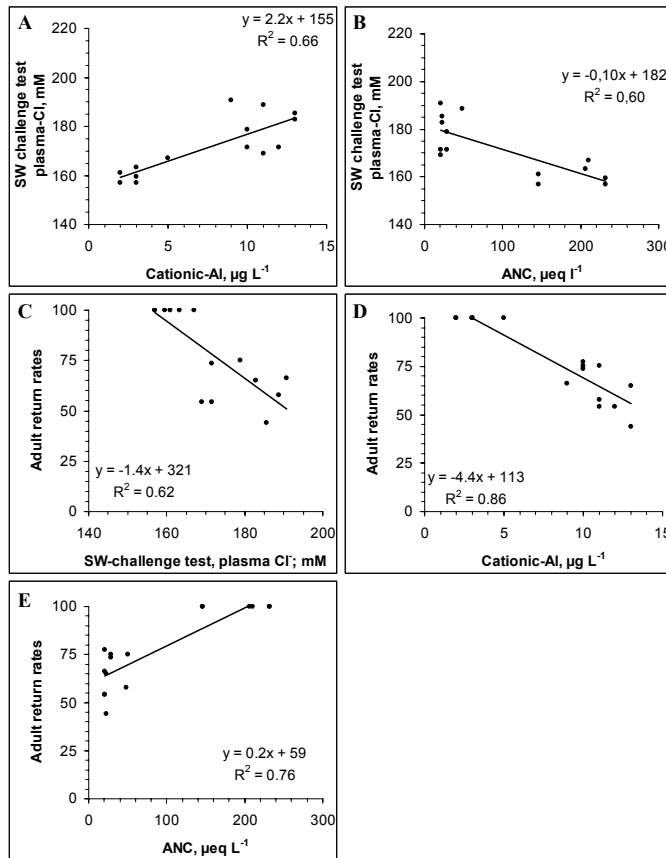
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**Fig. 7.** Relationships between (a) cationic Al (as LAI) and (b) ANC with respect to effects on hypo-osmoregulatory capacity (plasma Cl<sup>-</sup>). Figures (c)–(e) Relationship between hypo-osmoregulatory capacity, cationic Al (as LAI) and ANC with respect to adult return rates. Linear relationships are entered into the graphs whenever significant.

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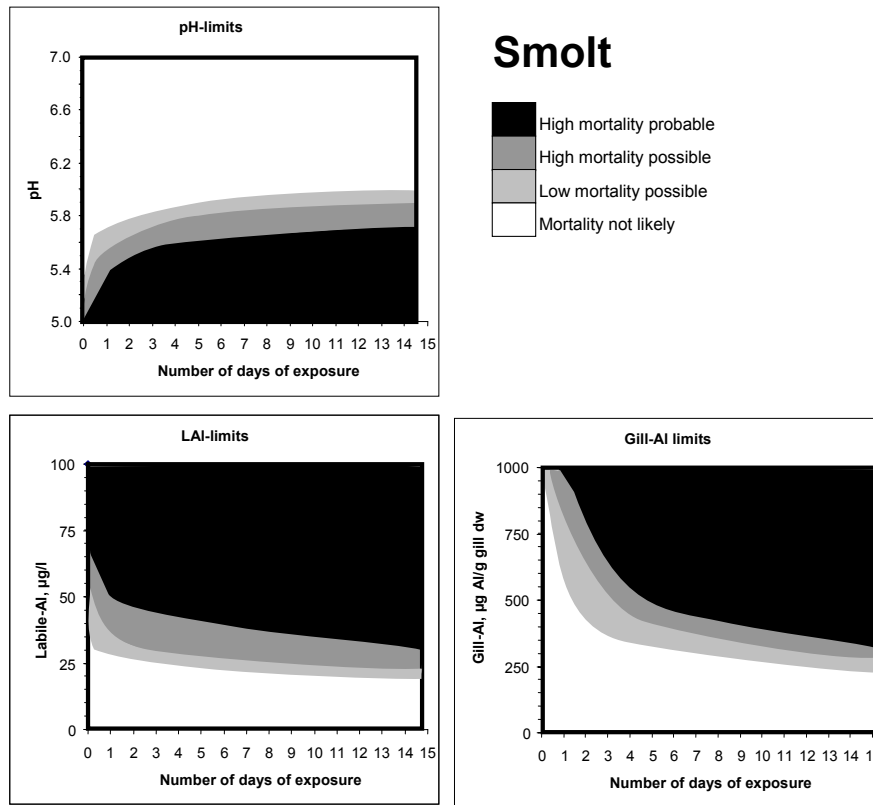
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**Fig. 8.** Expected mortality levels for smolt of Atlantic salmon with respect to pH, cationic Al (as LAI) and gill Al in relation to exposure duration. After: Kroglund and Rosseland, 2004.

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