Development of saltwater tolerance in Atlantic salmon *Salmo salar* in various water qualities in Suldalslågen:

Effects of variation in pH and dissolved aluminium

Iver Kongssund

Faculty of Applied Ecology and Agricultural Sciences

Master in Applied Ecology

2018

Applied Ecology

Evenstad, April 26, 2018

Iver Kongssund

Abstract

Kongssund, Iver (2018). Development of saltwater tolerance in Atlantic salmon *Salmo salar* in various water qualities in Suldalslågen: Effects of variation in pH and dissolved aluminium. 65 pages including references.

Key words: *Toxicity, aluminium, acidification, Atlantic salmon, smoltification*

1. Aqueous aluminium is described as the principal toxicant killing fish in acidified waters, along with low pH. A raising concern has been the possible effect this toxicant have on the declining Atlantic salmon population and their ability to adapt to changes in salinity.

2. River Suldalslågen in Suldal municipality has been subject to such a decline in salmon population, and numerous studies have been conducted trying to identify the role of water quality to both the smoltification process and survival. The aim of this study has been to describe the development of seawater tolerance in Atlantic salmon exposed to different water qualities in Suldalsvassdraget in spring and throughout the migration period of smolts. To achieve this, water chemistry parameters were collected, as well as physiological conditions from fish exposed to different water qualities and seawater challenge tests, during the entire period. Collected data was analysed and statistically modelled with GLMMs. An additional aim was to answer the applicability of seawater challenge testing as a tool for identifying an ongoing smoltification process and as predictor for water quality criteria in Atlantic salmon.

3. The results suggest that the Atlantic salmon in Suldalslågen never developed a satisfactory seawater tolerance, and that hatchery-produced fish from Ritland hatchery never could be characterized as smolt, prior to or during the time of migration. Thus, I have only to a limited extent achieved the aim of describing the development of seawater tolerance. The results further exposes aluminium in Suldalslågen not to be responsible for non-developing seawater tolerance in salmon, but on a contrary acting as a possible positive "stimulator" in physiological processes associated with osmoregulation in fish, where these processes, for some reason, is not developing normally.

4. Finally, this study suggest seawater challenge testing to be limited in predicting water quality criteria in Atlantic salmon, and give a cautionary notation to the applicability of hatchery-reared fish and possible future economic and socioeconomic consequences.

Contents

1. Introduction

The migration between freshwater and the marine environment is for diadromous fish species a strategy of adaptive value, a behaviour inherited for generations to increase individual fitness through the different stages of life cycle (Lucas & Baras, 2001). As for a variety of different species, the Atlantic salmon (*Salmo salar*) experiences challenging physiological transformations when migrating from familiar salt-free to novel salt-rich environments. The life cycle mainly includes spawning in freshwater followed by a migration to the sea, where the foundation for growth is made due to the presence of rich food resources (Klemetsen et al., 2003). Salmon display a wide range of variability in freshwater habitat use (Gibson 1993, 2002; Heggenes et al. 1999) both within and among populations. The species show a diverse life history with differences in sea-age maturity (Meerburg 1986; Power 1986; Riddell 1986; Randall et al. 1986; Jonsson et al. 1991a) and even exclusively freshwater resident populations (Klemetsen et al., 2003). Anadromous populations in Norway has shown a difference between individuals from a particular year-class in time of smoltification, ranging from 1-8 years before they migrate to the sea for the first time (L'Abee-Lund et al. 1989). After reaching saltwater, they typically spend 1-2 years feeding and growing in the ocean before returning to freshwater to spawn (Klemetsen et al., 2003).

Before migrating to the sea, the anadromous species have to undergo an extensive change in life history traits, to be adapted to their new environment. This process is referred to as the process of smoltification. In Atlantic salmon, this process alters the changes in physiology, morphology and behaviour to adapt the fish to a life in seawater (McCormick & Saunders, 1987; Hoar, 1988). The completion of smoltification is influenced by several environmental factors including temperature (Solbakken et al., 1994) and photoperiod (McCormick et al., 1987; Saunders & Harmon, 1990; Duston & Saunders, 1992) in addition to size (Boeuf et al., 1985). During the acclimation to seawater, the smolts experience hyper-osmotic stress resulting in an accumulation of increased plasma ion concentrations (McCormick et al., 1989) along with tissue hydration (Blackburn & Clarke, 1987), followed by an adjustment period. The duration of this period varies with smolt status (McCormick et al., 1989; Sigholt & Finstad, 1990), size (Bjerknes et al., 1992) and temperature (Sigholt & Finstad, 1990).

The stress terminology is often used in context with what is referred to as "*compensatory physiological changes*" as a result of inner processes in the organism, like the mentioned smoltification, maturation and disease (Reimers & Døving, 1992). Stress is defined as the sum of all physiological responses an animal is using to maintain a normal level of metabolism, following physical and or chemical alterations in the environment (Reimers & Døving, 1992). Stress is an adaptive physiological response which maintains homeostasis and increase the individual fitness. A protracted and chronic exposure of stress will do the opposite, and be maladaptive, resulting in reduced growth, reproduction and survival (Reimers & Døving, 1992).

The primary reaction to stress can be divided into two categories; 1) increased excretion of catecholamine and 2) increased excretion of corticosteroids. These two responses leads to numerous physiological changes which alters metabolism from what is referred to as an "*anabolic"* state (absorption and storage of energy) to a *"catabolic"* state (decomposition and use of energy storage) (Reimers & Døving, 1992). Increased excretion of catecholamines (adrenaline and noradrenaline) can occur minutes after exposure to a stress factor, and can be sustained for a couple of hours, depending on strength and stimuli. An increased plasma level in catecholamines affect circulation, respiration, osmoregulation and metabolism. The cardiac output increases and resistance in gills is reduced by dilating blood vessels. The respiratory surface in gills increases by changing the usual passive lamellae to active, creating a possible increased oxygen consumption (Reimers & Døving, 1992). This increased active gill surface will generate a higher water consumption and loss of ions in freshwater fish, while increase excretion of water and a passive ion consumption in saltwater fish. This elevation of oxygen consumption is a clear adaptive response to prepare an individual for an escape or a fight, but can lead to a maladaptive osmoregulation effect (Reimers & Døving, 1992).

During stress, the *adrenocorticotrophic hormone* (ACTH) stimulates excretion of cortisol and cortisone in fish. Cortisol has an osmoregulatory function, especially in saltwater adaptation in euryhaline fish populations (Reimers & Døving, 1992). Cortisol can induce ion consumption in freshwater and ion excretion in saltwater, in interaction with other hormones. An elevated level of cortisol over a longer period of time, will lead to a compromised immune system (Reimers & Døving, 1992). Changes in physiological parameters can reveal type or magnitude of stress, identify a stress factor or the effect of a stress factor. Variations can be caused by species, gender, time of day or year, and more. If exposure to a stress factor

only causes a limited amount of stress, the physiological parameters will then restore back to normal levels (Reimers & Døving, 1992). When assessing level of stress, the most common measurements are 1) haematological, 2) blood chemical, 3) tissue chemical altercations, or 4) response in the entire organism. The most common haematological parameters are hematocrit, haemoglobin, number of red blood cells and the number of different white blood cells. Stress hormones like adrenaline and cortisol are the preferred indicators of stress in the literature (Reimers & Døving, 1992). The benefit of these indicators is that they directly measure the *primary response* of stress factors (Reimers & Døving, 1992).

Toxic chemicals represent an important stress factor for fish by either a direct toxic effect or by transfer through the primary effectors adrenaline and cortisol. The effect of a toxic compound can be divided into several levels. If smoltifying Atlantic salmon is exposed to acidic aluminous water, the reduced level of gill Na^+, K^+, -ATPase activity will lead to a reduced capability to transport ions (Sigholt et al., 1995; Reimers & Døving, 1992). Increase of this particular enzyme during the smoltification is necessary for the fish to void ions when entering the sea, but also to maintain ion balance during freshwater residence (Reimers & Døving, 1992). Simultaneously, there is a loss of homeostasis with a strong disturbance of ion balance, and fish are not able to develop saltwater tolerance (Reimers & Døving, 1992). Hence, contamination causes disturbances in basal processes that prevent fish from smoltifying, thus the ability to migrate and survive in the ocean (Reimers & Døving, 1992).

As mentioned, aluminium can act as a toxic compound. Dissolvence of different aluminium sources in water is highly dependent on pH, temperature and the type and amount of ligands present. Dissolved in water, aluminium will spread by the hydrolysis equilibrium, and similar to the dissolvence of aluminium, the Al-hydrolysis is dependent on both pH and temperature (Reimers & Døving, 1992). Aluminium will therefore act in different physical and chemical forms in natural water, depending on the predictors mentioned. The many forms of aluminium can have different level of toxicity for fish populations. Aluminium bound to organic compounds is not toxic to fish, and if humus substances or citrate are added to acidic aluminous water, the toxicity of aluminium will decrease (Reimers & Døving, 1992). Inorganic aluminium compounds with low molecular weight, is regarded as the most toxic, in addition to the level of polymerisation. The gill surface in Atlantic salmon is covered by a layer of mucus which contains numerous molecules with a negative charge. Al-hydroxides with a positive charge will bind to the negatives, resulting in continued Al-polymerisation and formation of large particles on the gill surface, opposed to freely in the

water (Reimers & Døving, 1992). When pH is higher than 4.0 a formation of hydroxide compounds of aluminium will start, as a result of $Al(H_2O)_6^{3+}$ (usually written Al^{3+}) deprotonation (release of H^+-ions). The actual Al^{3+} -ion could also possibly bind to the negatives on the gill surface.

Aluminium as a toxic compound, is primarily affecting mechanisms related to the gills causing imbalance in regulation of ions and issues with respiration. The result of Al-polymerisation in gills can lead to densification between the secondary lamellae, and consequently reduce water flow in the respiratory surface (Reimers & Døving, 1992). Hypoxia or reduced availability of oxygen, is recognised as the main reason to mortality of salmonids at relative high levels of pH (5.5 – 6.5). Within this range, the Al-polymerisation would be favoured. Additionally, temperature as an important predictor to the Al-hydrolysis will increasingly stimulate the ventilation frequency in Atlantic salmon with higher temperatures. Al-polymerisation will therefore be recognised as one of the main factors explaining hypoxia as reason for fish mortality in Al-rich water, with high levels of pH (5.5 – 6.5) and temperature (10-20°C) (Reimers & Døving, 1992). The degree of toxicity and the magnitude of impact on either fish survival or the ability to develop seawater tolerance is a complex interaction between several explaining factors. Species and stage of development are also factors to consider. In Atlantic salmon, the most sensitive periods are moments after hatching and during the smoltification process (Reimers & Døving, 1992).

Despite an extensive quantity of available information on Atlantic salmon, a raising concern for some years has been the continued decline of wild stocks in the North Atlantic (Mather et al. 1998; Parrish et al. 1998; Potter & Crozier 2000). Generally, it is believed that the cause of decline is connected to events in the marine environment (Friedland et al. 1998; Jacobsen & Hansen 2000; Potter & Crozier 2000; Montevecchi et al. 2002), however causation could be initiated in freshwater with final consequences when fish are at sea (Randall et al. 1989; Walters & Ward 1998; Fairchild et al. 1999).

In the early 1990s Norwegian lakes and rivers were severely influenced by acidification (Henriksen & Hesthagen, 1993), leading to a decline and elimination of several fish populations (Muniz, 1984; Rosseland, Skogheim & Sevalrud, 1986; Hesthagen & Hansen, 1991). In southern parts of Norway, Atlantic salmon populations were seriously affected, being virtually extinct in 25 rivers (Hesthagen & Hansen, 1991). Aqueous aluminium is described by several authors as the principal toxicant killing fish in acidified waters, along

with low pH (Burrows 1977; Driscoll et al. 1980; Howells et al. 1994; Gensemer & Playle 1999).

One of the river systems that experienced a dramatic decline of the salmon population in the early 90s, is Suldalsvassdraget in Rogaland county (Figure 1). The reason for the affected salmon population could be complex, including several explaining factors both outside and within the watercourse (Kaasa et al. 1998). Throughout the last two decades, numerous studies have been conducted to describe limiting factors in Suldalslågen and to what extent acidification and regulations have had an impact on the water quality (Heggberget et al. 1994; Kaste et al. 1995; Blakar, 1995; Kaasa et al. 1998; Blakar & Haaland, 2000). Some of the later research (Kroglund et al. 1998a, b, c; Øxnevad & Poléo, 1998; Finstad et al. 1999, 2000; Fiske et al., 2001; Kroglund & Finstad, 2001; Poléo et al. 2001a, 2002; Strand et al., 2000, 2001, 2003; Schjolden et al., 2001) concludes acidification not to be the sole responsible factor for the decline in salmon population. A remaining question of importance is if acidified Al-rich water from the Suldalslågen watersheds could possibly harm the salmon population, without detectability during the freshwater residence during the life cycle. A key factor in this regard, is whether the death of salmon at sea is caused by a reduced saltwater tolerance as a result of the exposure to the perceived poor water quality (Kroglund & Finstad, 2001, 2003), or by exposure to sea lice (*Lepeophtheirus salmonis*) when smolts migrate from the watercourse (Kaasa et al., 1998; Grimnes et al. 2000; Holst & Jacobsen, 1999).

Kroglund et al. (1998a, b) argues a possibility of affected wild salmon caused by water quality. It has been suggested through measurements of fish response in experiments, that negative effects of water qualities is not to be excluded. Additionally, a reduced saltwater tolerance in both wild- and hatchery-produced fish from Suldalslågen has been reported (Kroglund et al., 1998b; Finstad et al., 1999, 2000; Strand et al., 2000, 2001) to have a correlation with low water quality. Finstad et al. (1999, 2000) reports a reduced ability in osmoregulation in seawater challenge tests, with a satisfying ability in freshwater. With no detection of a decline in density of juveniles in Suldalsvassdraget, it is argued that the accumulation of Al on gills still could reduce the saltwater tolerance, and further affect marine survival (Kroglund & Staurnes, 1999). In this context, several studies have argued for low Al-concentrations to have a negative impact on the Atlantic salmon in Suldalslågen (Kroglund et al. 1998a, b; Kroglund & Finstad 2001, 2003).

Despite the research presented, a number of studies points to the opposite. Some argue acidification as not to be a real threat for the salmon population in Suldalslågen (Øxnevad & Poléo 1998, Poléo et al. 2001a, 2002, Jensen et al. 2001, 2002, 2003, Schjolden et al. 2001), and that the concentration of Al is likely to be too low to cause reduced survival in smolts in the ocean. However, none of these studies has specifically looked at the development of seawater tolerance in Atlantic salmon in Suldalslågen. Poléo et al. (2001b) reported in a preliminary study that salmon exposed in acidified Al-rich water showed a higher seawater tolerance than salmon exposed to limed water, thus concluded the seawater challenge test (Clarke & Blackburn, 1977) to give a poor estimate of fish health status. This study was conducted at the end of the migration period, hence the seawater tolerance was not registered over time. The aim of this present study has therefore been to identify the development of seawater tolerance in Atlantic salmon (presmolt) exposed to different water qualities in Suldalsvassdraget in spring and throughout the migration period of smolts. To achieve this, physiological conditions taken from blood samples were compared using generalized linear mixed models (Bolker et al. 2009), as well as water chemistry parameters for aluminium. This study builds on experiments and unpublished data conducted by Poléo et al. in 2002. Based on the likely assumptions in the earlier research of Poléo et al. (2001b), I test the following hypothesis that;

i. *The development of seawater tolerance in Atlantic salmon is not negatively affected by water quality, hereunder acidified aluminous water.*
   I predict a compromised ability in salmon to adapt to changes in salinity, not to be affected by presumed poor water quality.

ii. *Stress response expressed as changes in physiological conditions is not affected by acidified aluminous water in Suldalsvassdraget.*
   I expect the toxicity level of aluminium in Suldalsvassdraget to be too low to affect variation in physiological conditions as plasma cortisol, plasma chloride and hematocrit.

Finally, I evaluate the applicability of seawater challenge testing as a tool for observing smoltification and the additional future recommendations for management implications.

2. Methods

2.1 Study area

The study site is situated in Suldalsvassdraget, Rogaland county, Norway (59°59′30″N 6°51′13″E) (Figure 1). The source of the river Suldalslågen is Lake Suldalsvatnet (69m.a.s.l.) and the river ends in Sandsfjorden at Sand. The main river course runs through Suldal municipality and is 22, 3 km long. The watersheds covers 1462,7km^2 with numerous tributary streams and creeks (NVE Atlas).

Four stations were placed along the watercourse Suldalsvassdraget; at Suldalsvatnet (Osvad), at Prestabekken (Prestvika), at Steinsåna, and lastly at Foss – approximately 300 meters upstream the water exit of Fossåna. At the location of Foss station, three different water qualities were studied (Table 1). In total, six different water qualities were used in the experiments, and for each water quality a field setup was created (see below).

Table 1. Stations with water qualities from the watercourse Suldalsvassdraget used in the field experiments. pH values and Al-concentration, µg Al /l (mean) is obtained from Blakar & Haaland (2000), and in Prestabekken from Poléo et al. (2001a).

Station	Water quality	pH	Al_{tot}
Osvad	Lake Suldalsvatnet – natural acidic Al-poor	6.3	30
Prestvika	Creek Prestabekken – natural non-acidic water	6.4	50
Steine	Creek Steinsåna – limed water	6.9	50
Foss	Creek Fossåna – natural acidic Al-containing	5.7	80
	River Suldalslågen – limed water	6.3	30
	River Suldalslågen +Al – limed water*	6.3	30

*River Suldalslågen+Al is more or less identical to River Suldalslågen, with an Al-enrichment.

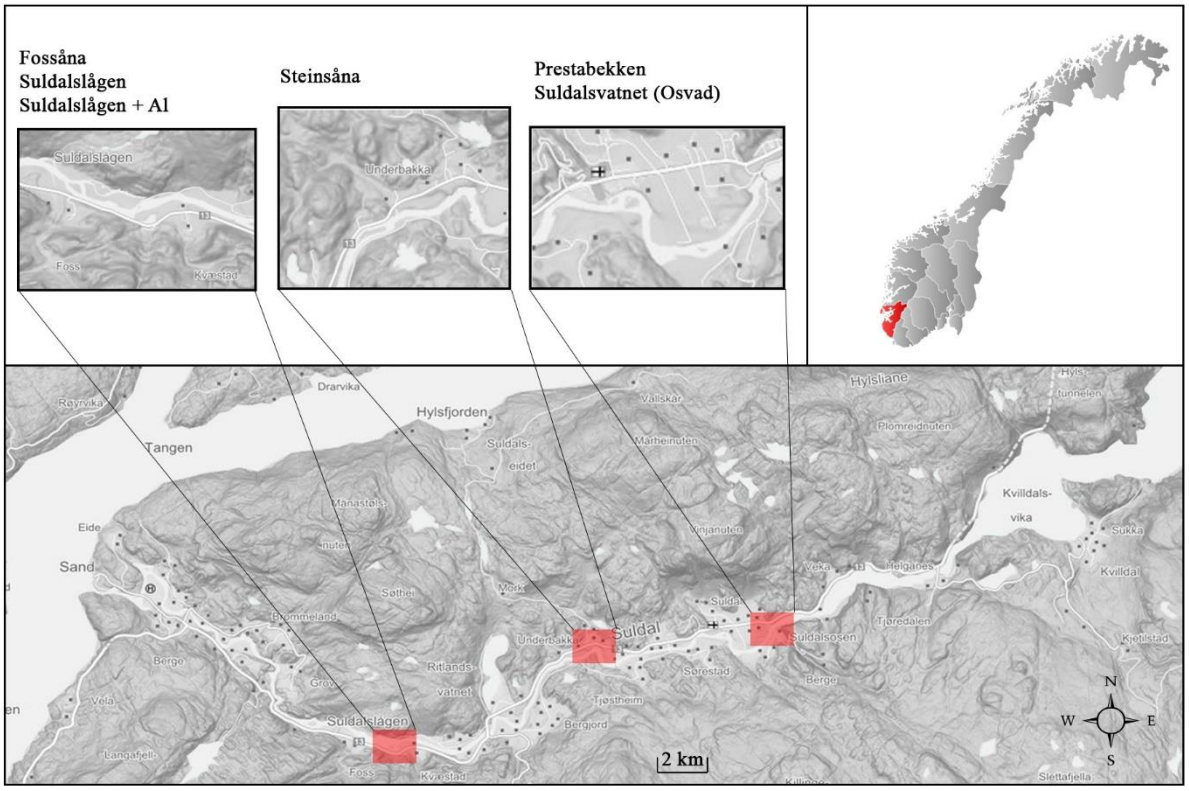

Figure 1. The six different sampling locations/water qualities in Suldalsvassdraget, Rogaland county – Norway. The map is created from Norgeskart with Adobe Photoshop CS6 version 13.0.

2.2 Experimental species

Atlantic salmon (*Salmo salar*) parr of the River Suldalslågen stock, ranging from 6.7 to 17.0 cm (mean = 11.7 cm) in length, were obtained from the Ritland hatchery and transported in water filled plastic bags to the different stations where the experiments were conducted. The salmon were then transferred into separate fish tanks (approximately N=375 in each tank) and acclimated for one week prior to the experiments that begun on the 5. Mars 2002. The exception was the tank for water exposure from Fossåna, where the fish were inserted on the 8. Mars due to issues with frost and consequently uncertain water supply. As a result, the initial seawater test for Fossåna was conducted a week later than for the other water qualities. The fish were not fed during the experiments, although the water pumps made sure of accessibility of substrate and benthos in the chambers. Consequently, no fish were starving, and the condition of the fish did not change during the experiments.

2.3 Experimental setup

At each station there were placed 500 litres fish tanks by the water locations. The setup consisted of an open system made out of fiberglass with a continuous water flow, and a spillway at the bottom (Poléo et al., 2001b). Water pumps lowered into the water source at each station filled the tanks with water at 20 l/min giving a water supply of 5.3 litres per gram fish per day. Sprague (1973) recommends a water supply of minimum 2.0 l/(g/d) for similar studies. The experimental setup was constructed to prevent changes in the level of water flow at the locations. All tubes and transitions were made of PVC and silicone. Each tank was covered with a top board including a 20 × 20 cm "window" in the centre, so the fish could register changes in daytime and to protect the fish from unnecessary disturbances (Figure 2). Water from Suldalsvatnet, Prestabekken, Steinsåna and Fossåna was led directly into their respective fish tanks while the water from Suldalslågen was directed into two different tanks (Suldalslågen and Suldalslågen+Al), using a Y-connector. Untreated water from Suldalslågen was directed into the tank for the water quality "Suldalslågen". The aluminium-enriched water ($Al(NO_3)_3$-solution) called "Suldalslågen+Al" was directed into the exposure chamber via a 50 litres spillway tank. During the additive process the $Al(NO_3)_3$-solution (concentration 3.5 g/l (86.9 g $Al(NO_3)_3 \times 9H_2O$ dissolved in 25 litres of double-distilled)) was added with a peristaltic pump and the velocity of 4 ml/min. The water supply to the fish tank was adjusted to 4 l/min so that the amount of aluminium added was 250 µg/l. The additive process lasted for 39 hours and 25 minutes (from 19. April, 15:20 – 21. April, 06:45). During this period the pH in the water "Suldalslågen+Al" decreased from 6.3 to 5.8.

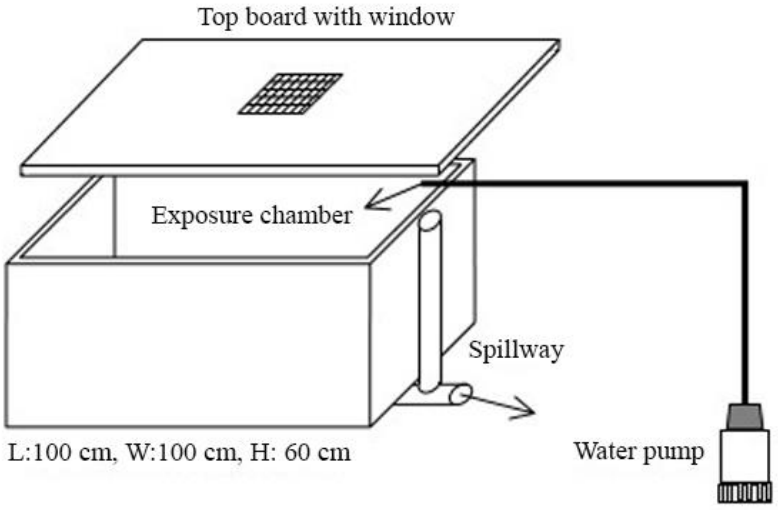

Figure 2. Sketch of the exposure chamber located at each station/water quality during the experiments. Created with Adobe Photoshop CS6 version 13.0.

2.4 Experimental protocol

The present study was performed as an experimental study, in the spring of 2002 (26. February – 14. June) and started with the transmission of Atlantic salmon parr (N=375) in each fish tank at every station. Once a week throughout the whole experimental period, seawater testing was conducted on 15 specimens from each water quality. Following the seawater test, blood- and gill samples were taken from the remaining surviving fish. Equivalent testing was performed on 10 specimens from each fish tank as a control sample on the physiological condition of the seawater tested fish. Throughout the experimental period, daily measurements were taken of water temperature, pH and water conductivity from the fish tanks' spillway. A weekly aluminium fractionation of the water qualities was also performed. In addition, an extra water sampling was performed with a consecutive aluminium fractionation associated with a flood in Fossåna.

2.5 Seawater challenge test

The seawater challenge testing (Clarke & Blackburn, 1977) was performed in 50 litres black plastic buckets (mortar bucket), immersed in tanks placed at the spillway of the fish tanks. The drainage from the fish tanks made sure of a temperature in the seawater buckets to be equivalent to the temperature in the fish tanks. The plastic buckets were filled with water from the applicable water source and added a salt mixture "Instant Ocean" to a salinity of 33.0 ± 0.3‰ . The salinity was measured with an YSI 30M/10FT Conductivity Meter which also measures the water temperature. The artificial seawater was bubbled with oxygen through an aquarium pump. The water's O_2-saturation was measured with a WTW CellOx 325 oxygen electrode connected to a WTW Oxi 340 Oximeter with the option of a salinity preset. 15 specimens were transferred into the "seawater buckets" at every seawater challenge test, and the buckets were covered with black plastic. After 24 hours, the surviving fish was transferred into a bucket containing a mixture of water and 7 ppm metomidate hydrochloride (anesthetic). Measurements of temperature, salinity and O_2-saturation in the artificial seawater were conducted both before and after the seawater challenge test.

2.6 Water chemistry

The pH was measured with a Radiometer pH-meter-29 with a GK 2401 C combined glass/reference electrode. Radiometer pH-buffer 4.0 and 7.0 was used to calibrate the electrode. The water was sampled in bottles made of polyethylene that were previously washed in water from which the sample was taken. The pH-value was registered to closest 0.1 pH-unit when the pH-meter varied less than 0.05 pH-unit/min. The standard deviation for measured pH is ± 0.04 pH-unit. Temperature and conductivity of the water was measured with an YSI 30M/10FT Conductivity Meter.

2.7 Aluminium

Cation exchange (Driscoll, 1984) combined with ketone extraction (Barnes, 1975) was used to distinguish different aluminium fractions. This is a suitable fractionation method for aluminium in the field (Sullivan et al., 1986; Lydersen et al., 1994). The standard deviation of the method is estimated to be approximately 1% of the mean (Sullivan et al., 1986) and the detection limit is 13 µg Al/l (Vogt et al., 1994). Upon extraction of a water sample,

aluminium is complexed with 8-hydroxyquinoline (HQ) (C_9H_7NO) and the Al-HQ complex is extracted into an organic phase of methyl isobutyl ketone (MIBK). The extraction time is 20 seconds, as Barnes (1975) recommends. The extracts were stored at 4°C for at least 24 hours before the amount of aluminium was analysed spectrophotometrically (Shimadzu UV-1201 spectrophotometer) at 395 nm (Tikhonov, 1973; Bloom et al., 1979). Absorbance was also measured at 600 nm to correct for iron interference (Sullivan et al., 1986). The amount of monomeric Al compounds (Al_a) was determined by direct extraction of an untreated water sample (Driscoll, 1984). In cation exchange, positively charged compounds are retained in the ion exchange column while negative and uncharged compounds escape. Cation exchangers on Na^+ form was used (Amberlite IR-120, 10 ml ion exchange resin in the column). In order to avoid changes in the Al fractions due to changes in pH during ion exchange, the pH of the ion exchange was adjusted so that the pH did not differ by more than 0.5 pH from the pH of the water sample (Driscoll, 1984). The fluid velocity through the column was 3.8 ml/min per ml of ion exchange resin. The ion exchange resin was prepared with 60 ml of 10^{-4} M NaCl between each water sample and the 60 ml of water sample was always run through the ion exchanger before it was collected for analysis (extraction). Based on the extraction time (20 sec), aluminium is extracted from an ion-exchanged water sample defined as non-labile aluminium. In natural water, this fraction is often defined as monomeric organic aluminium (Al_o) (Driscoll, 1984). Monomeric inorganic aluminium (Al_i) is then left in the ion exchange, and can be calculated as the difference between Al_a and Al_o. Colloidal, stable organic and hydroxyl organic Al compounds are not extractable over 20 seconds. Therefore, the total concentration of aluminium (Al_r) in the samples was determined after extraction of water samples acidified with HNO_3 to pH 1.0 and at least 24 hours storage. Table 2 gives an overview of the fractions that were analysed or calculated.

Table 2. Description of various Al fractions that were measured and calculated.

Al_r:	Total aluminium, determined by extraction of water sample acidified to pH 1 with HNO_3, after at least 24 hours of storage.
Al_a:	Total monomeric aluminium, determined by extraction of untreated water sample.
Al_o:	Organic monomeric aluminium, determined by extraction of the eluate from a cation-exchanged water sample.
Al_i:	Inorganic monomeric (or labile) aluminium, calculated as the difference between Al_a and Al_o.

2.8 Fish sampling and Analysis

When sampling control fish, 10 specimens were transferred from the fish tanks to a bucket containing 7 ppm metomidate hydrochloride solution (anesthetic), followed by an extraction of a blood- and a gill sample from each fish. When sampling the saline-tested fish, all the surviving individuals were anesthetized. The gill samples were frozen for possible future aluminum alloy analyzes. The blood sample was taken from the dorsal vein, behind the anal fin, using a heparinized 2 ml disposable syringe. The blood was transferred from the syringe to 50 µl capillary tube. The capillary tubes were sealed with plastic liners at one end and centrifuged for 3 minutes in a microhematocrit centrifuge TYPE 346. After centrifugation, hematocrit was measured and plasma isolated for later plasma chloride and plasma cortisol analyzers.

2.9 Hematocrit and plasma chloride

Hematocrit was measured and calculated as the average percentage of blood cells of total blood, based on a maximum of 5 capillary tubes from each fish. Plasma chloride was analyzed on a sample from each fish using a Radiometer CMT 10 chloride titrator. The titrator has an accuracy of ± 0.5%. 20 µl of plasma was used per measurement.

2.10 Plasma cortisol

Plasma cortisol was analyzed by radioimmunoassay (RIA) (Simensen et al., 1978; Olsen et al., 1992). Liquid scintillation (Packard TriCarb 1500) was used to detect radioactivity in the samples. The mean detection limit (± s.d.) was 0.39 ± 0.37 ng/ml. The interassay coefficient of variation was 13.2% at 37.4 ng/ml and 45.3% at 1.9 ng/ml. The interassay coefficient of variation was 7.1%. The non-specific binding of the antibody was 0.6 ± 0.1% of the total activity. Samples with lower cortisol concentrations than the detection limit were given a cortisol value similar to that.

2.11 Statistical analyses

Fish survival

To explain the variation in fish survival across different water qualities, variables were analysed using a longitudinal design (Figure 9). Number of weeks (10-24) were treated as categorical variables, because I did not necessarily expect a linear relationship between the two variables. 'Station' (1-6) refers to water quality/location and were also treated as a categorical variable. As independent variables, I used 'Temperature', the much described toxic (Grande et al. 1978; Poléo et al. 1997; Reimers & Døving, 1992) 'Ali' (inorganic monomeric aluminium) and 'pH'. Additionally, I added the interaction between 'pH' and 'Ali' to the model as well as the interaction between 'Temperature' and 'Ali', as I expected an interacting relationship between the two variables, with possible effect on fish survival (Table 3). To account for possible overdispersion 'ID' (observations) were treated as a random effect. To account for unexplained variance 'station' was also nested as a random effect (Table 3). For count data and GLMMs (Generalized Linear Mixed Models), a common concern is overdispersion, underdispersion and zero-inflation (Martin et al. 2005; Richards, 2008; Bolker et al. 2009). I used the package DHARMa (Hartig, 2018) plotting residuals against predictors to derive an expression of the different models regarding these concerns. All fixed variables were scaled.

I performed all analyses in R version 3.4.3 (R Development Core Team 2017). To run the mixed models I used the package LME4 (Bates et al. 2014). I used the Akaike Information Criterion (AIC) and Delta AICc (ΔAIC) to compare final models for fish survival.

To further explain variation in fish survival across different water qualities, I ran a GLMM of a full model (Model D – Table 3) for each station (Table 4). By modelling the full model for each station I would achieve a conclusion to what predictors having an effect on survival in their respective location (Table 4). The difference in fish survival between the various water qualities were analysed using a GLM (Generalized Linear Model). The fixed variables were the categorical 'Station' and 'week'.

Water chemistry and physiological conditions

Means and standard deviations (s.d) were calculated for all water chemistry values, while means and standard errors (s.e.m) were calculated for all physiological values. To explain the variation in pH across the different water qualities, I ran a GLM across all stations (Figure 5). Explaining the variation in Al_r (total aluminium) and Al_i (inorganic monomeric aluminium) across the different stations were done similarly (Figure 7). The variation in physiological condition between the water qualities were analysed using a GLMM. The response variables in the physiological models were either 'Hematocrit', 'Plasma chloride' or 'Plasma cortisol', with the categorical 'Station' and 'week' as fixed predictors. To account for possible overdispersion 'ID' (observations) were treated as a random effect.

3. Results

3.1 Water level

During the trials, there were several periods with high water levels in Suldalslågen (Figure 3).

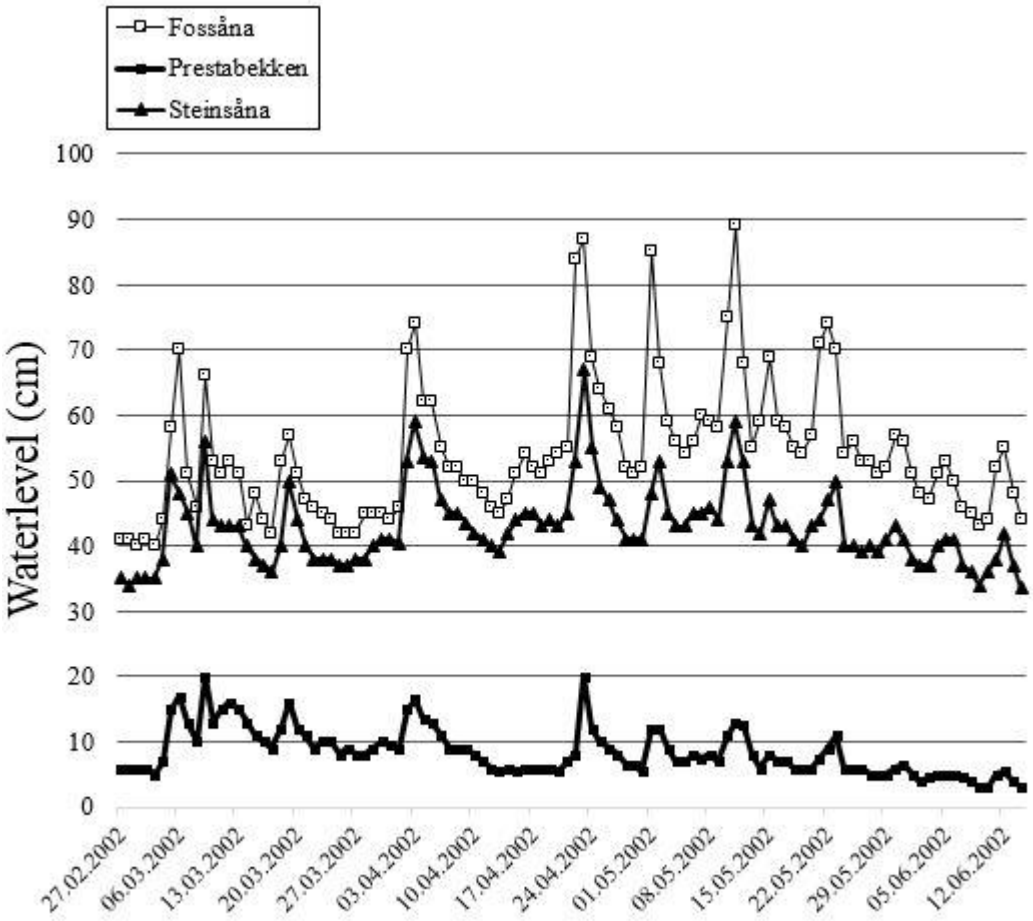

Figure 3. Water levels during the trial period in Fossåna, Prestabekken and Steinsåna.

3.2 Temperature

In the beginning of the experiments, the water temperature at the different stations were low (0.0 - 4.3°C), and Fossåna and Steinsåna were particularly cold. Throughout the experimental period the temperature rose to 8.3 – 16.2°C (Figure 4).

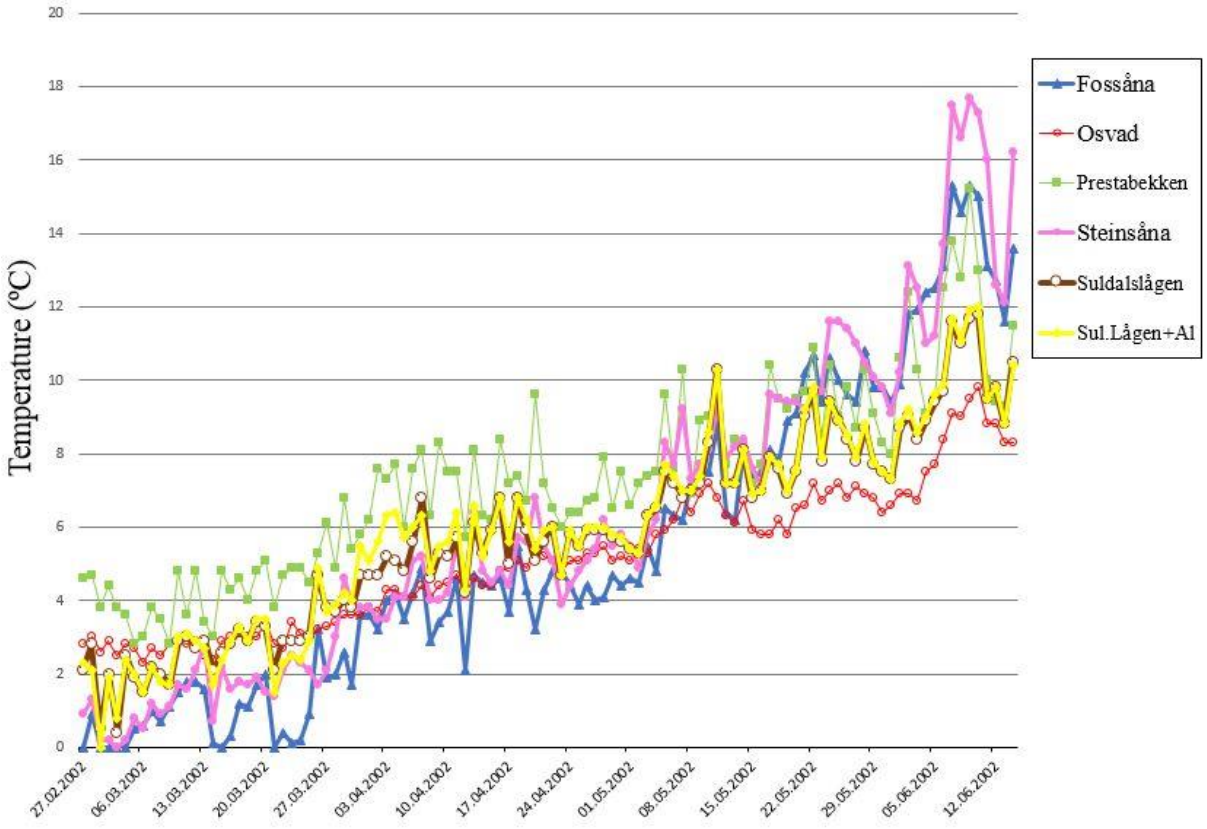

Figure 4. Water temperature at each station throughout the experimental period.

3.3 pH

In all the water qualities, there were an increase in the pH level throughout the experiments (Figure 6). In Prestabekken, Suldalsvatnet, Suldalslågen and Suldalslågen + Al, the level of pH were relative stabile within the timeframe of the experiments, showing levels of pH above 6.0 (Figure 6). Suldalslågen + Al show a considerably decrease of pH in the water quality on the 20. April, the day after the addition of aluminium. The biggest variation in pH were measured in Steinsåna and Fossåna (Figure 5). Steinsåna varied in pH between 6.2 and 6.9. Until the 12. May, the pH in Fossåna was low (5.2 – 5.8), before gradually rising from 5.8 to 6.1 (Figure 6). The result of a GLM show Fossåna to be significantly different in the variation of pH (GLM: $t_{83} = 1.68$, $p < 0.001$, AIC = -86.41, Figure 5) from all the other stations.

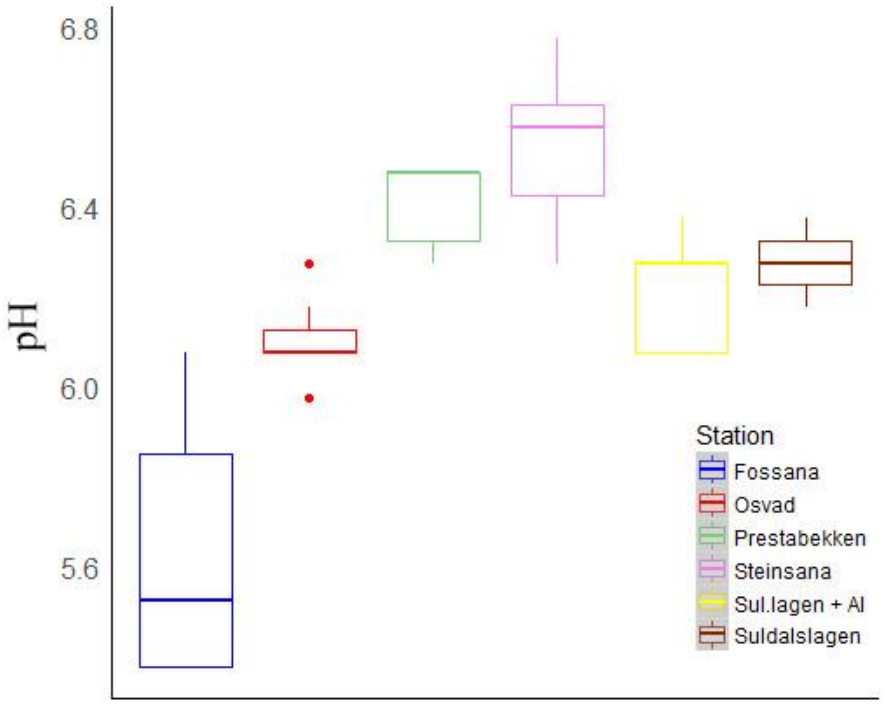

Figure 5. Result of a GLM with variation in pH level across each station, during the experimental period.

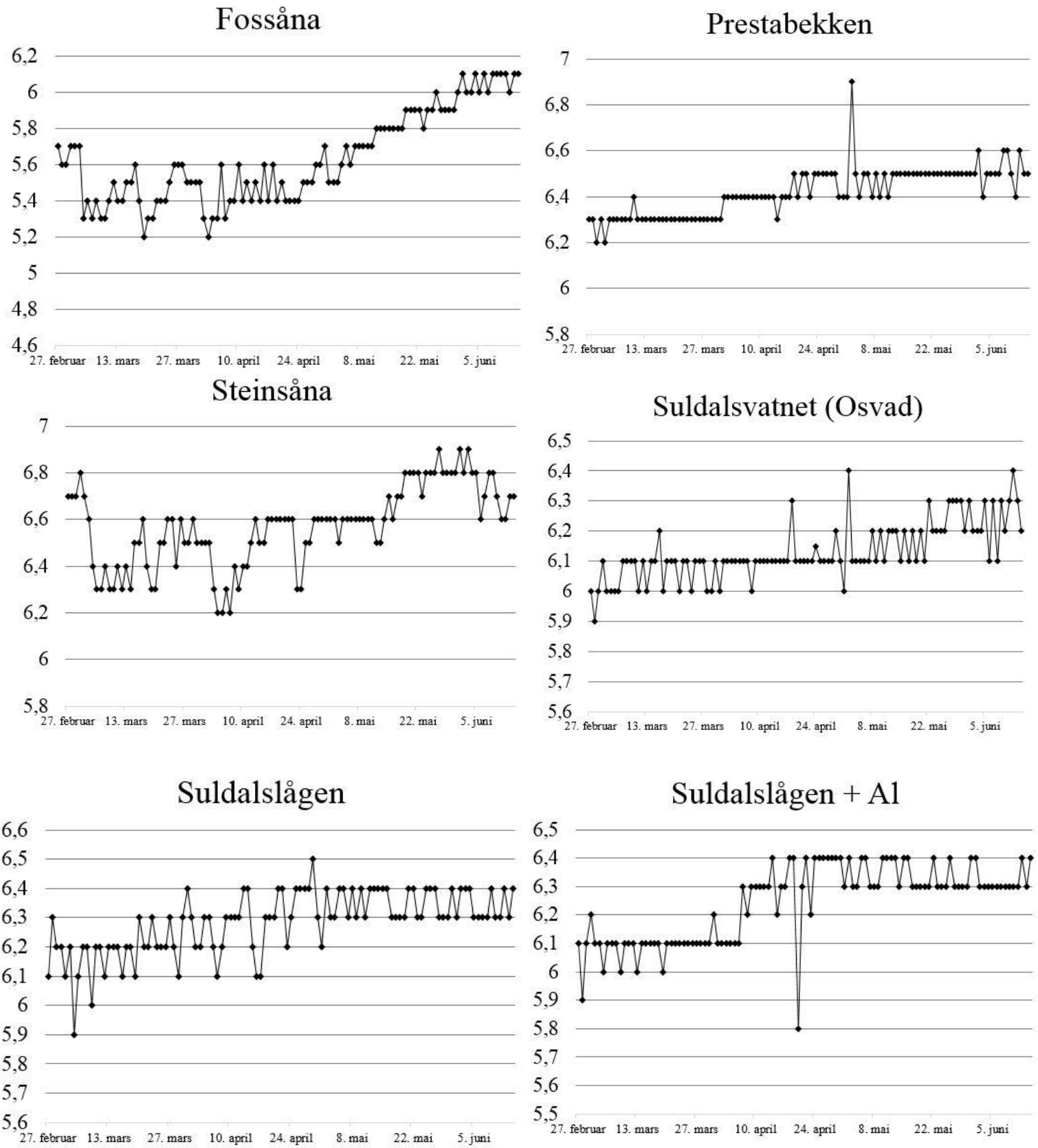

Figure 6. Variations in pH at each station throughout the experimental period.

3.4 Aluminium

There was a significant difference in variation of total aluminium (Al_r) in Fossåna from the other water qualities (GLM: t_{82} = 64099, p = 0.012, AIC = 843, Figure 7). Prior to the first week of May, Fossåna measured relative high concentrations of total aluminium (Al_r) (80 - 130 µg/l, Figure 8) with the largest variation. Compared to the other water qualities, Fossåna measured higher concentrations of inorganic monomeric aluminium (Al_i) (27 ± 13 µg/l (mean ± s.d.)) (GLM: t_{80} = 66.936, p = 0.0015, AIC = 236.5, Figure 8). In Suldalsvatnet (Osvad), Prestabekken and Suldalslågen, the variation in Al_r-concentration was between 20 - 70 µg/l, with mean concentrations roughly close to 40 µg/l (Figure 8). Mean Al_i-concentrations were just above 10 µg/l for the latter water qualities. On the 20. April when the Al-addition took place, the Al_r-concentration rose to 270 µg/l in Suldalslågen + Al (Figure 8). Additionally, Al_i-concentrations rose to 75 µg/l during the addition of aluminium.

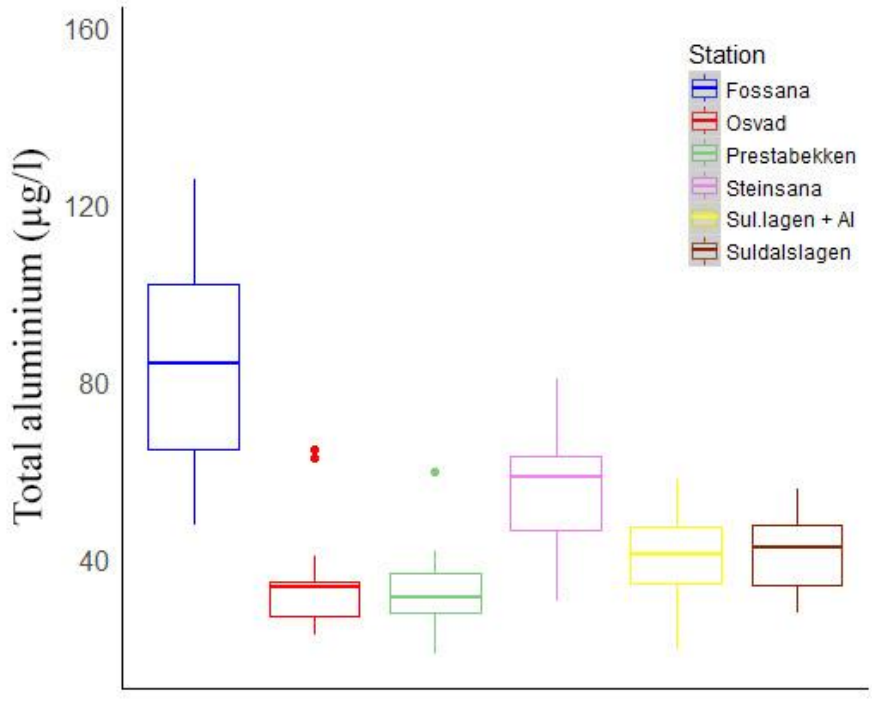

Figure 7. Result of a GLM showing variation in total aluminium (Al_r) across the different stations, throughout the experimental period.

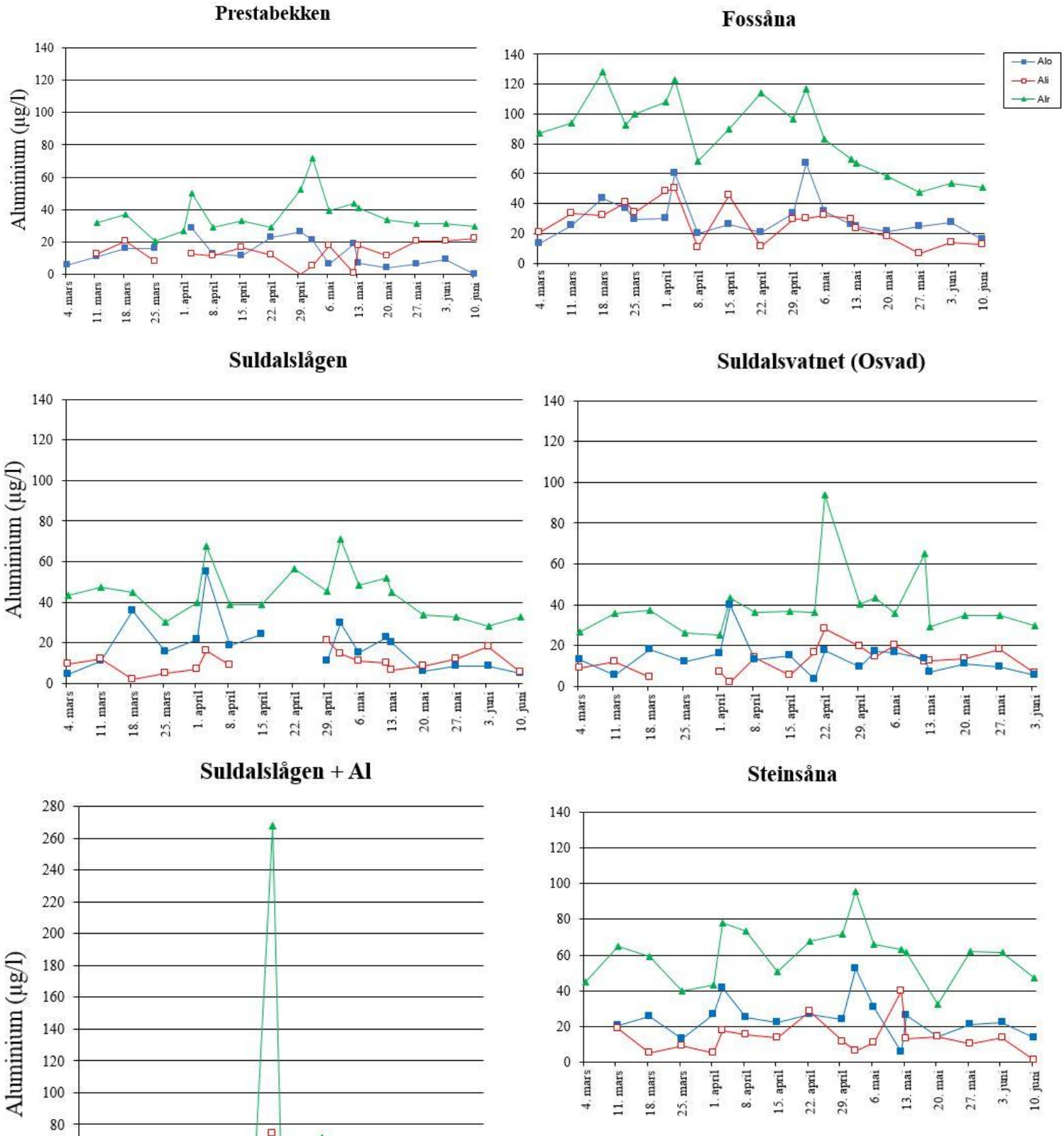

Figure 8. Aluminium concentrations of the most important Al-fractions, measured weekly in the different water qualities throughout the experimental period. Alr = total aluminium, Alo = organic monomeric aluminium, and Ali = inorganic monomeric aluminium. Breakage in line is due to unsuccessful measurements.

3.5 Seawater challenge test

3.5.1 Fish survival

There was a genereal common pattern for all the stations with pH, the interaction between pH and Al_i, and the interaction between temperature and Al_i showing a significant ($p < 0.001$) effect on variation in fish survival (Table 3, Figure 9). Additionally, week 19-24 were also showing a significant ($p < 0.001$) effect. Al_i and temperature as independent variables had no significant effect on the variation in fish survival. As station were treated as a categorical random variable the test only tells us that there is a difference in stations, but it gives no indications as to which station that differs from each other (For better vizualisation; see figure 11-16). All stations or water qualities experienced a decline in Atlantic salmon survival through the experimental period (Figure 9). For model selection, lowest AIC and Delta AICc (ΔAIC) is used in addition to models accounting for overdispersion and unexplained variance. Model D and E (Table 3) are very similar in AIC and vary little, both accounting for overdispersion (ID nested as random predictor). ΔAIC suggest substantial evidence for Model D. Model A and B has left out the accounting for overdisperion and are therefore not as suitable to make inference (eventhough Model B show ΔAIC = 1.22). Model A and B are essentially the same as C and D, with the absence of accounting for overdisperion (Table 3).

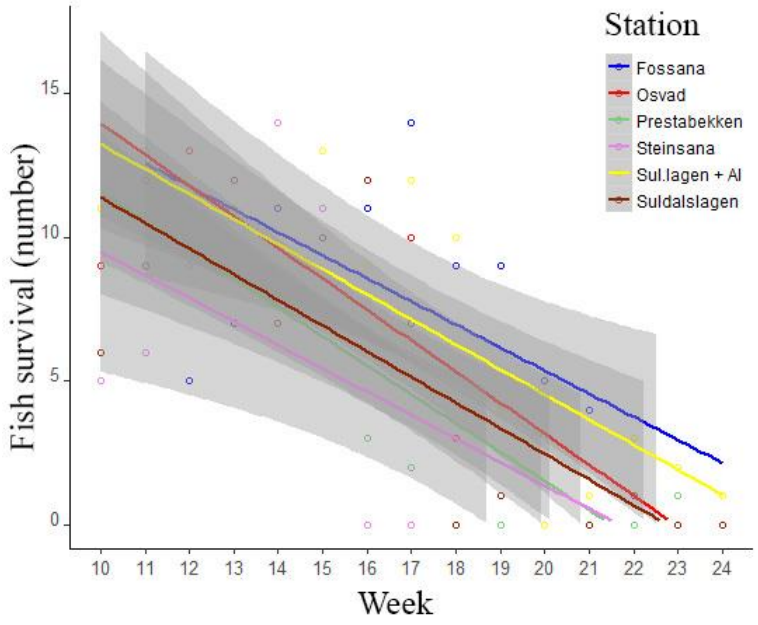

Figure 9. Relationship between time (week) and observed fish survival at each water quality (station). Grey areas show confidence intervals of each linear fit.

Table 3. List of test results for all models relating fish survival with random and fixed variables. Variables in '(1 |)' are random predictors and variables outside are fixed. An interaction between predictors is labelled with ':'.

Model		AIC	ΔAIC
A	**Response:** Fish survival (number) ~ (1 | station) + week + Ali + pH + Temp + week : Temp + Ali : pH + Temp : Ali	397.8	53.32
B	**Response:** Fish survival (number) ~ (1 | station) + week + Ali + pH + Temp + Ali : pH + Temp : Ali	382.5	1.22
C	**Response:** Fish survival (number) ~ (1 | station / ID) + week + Ali + pH + Temp + week : Temp + Ali : pH + Temp : Ali	399.8	59.39
D	**Response:** Fish survival (number) ~ (1 | station / ID) + week + Ali + pH + Temp + Ali : pH + Temp : Ali	384.3	4.71
E	**Response:** Fish survival (number) ~ (1 | station / ID) + week + pH + Temp + Ali : pH + Temp : Ali	382.8	0.00

All models are mixed models from the LME4 package in R. Stepwise reduction of models are presented. Model selection is based on lowest AIC and Delta AICc (ΔAIC). ΔAIC < 2 suggests substantial evidence for the model whereas values between 3 and 7 indicate that the model has considerably less support.

Predictors explaining survival at each station

The output from the mixed model show temperature to be the only significant ($p < 0.01$) predictor explaining variation in fish survival in both Suldalsvatnet and Suldalslågen + Al (Table 4, Figure 10). Fish survival decreased with the increasing temperature. In Fossåna, Al_i, pH and the interaction between Al_i and pH were significant ($p < 0.01$) in explaining variation in fish survival (Table 4, Figure 10). Survival of Atlantic salmon in Fossåna increased with increasing Al_i (Figure 10).

Table 4. List of summary results of a GLMM relating predictors and their significance level to fish survival at each station.

Station	Predictor	Estimate	SE	Z-value	p		AIC
Suldalsvatnet (Osvad)	Ali	-2.4263	1.7305	-1.402	0.160		
	pH	-5.9292	5.7819	-1.026	0.305		
	Temp	-2.1130	0.7666	-2.756	0.005	**	85.3
	Ali:pH	-8.8057	11.212	-0.785	0.432		
	Ali:Temp	-1.4229	1.3435	-1.059	0.289		
Fossåna	Ali	1.8623	0.6839	2.723	0.006	**	
	pH	-3.7715	0.9485	-3.976	< 0.001	***	
	Temp	0.3675	0.2671	1.376	0.168		68.8
	Ali:pH	1.5329	0.5708	2.686	0.007	**	
	Ali:Temp	0.03997	0.58933	0.068	0.946		
Steinsåna	Ali	-1.7028	6.4578	-0.264	0.792		
	pH	-7.76395	4.51234	-1.721	0.085	.	
	Temp	-0.8210	1.28515	-0.639	0.522		54.2
	Ali:pH	-0.04773	14.3994	-0.003	0.997		
	Ali:Temp	0.08115	2.50424	0.032	0.974		
Prestabekken	Ali	-1.3324	1.6319	-0.816	0.414		
	pH	6.3566	4.0654	-1.564	0.117		
	Temp	-0.2795	0.7132	-0.392	0.695		63.5
	Ali:pH	5.9114	7.2828	0.812	0.416		
	Ali:Temp	-1.0213	1.5396	-0.663	0.507		
Suldalslågen	Ali	0.2421	2.79870	0.086	0.931		
	pH	-5.4706	11.3951	-0.480	0.631		
	Temp	-1.3245	1.85476	-0.714	0.475		63.1
	Ali:pH	-23.286	22.0677	-1.055	0.291		
	Ali:Temp	1.93036	3.11837	0.619	0.536		
Suldalslågen + Al	Ali	-0.08842	0.22159	-0.399	0.689		
	pH	1.39092	1.34606	1.033	0.301		
	Temp	-0.80759	0.30705	-2.630	0.008	**	87.9
	Ali:pH	0.96534	1.93991	0.498	0.618		
	Ali:Temp	0.37620	0.50445	0.746	0.455		

*Significant codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1*

Survival in Atlantic salmon in Fossåna, Prestabekken and Suldalslågen + Al all show a tendency to increase with the higher amounts of Al_i present (Figure 10a). Fossåna was the only water quality showing a statistical significant relationship (Table 4). The remaining three water qualities show a negative tendency (insignificant) with increasing Al_i (Table 4, Figure 10a). All water qualities show a tendency to a negative linear relationship between fish survival and pH (Table 4, Figure 10b). Fossåna was the only water quality regarding a statistical significant effect of pH (Table 4). All water qualities show a tendency to decrease in fish survival with increasing temperature, giving Suldalsvatnet and Suldalslågen + Al as the only two stations with a statistical significant effect of temperature (Table 4, Figure 10c).

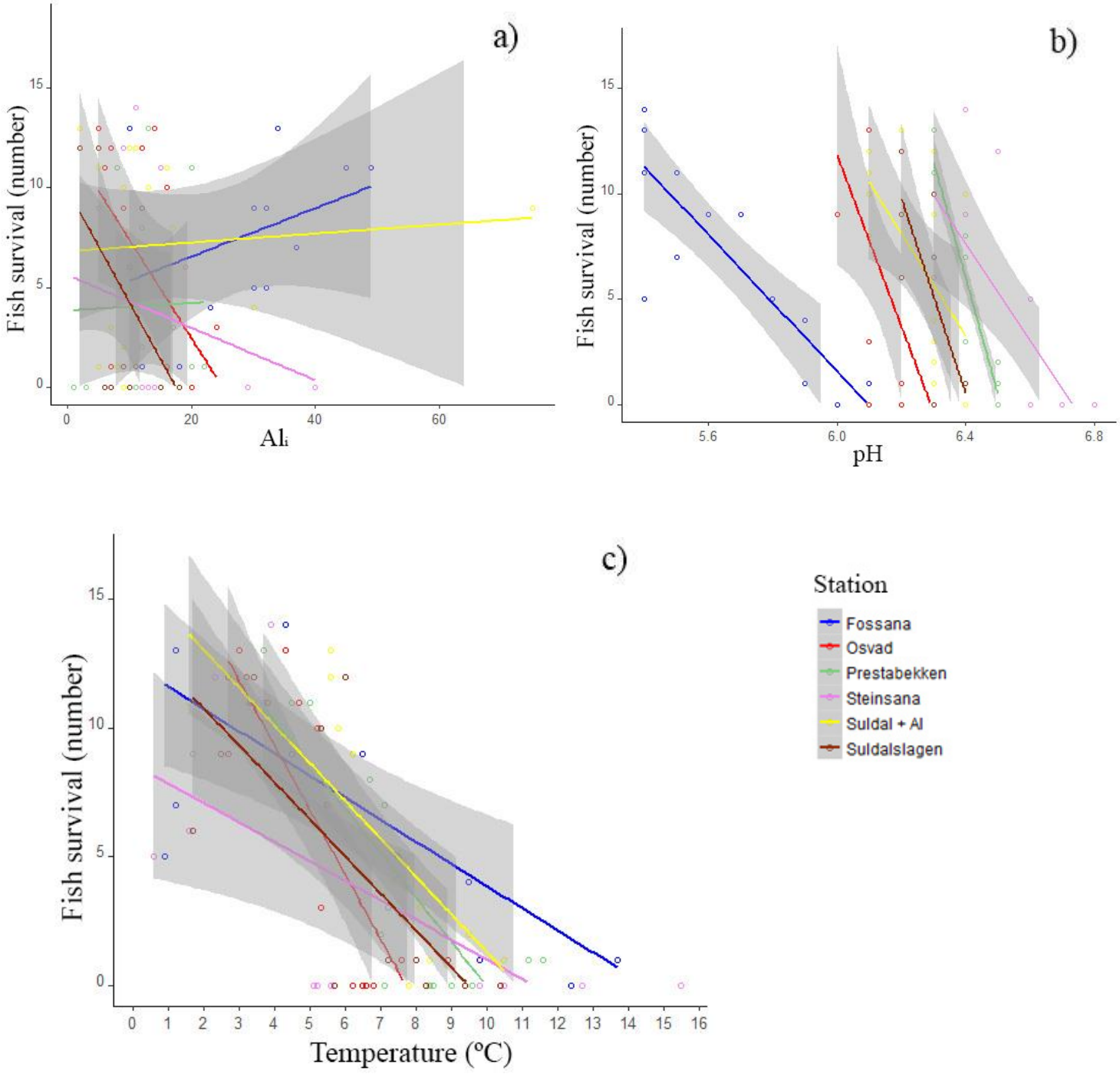

Figure 10. Relationship between observed survival in Atlantic salmon and Al_i (a), pH (b) and Temperature (c) at different water qualities (stations) during the experiments. Grey areas show confidence intervals of each linear fit.

Difference between stations

Fossåna is significantly different, with a longer period of survival than Prestabekken ($p < 0.01$), Steinsåna ($p < 0.001$) and Suldalslågen ($p < 0.05$) ($t_{69} = 527.39$, AIC = 452.93, Figure 9). Additionally, showing week 18-24 to be the period of difference ($p < 0.01$). Suldalsvatnet as the third most successful water quality (Figure 11) is also significantly different, with a longer period of survival than Prestabekken ($p < 0.01$), Steinsåna ($p < 0.01$) and Suldalslågen ($p < 0.05$) ($t_{55} = 485.44$, AIC = 389.2, Figure 9), with week 19-24 being the period of difference ($p < 0.001$). The water quality Suldalslågen + Al differs in survival in Atlantic salmon with a longer period of survival than both Prestabekken and Steinsåna, in week 16 ($p < 0.05$) and week 18-24 ($p < 0.01$) ($t_{28} = 215.96$, AIC = 234.28, Figure 9).

For all the water qualities, the survival in Atlantic salmon were highest in the beginning of the experimental period. In mid-April (week 15), there was an increase in mortality, followed by low survival at the end of the period (Figure 11).

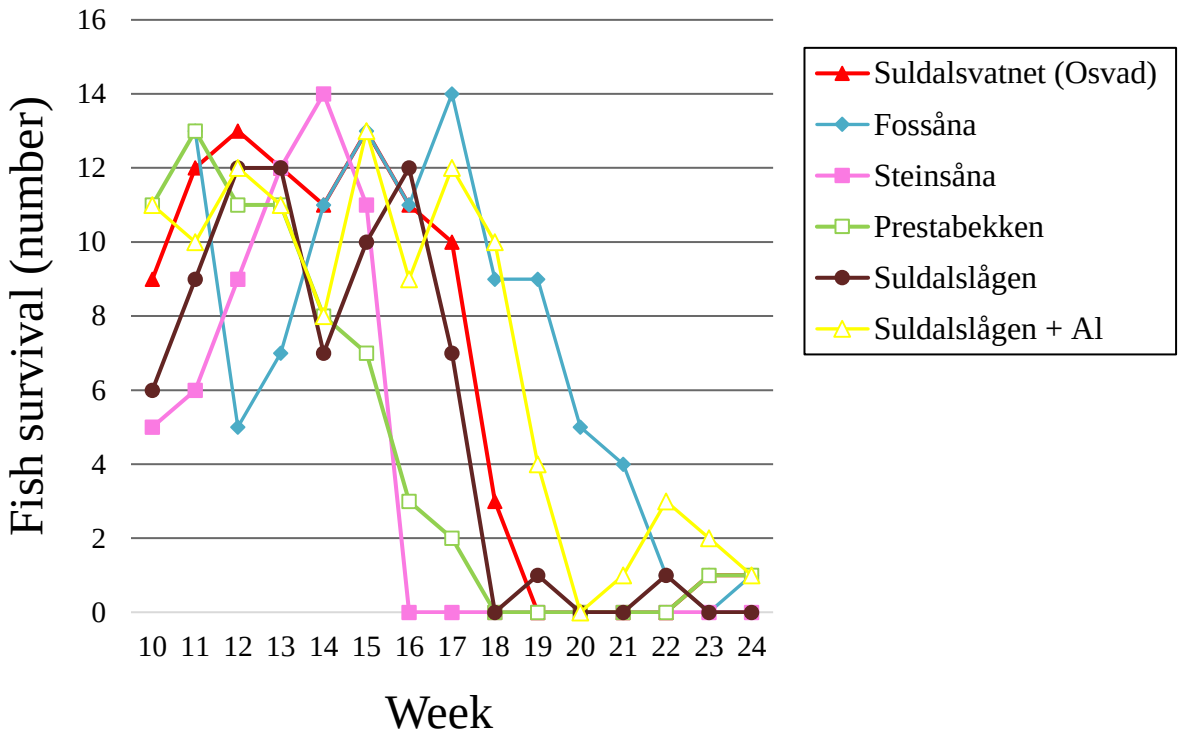

Figure 11. Weekly survival in Atlantic salmon exposed to different water qualities in seawater challenge tests.

Considering survival in all the water qualities could be somewhat difficult to interpret, and by looking at the result in a different context could clarify things a bit more. Visualizing fish survival in Fossåna and Steinsåna, presumably the worst and best water quality, show a longer period of a relative good survival in Fossåna compared to Steinsåna (Figure 12). After week 16, there were no surviving fish exposed to water from Steinsåna. In Fossåna, the only occasion with 100% mortality was June 5. The mixed model (Table 4) showed survival in Fossåna to be explained by the amount of inorganic monomeric aluminium (Al_i) and pH present, and the interaction between the two.

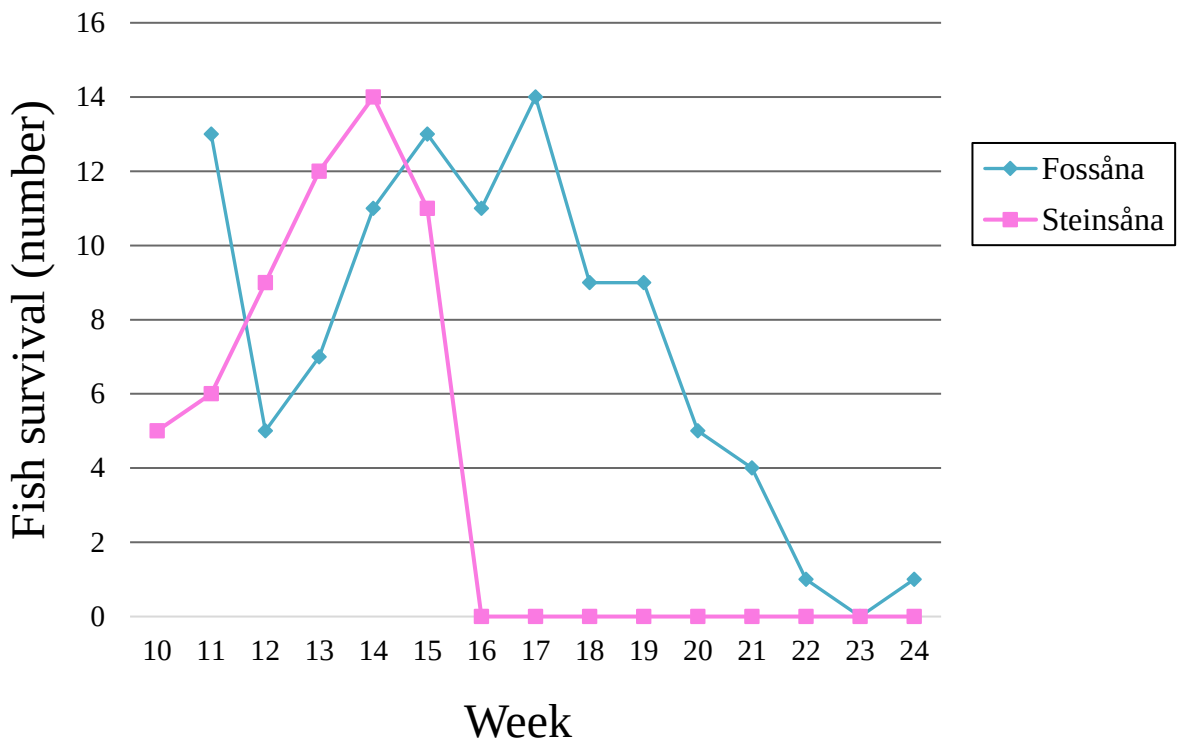

Figure 12. Weekly survival in Atlantic salmon, exposed to Fossåna and Steinsåna in the seawater challenge tests (n=15).

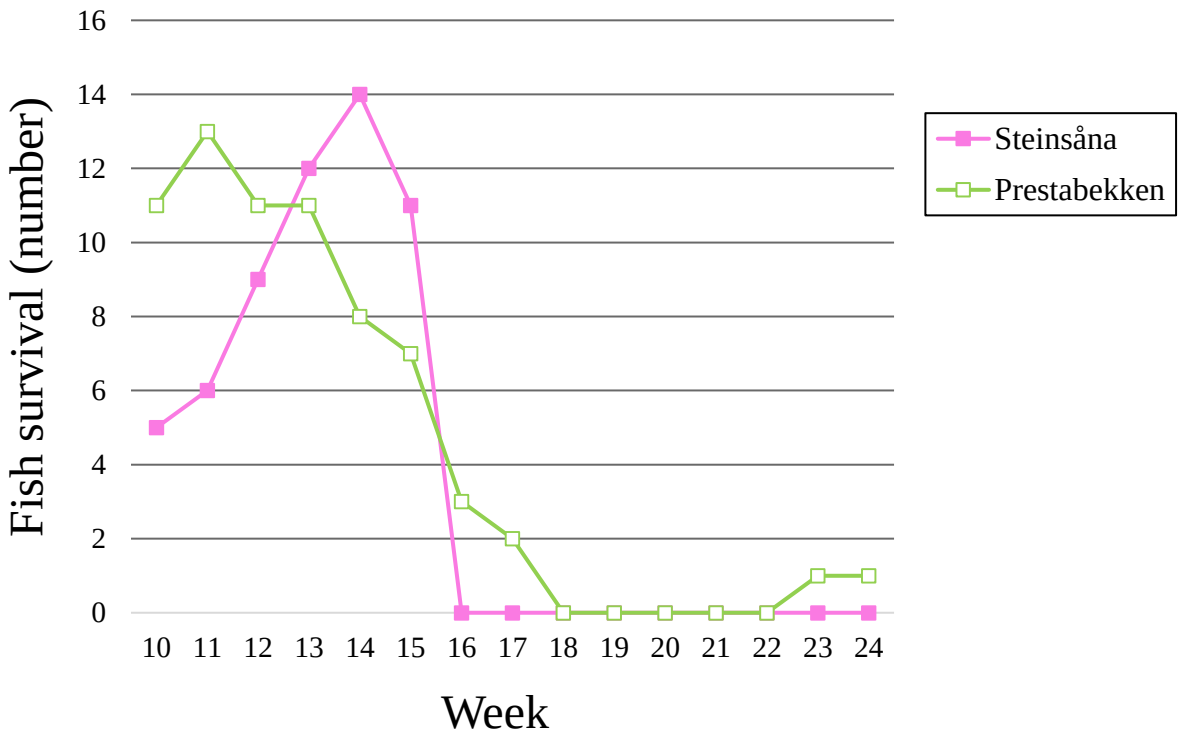

Figure 13. Weekly survival in Atlantic salmon, exposed to Prestabekken and Steinsåna in the seawater challenge tests (n=15).

Fish exposed to water with high levels of pH, Steinsåna (mean=6.6) and Prestabekken (mean=6.4) show a similar survival through the seawater challenge tests (Figure 13). In the beginning of the period there was a difference in fish exposed to water from Prestabekken, showing higher survival rate before gradually decreasing with no survivors on the 30. April (week 18). Only two individuals survived water from Prestabekken in June. Fish exposed to water from Steinsåna showed low survival in the beginning of the period, followed by an increase to almost 100% survival on April 3. After peaking, there were no survivors in week 16 (Figure 13).

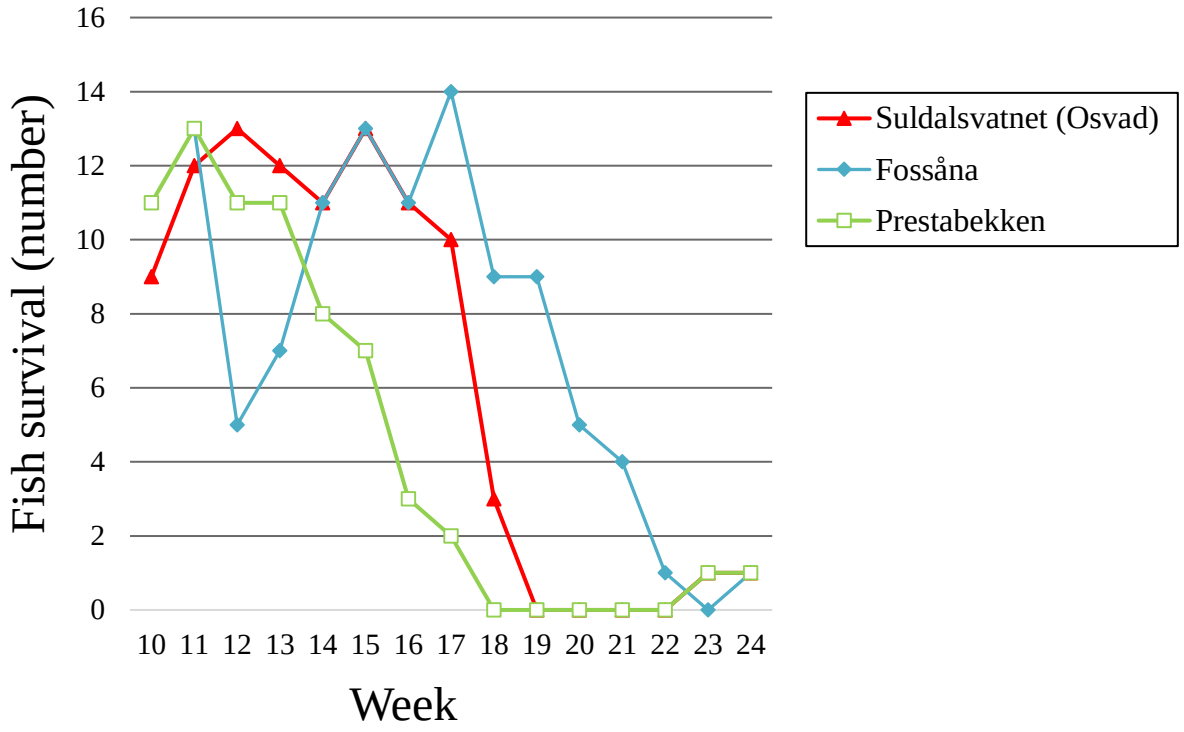

Figure 14. Weekly survival in Atlantic salmon, exposed to Suldalsvatnet, Fossåna and Steinsåna in the seawater challenge tests (n=15).

Comparing the water qualities with no liming, the survival in Suldalsvatnet, Fossåna and Prestabekken varies (Figure 14). With the exception of a short period in the beginning of the tests, Fossåna exposed fish showed the longer period with higher survivals. Suldalsvatnet and Prestabekken were similar in survival response, with Prestabekken having the shorter period of survival.

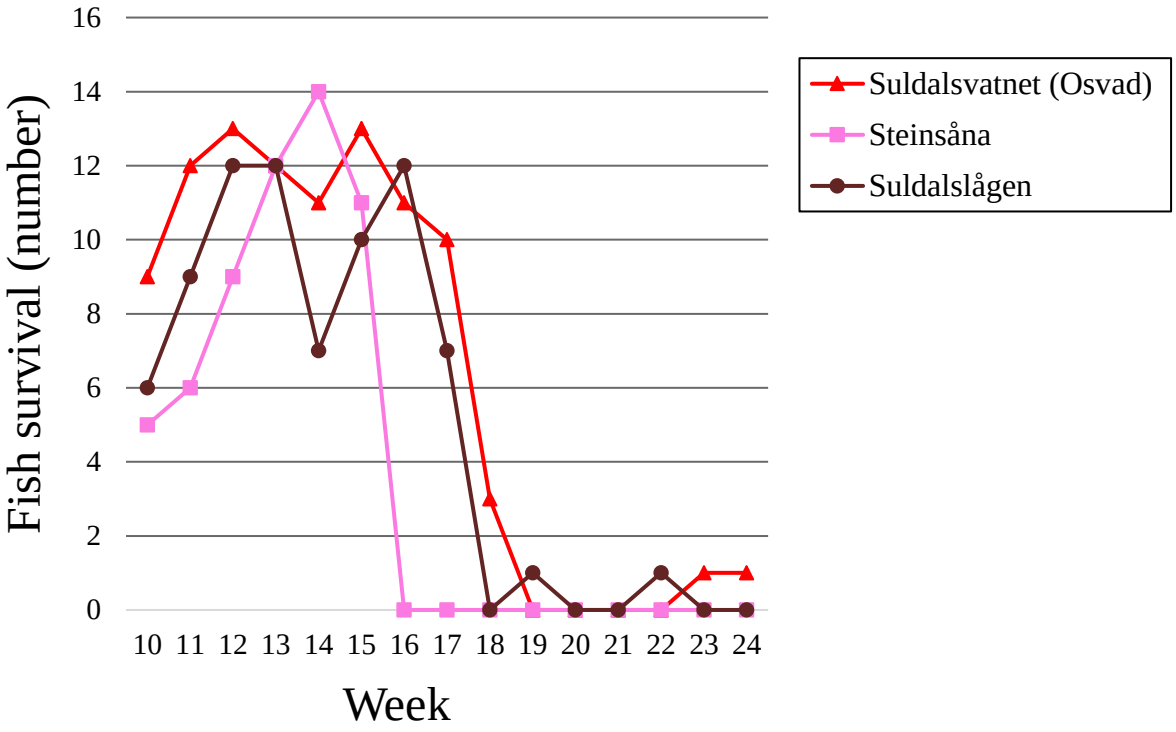

Figure 15. Weekly survival in Atlantic salmon, exposed to Suldalsvatnet, Steinsåna and Suldalslågen in the seawater challenge tests (n=15).

Comparing water qualities by liming gradient also show a difference in survival (Figure 15). Suldalsvatnet (no liming) and Suldalslågen (moderately limed) are similar in survival response through the seawater challenge tests. The results indicates that Steinsåna (heavily limed) exposed salmon experienced a substantial shorter period of survival than the latter two (Figure 15).

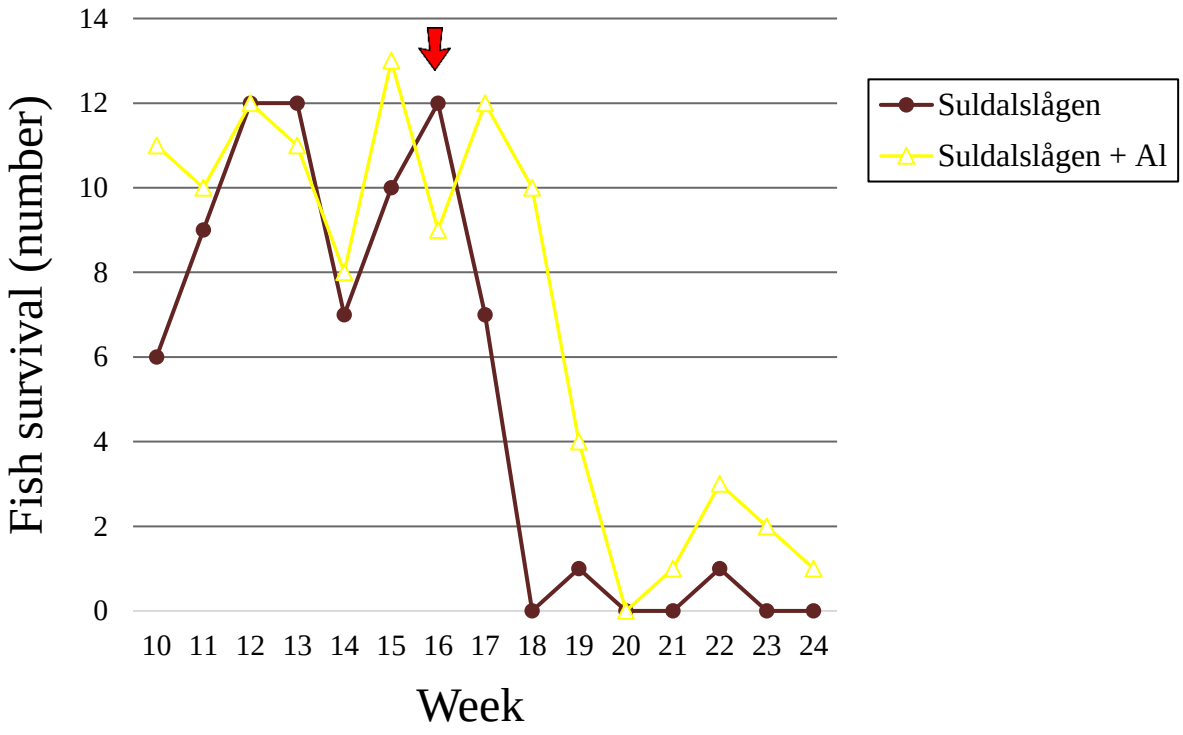

Figure 16. Weekly survival in Atlantic salmon, exposed to Suldalslågen and Suldalslågen + Al in the seawater challenge tests (n=15). Red arrow indicates time of the aluminium addition.

Fish exposed to water from Suldalslågen with and without aluminium show a very similar survival response (Figure 16). The results show little difference in the first half of the period, where the water qualities are identical. At the time of the heavy addition of aluminium, the survival of the fish in the +Al group were higher (Figure 16). The results indicates that salmon exposed to aluminium experience higher seawater tolerance.

Throughout the experimental period, all water qualities experienced observed mortality of fish in the seawater challenge tests (Figure 17). Highest survival was observed in Fossåna (49%) and Suldalslågen + Al (47%), while fish exposed to Steinsåna (presumed best water quality) showed nearly half the survival (25%). Third highest survival observed was in Suldalsvatnet (43%), and in Prestabekken the survival was 30%. In Suldalslågen the overall survival was 34%. Comparing survival in Suldalslågen with Suldalslågen + Al, show little difference prior to the addition of aluminium (68 and 74%). Following the addition of aluminium, only 9% of the fish exposed to Suldalslågen water survived, while 32% survived in Suldalslågen + Al.

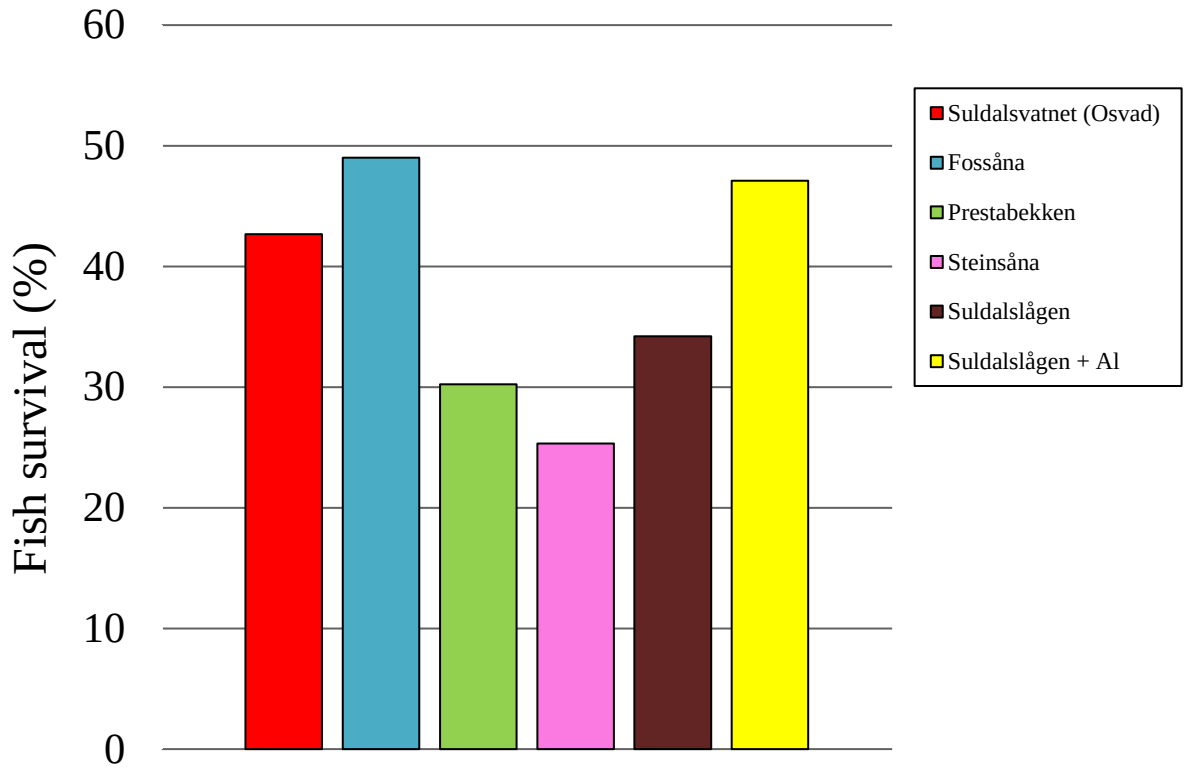

Figure 17. Total survival (%) in Atlantic salmon in seawater challenge tests, during the experimental period.

3.5.2 Physiological response

Control fish

The results of the GLMMs show Fossåna to be significantly different from all the other water qualities with higher hematocrit and plasma chloride levels through time (week) ($p < 0.001$, Figure 18). Prestabekken and Steinsåna also differ in plasma chloride ($p < 0.01$, Figure 18), with Steinsåna being the more elevated. Plasma cortisol levels in Fossåna exposed fish were slightly higher than fish exposed to water from Suldalsvatnet ($p = 0.016$, Figure 18). In addition, observed plasma cortisol levels in Suldalsvatnet exposed fish varied from Suldalslågen ($p < 0.01$) and slightly from Prestabekken and Suldalslågen + Al ($p < 0.05$), showing lower levels in Suldalsvatnet (Figure 18).

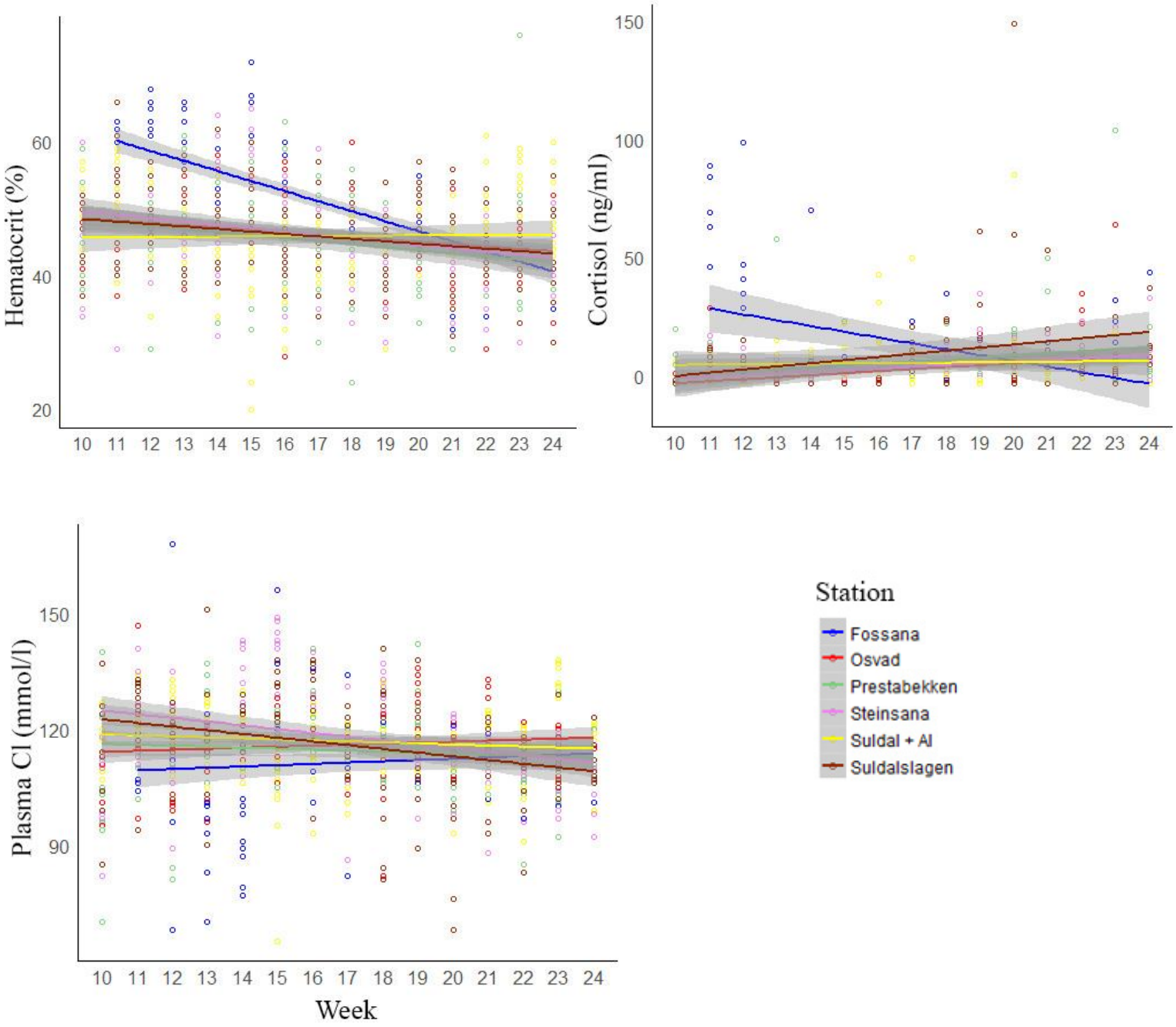

Figure 18. Physiological response in Atlantic salmon (control group) when exposed to different water qualities, through time (week). Grey areas show confidence intervals of each linear fit.

Seawater challenge test

Fish exposed to Fossåna showed a difference in variation of hematocrit levels (higher) through time, compared to all the other water qualities (GLMM: $p < 0.001$, Figure 19), assuming still presence of survivors (until first half of May; Figure 20-25). When comparing plasma chloride, Fossåna exposed fish varied from Steinsåna and Prestabekken with higher levels ($p < 0.01$) and slightly from Suldalsvatnet, Suldalslågen and Suldalslågen + Al ($p < 0.05$). Plasma cortisol in Fossåna exposed fish were significantly higher than in Prestabekken ($p < 0.001$). Suldalsvatnet exposed fish were barely higher in plasma cortisol than in Prestabekken ($p = 0.03$), Prestabekken levels were lower than in Steinsåna and Suldalslågen ($p < 0.01$) and Steinsåna exposed fish showed slightly higher ($p = 0.03$) levels of plasma cortisol than in Suldalslågen (Figure 19).

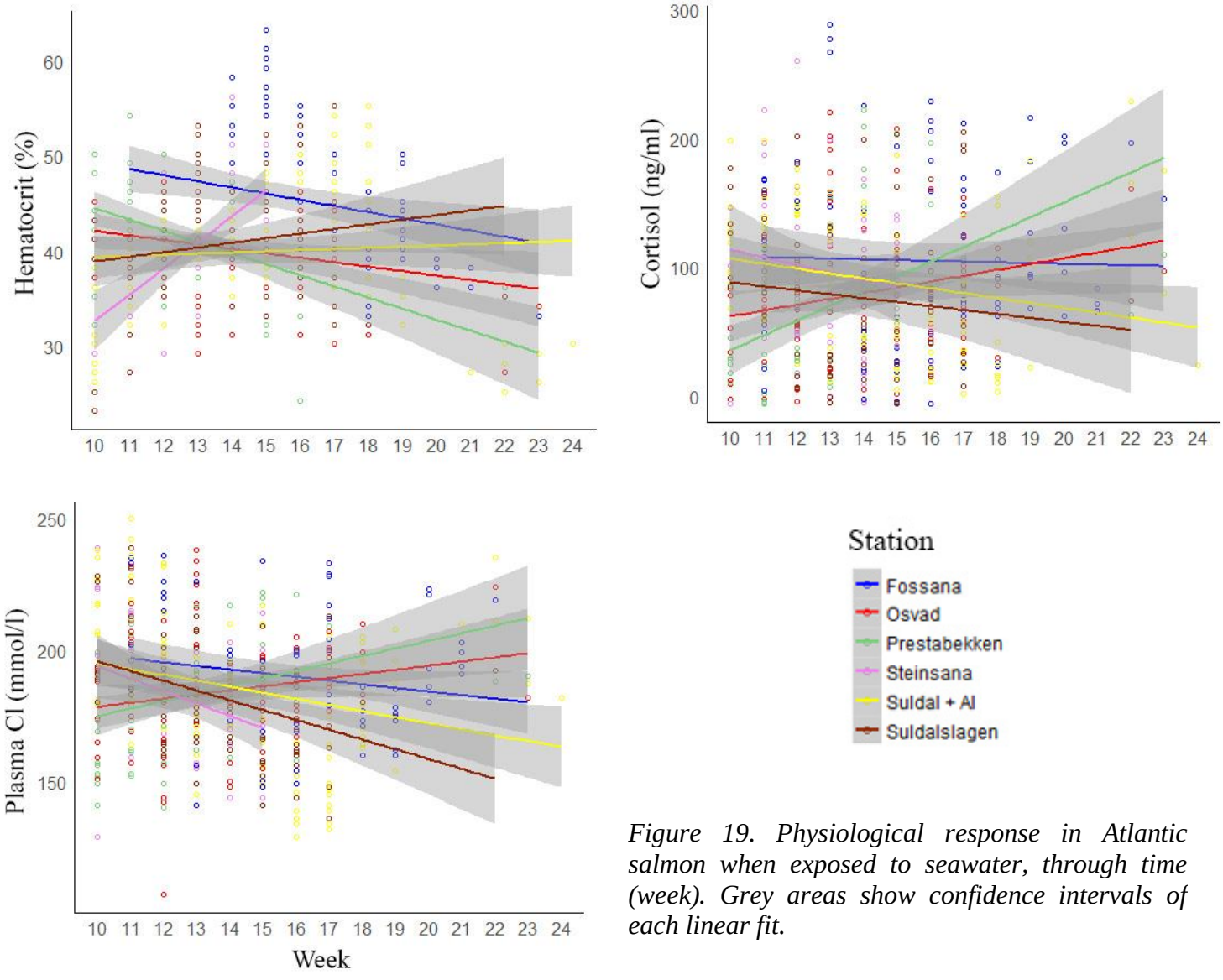

Figure 19. Physiological response in Atlantic salmon when exposed to seawater, through time (week). Grey areas show confidence intervals of each linear fit.

Fossåna

Plasma chloride levels in fish exposed to Fossåna were mostly between 111 ± 7 and 131 ± 8 mmol/l (n=10) (white) throughout the experimental period, with the exception of 28. Mars and 4. April where measurements were low (100 ± 8 and 92 ± 6 mmol/l (n=10)) (Figure 20). Plasma chloride levels in surviving fish from seawater exposure (black) were elevated during the period (between 176 ± 6 (n=9) and 227 ± 6 mmol/l (n=5)), peaking in Mars and April (Figure 20).

The level of hematocrit in the blood cells was clearly elevated in the beginning of the period (between 53 ± 4 and 64 ± 4%, n=10) until 10. April, followed by a gradual descent towards approximately 45% throughout the remains of the period (Figure 20). The surviving fish in the seawater challenge test (black) showed relatively normal levels of hematocrit in the first three weeks, approximately 45% (Figure 20). Between 4. – 28. April there was a notable elevation (between 48 ± 4 (n=14) and 57 ± 4% (n=13)), followed by a decrease that varied between 31 (n=1) and 44 ± 4% (n=9) in the end.

During the two first weeks post insertion date, the plasma cortisol levels in the blood cells were highly elevated, respectively 73 ± 8 and 53 ± 11 ng/ml (n=10), which indicates fish experiencing stress (white). After this mentioned elevation, the plasma cortisol concentrations decreased and varied between 0 and 18 ± 7 ng/ml (n=10) for the remaining period (Figure 20). Surviving fish from the seawater challenge test (black) experienced an elevation with high variations in plasma cortisol concentrations (between 58 ± 14 (n=13) and 208 ± 17 ng/ml (n=7)). Like in Suldalsvatnet and Prestabekken, the plasma cortisol levels in Fossåna fish rose in the beginning of the period, but from a notable higher level (>100ng/ml), reaching 200 ng/ml in Mars. Following this peak, the mean concentration decreased to 76 ± 16 ng/ml (n=11) in the beginning of April, persisting for a few weeks before varying between 80 and 140 ng/ml throughout the rest of April and May (Figure 20).

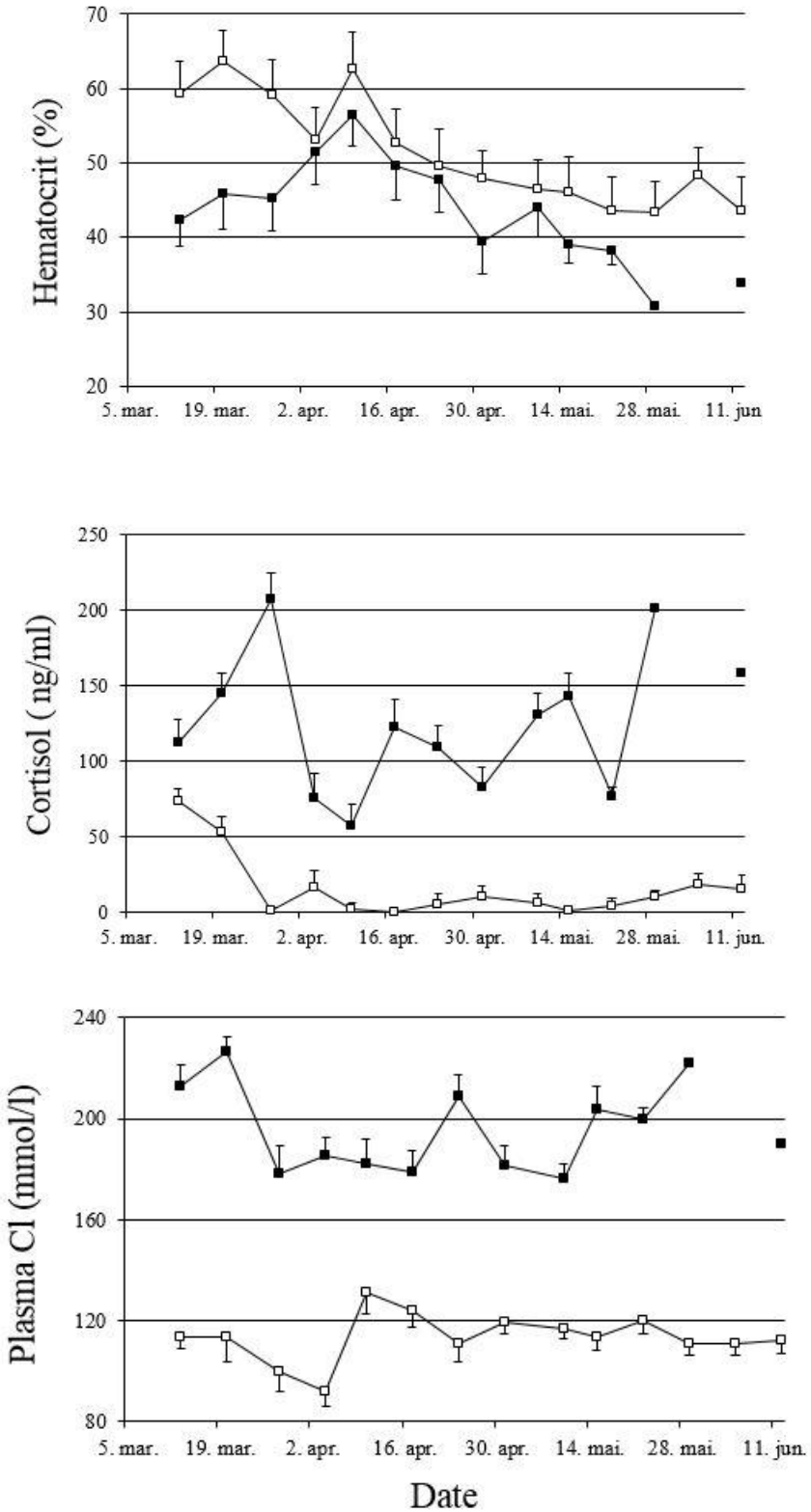

Figure 20. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Fossåna (white) (n=10), and Fossåna exposed salmon after 24 hours in the seawater challenge test (black) (n between 1 and 14, breakage in line due to 100% mortality).

Suldalsvatnet

Plasma chloride levels were between 110 ± 6 and 129 ± 5 mmol/l (mean ± s.e.m., n=10) throughout the experiments in fish exposed to from Suldalsvatnet at Osvad (Figure 21). Surviving fish from the seawater exposure experienced elevated levels of plasma chloride, varying between 166 ± 10 (n=13) and 227 mmol/l (n=1). Highest and lowest notation were both observed in Mars.

The level of hematocrit in the blood cells was relatively stable in the experiments. In the first half of the period, the levels of hematocrit were somewhat higher (between 47 ± 5 and 51 ± 5%, n=10) than in the second half (between 43 ± 5 and 47 ± 4%, n=10) (Figure 21). Surviving fish from seawater tests, exposed to water from Suldalsvatnet showed normal levels of hematocrit, roughly 40%.

Plasma cortisol concentrations varied between 0 and 24 ± 7 ng/ml (n=10). From second half of April the concentrations of cortisol in the blood cells rose to its peak on the 28. May (Figure 21). The seawater tested fish experienced high levels of plasma cortisol through the period (between 38 ± 12, (n=9) and 167 ng/ml (n=1)), with a high variation between weeks (Figure 21).

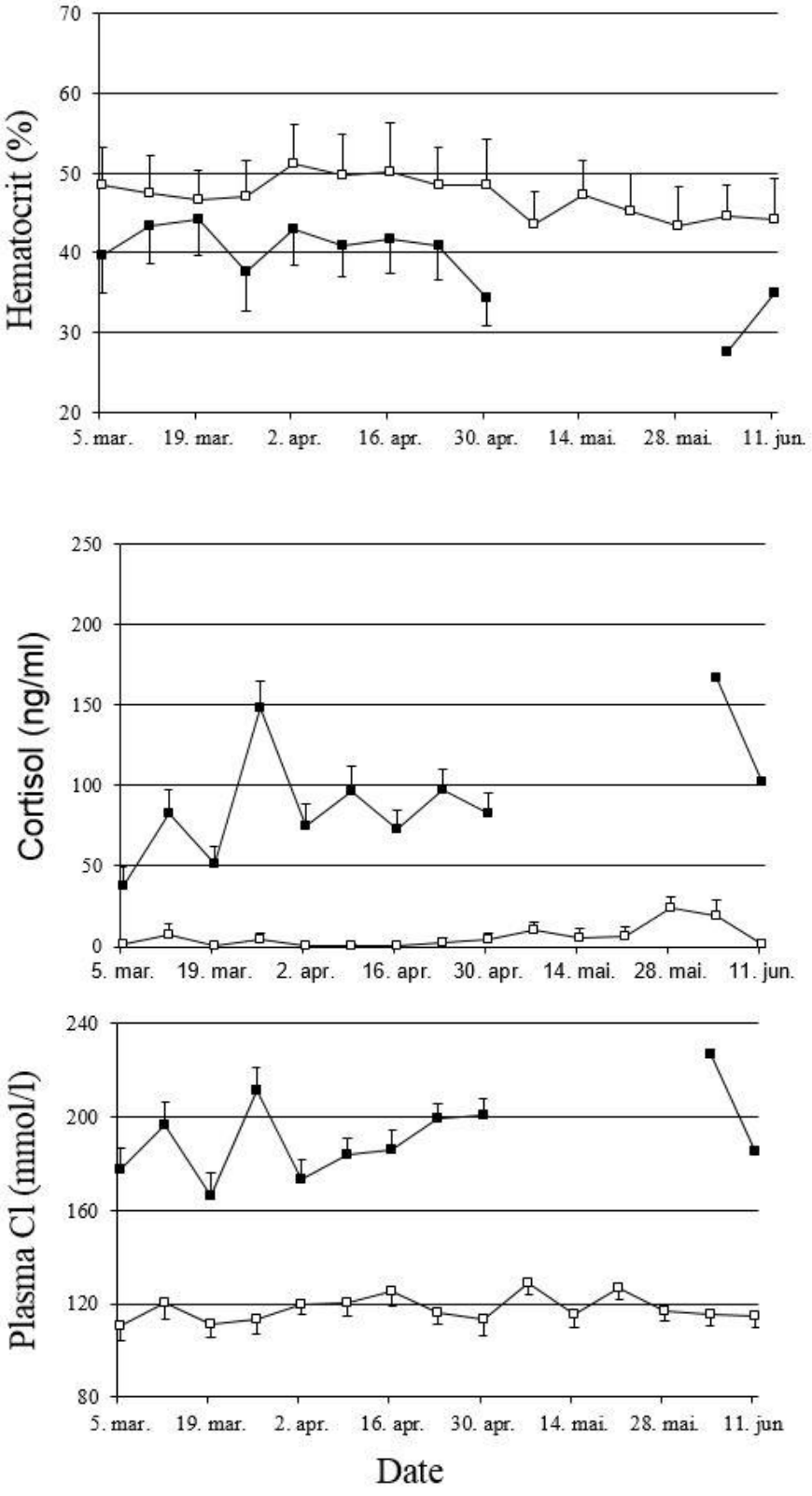

Figure 21. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Suldalsvatnet (white) (n=10), and Suldalsvatnet exposed salmon after 24 hours in the seawater challenge test (black) (n between 1 and 13, breakage in line due to 100% mortality).

Prestabekken

Plasma chloride levels were between 109 ± 9 and 128 ± 6 mmol/l (n=10) for fish exposed to water from Prestabekken, during the experiments (Figure 22). For seawater tested individuals the levels were elevated and varied between 170 ± 9 (n=11) and 205 ± 7 mmol/l (n=2). Highest and lowest notations were observed on 9. April and 5. Mars, respectively (Figure 22).

The level of hematocrit in the blood cells was relatively stable, with the first half of the period showing somewhat higher measurements (between 45 ± 6 and 54 ± 5%, n=10), than the second half of the period (between 40 ± 5 and 48 ± 7%, n=10) (Figure 22). Prior to 9. April, the surviving seawater tested fish showed normal hematocrit levels between 39 ± 5 (n=7) and 45 ± 5% (n=11). Later, hematocrit levels were fairly low in the surviving fish at Prestabekken with 30% (n=1) and 37% (n=1) (Figure 22).

Plasma cortisol concentrations in the blood cells were stable during the experiments 1 ± 1 and 25 ± 9 ng/ml (n=10), with a slight tendency of an increase in the end (>20 ng/ml) (Figure 22). Surviving seawater tested fish at Prestabekken showed considerable increase in plasma cortisol from the beginning of the experiments (20 ± 8 ng/ml, n=11) until peaking in the end of Mars (164 ± 16 ng/ml, n=8). In April, the cortisol levels varied between 100 and 150 ng/ml.

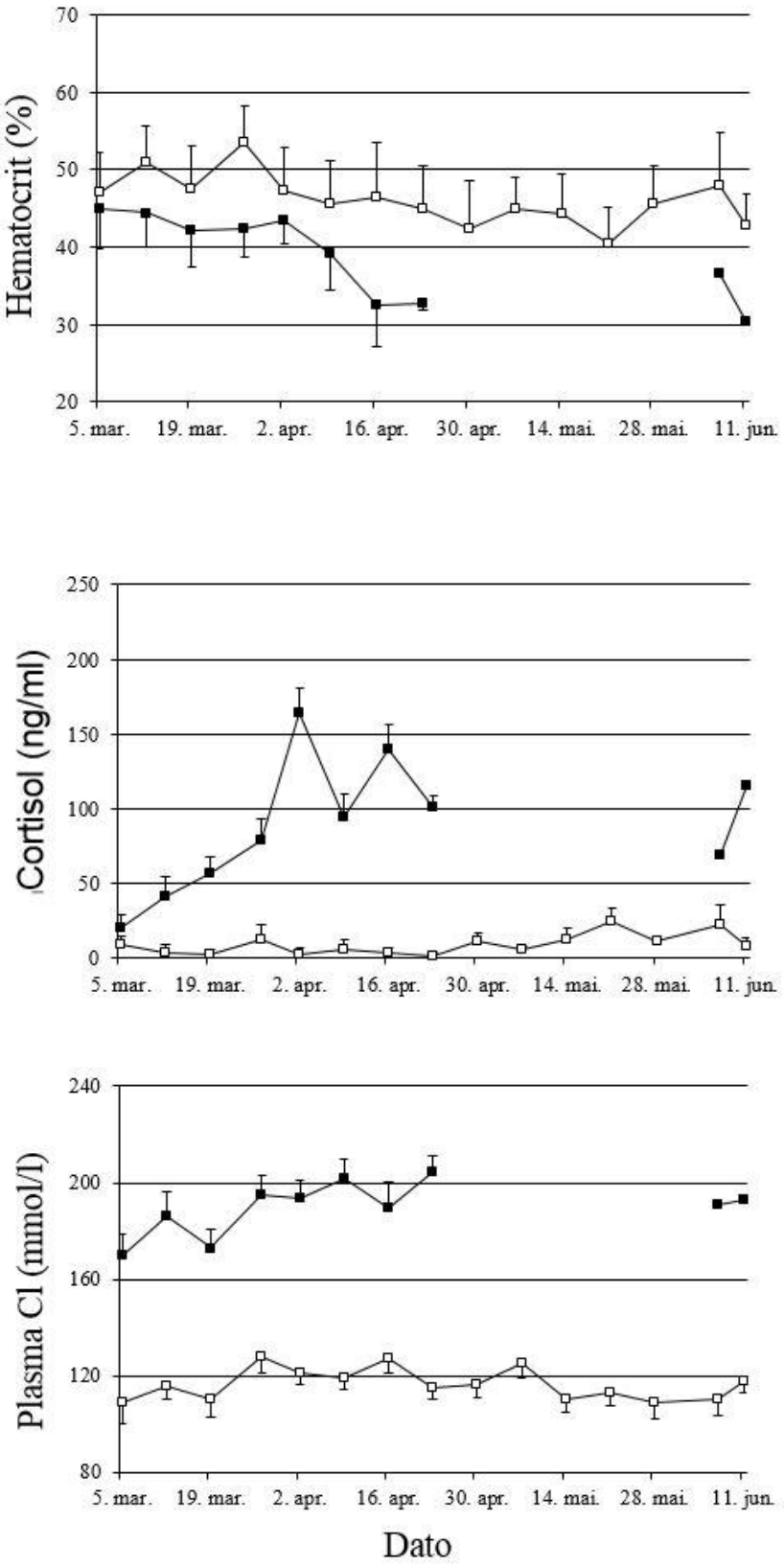

Figure 22. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Prestabekken (white) (n=10), and Prestabekken exposed salmon after 24 hours in the seawater challenge test (black) (n between 1 and 13, breakage in line due to 100% mortality).

Steinsåna

Plasma chloride levels varied between 109 ± 6 and 141 ± 6 mmol/l (n=10) (Figure 23), for Steinsåna exposed fish. Surviving seawater tested fish experienced elevated levels of plasma chloride until the 10. April (175 ± 8 (n=12) and 206 ± 13 mmol/l (n=5)).

The level of hematocrit in the blood cells were relatively stable during the experiments, with the first half of the period showing somewhat higher measurements (between 47 ± 4 and 55 ± 6%, n=10), than the second half (between 42 ± 5 and 48 ± 4%, n=10) (Figure 23). The surviving seawater tested fish in Steinsåna experienced an increase in hematocrit levels until the 3. April from 29 ± 3 (n=5) to 45 ± 5% (n=14) (Figure 23).

Plasma cortisol concentrations in the blood cells were fairly low and stable, between 0 and 14 ± 6 ng/ml (n=10)(Figure 23), during the experiments in Steinsåna. In surviving seawater tested fish, there was observed high levels of cortisol, starting with an increase from 112 ± 16 ng/ml (n=5) on the 6. Mars to 150 ± 17 ng/ml (n=6) on the 13. Mars. Later in Mars, the cortisol levels dropped to 80 ± 15 ng/ml (n=12), followed by a second increase to just above 100 ng/ml (Figure 23).

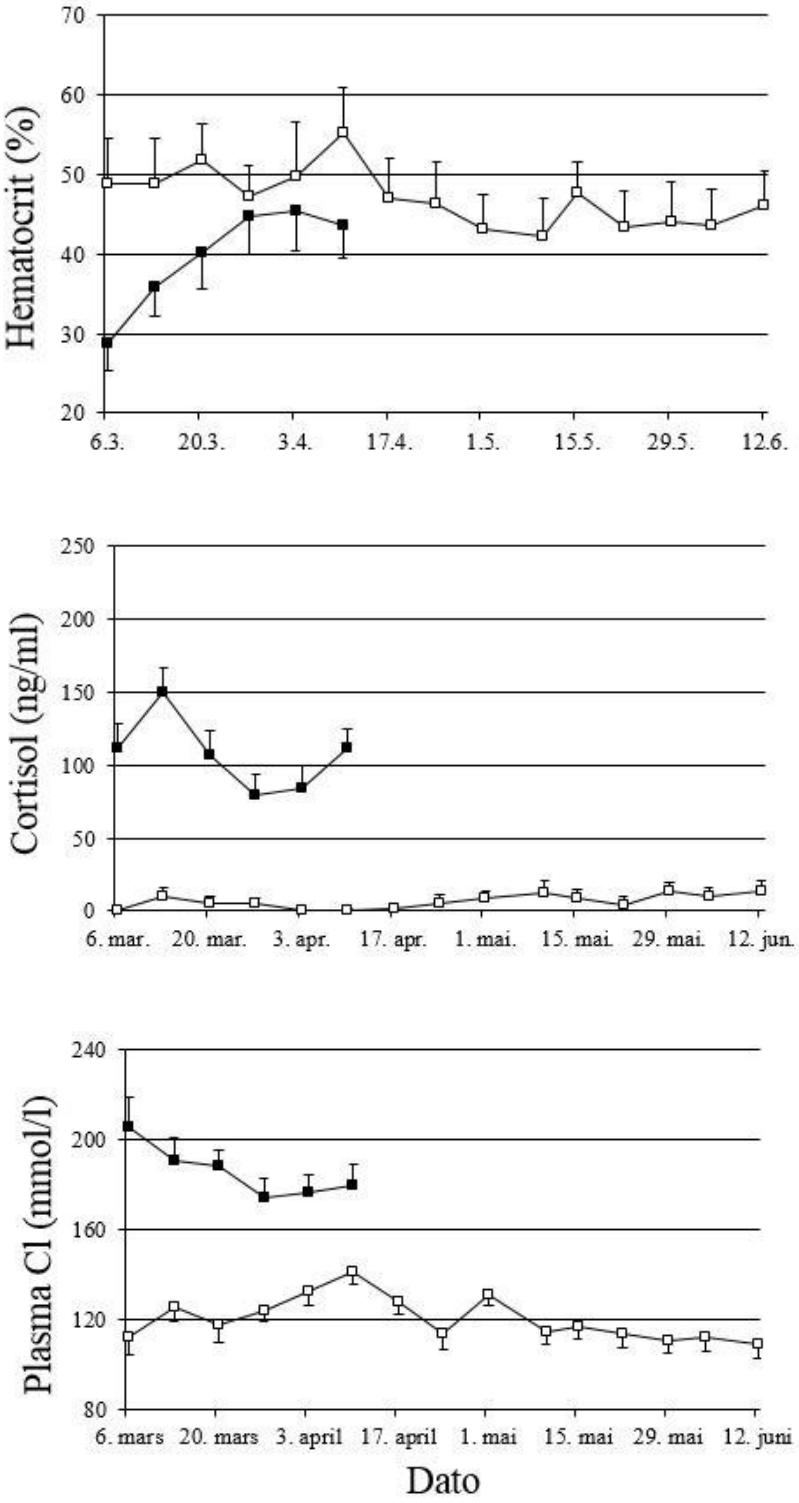

Figure 23. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Steinsåna (white) (n=10), and Steinsåna exposed salmon after 24 hours in the seawater challenge test (black) (n between 5 and 14, breakage in line due to 100% mortality).

Suldalslågen

Plasma chloride levels were stable between 110 ± 6 and 131 ± 5 mmol/l (n=10) during the experiments, with the exception of 16. May (100 ± 8 mmol/l (n=10) (Figure 24). The surviving seawater tested fish experienced an elevated level of plasma chloride through the experimental period, varying between 173 ± 9 (n=10) and 213 ± 10 mmol/l (n=9) (Figure 24). Highest and lowest notation were observed in the second week and in April (Figure 24).

In Suldalslågen exposed fish, the blood's hematocrit levels were stable during the experiments 43 ± 5 and 52 ± 6% (n=10) (Figure 24). The surviving seawater tested fish showed relative low hematocrit levels between 33 (n=1) and 47 ± 4% (n=12), with an increase in levels from the start of the period until late Mars (Figure 24).

Plasma cortisol concentrations in Suldalslågen exposed fish were somewhat elevated early in the experiments, followed by a decline towards 0, during the first three weeks in April (Figure 24). On the 25. April, there was a notable increase in cortisol concentrations, lasting until the 16. May (46 ± 16 ng/ml (n=10)). Following this peak, the cortisol decreased to levels below 10 ng/ml in the end of May. Surviving seawater tested fish experienced high levels of cortisol in the blood cells during the experiments in Suldalslågen (between 53 ± 14 (n=12) and 135 ± 12 ng/ml (n=6)). Initially, the cortisol concentrations decreased from 135 ± 12 ng/ml (n=6) on the 6. Mars, to 53 ± 14 ng/ml (n=12) on the 29. Mars (Figure 24). In April, cortisol levels gradually rose to just above 100 ng/ml (Figure 24).

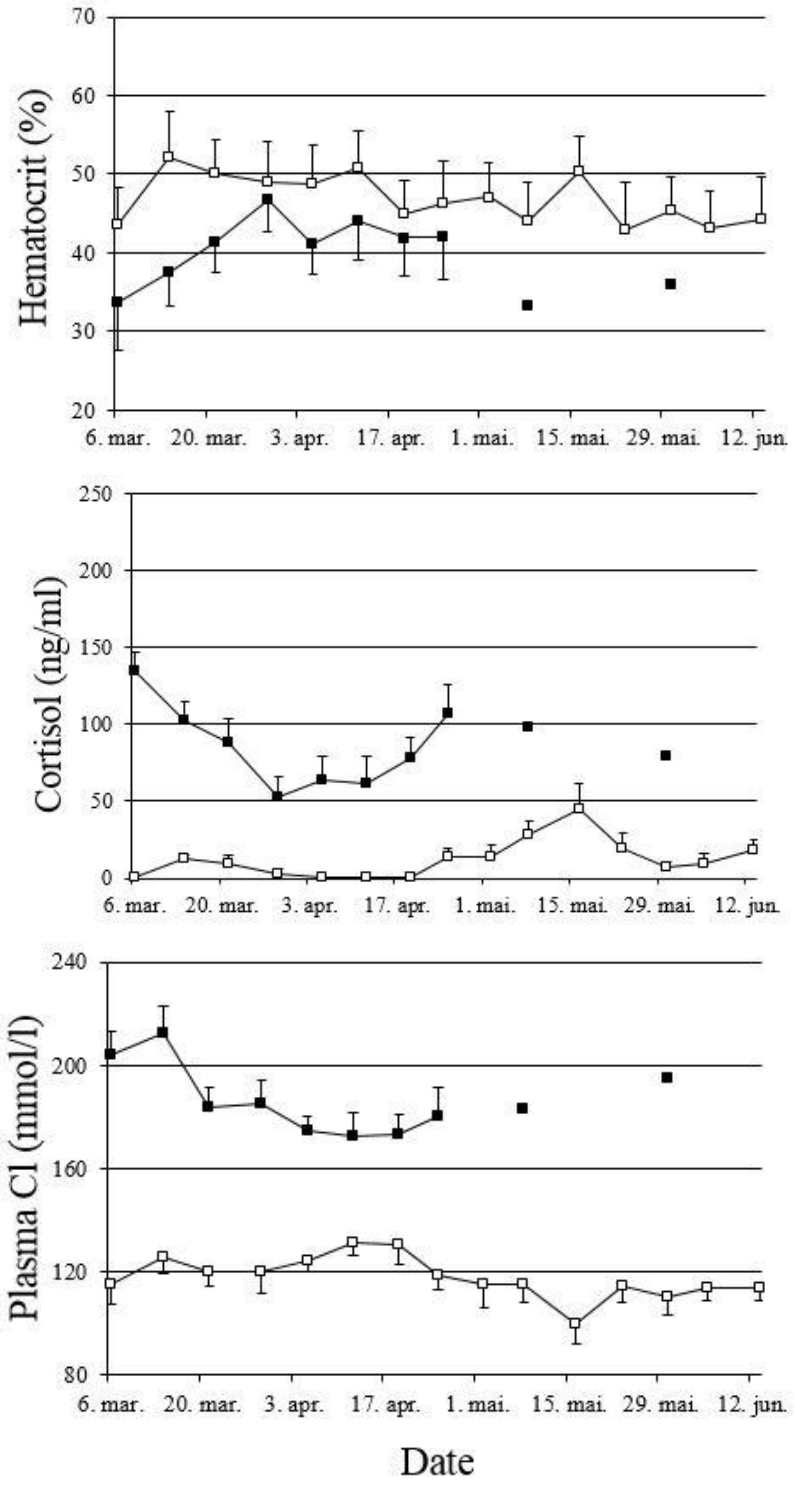

Figure 24. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Suldalslågen (white) (n=10), and Suldalslågen exposed salmon after 24 hours in the seawater challenge test (black) (n between 1 and 12, breakage in line due to 100% mortality).

Suldalslågen + Al

Plasma chloride in Suldalslågen + Al exposed fish were stable between 112 ± 6 and 134 ± 4 mmol/l (n=10) during the experiments, with the exception of 16. May (106 ± 8 mmol/l (n=10)) – as in Suldalslågen (Figure 25). Surviving seawater tested fish experienced a decline in plasma chloride during the first half of the period, from 215 ± 9 (n=11) to 154 ± 9 mmol/l (n=9). From 25. April until 30. May, plasma chloride rose from 158 ± 9 (n=12) to 222 ± 7 mmol/l (n=3), followed by drop at the end of the experiments (185 mmol/l (n=1)) (Figure 25).

The level of hematocrit in the blood cells were relatively stable for Suldalslågen + Al exposed fish. Until the 8. May, hematocrit was observed to be higher (between 45 ± 5 and 53 ± 5%, n=10) than in the remaining period (between 39 ± 6 and 46 ± 4%, n=10) (Figure 25). Until the 2. May, there was a stable increase in hematocrit in the blood cells for the surviving seawater tested fish, from 34 ± 5 (n=11) to 47 ± 5% (n=9) (Figure 25). In the end, observed hematocrit were fairly low between 29 ± 3 (n=3) and 36 ± 2% (n=4) (Figure 25).

Plasma cortisol concentrations varied between 0 and 24 ± 12 ng/ml (n=10) during the experiments in fish exposed to Suldalslågen + Al water (Figure 25). Surviving seawater tested fish showed stable cortisol concentrations during the three first weeks (125-140 ng/ml), before gradually decreasing until the end of Mars, and beginning of April (42 ± 10 ng/ml (n=9) on 18. April) (Figure 25). This was the day prior to the aluminium exposure. In May, the cortisol concentrations increased and on 8. May it was observed at 114 ± 16 ng/ml (n=4). The few surviving individuals in the last part of May experienced cortisol levels above 100 ng/ml, while a single individual surviving the 13. June showed concentrations of cortisol to be as low as 30 ng/ml (Figure 25).

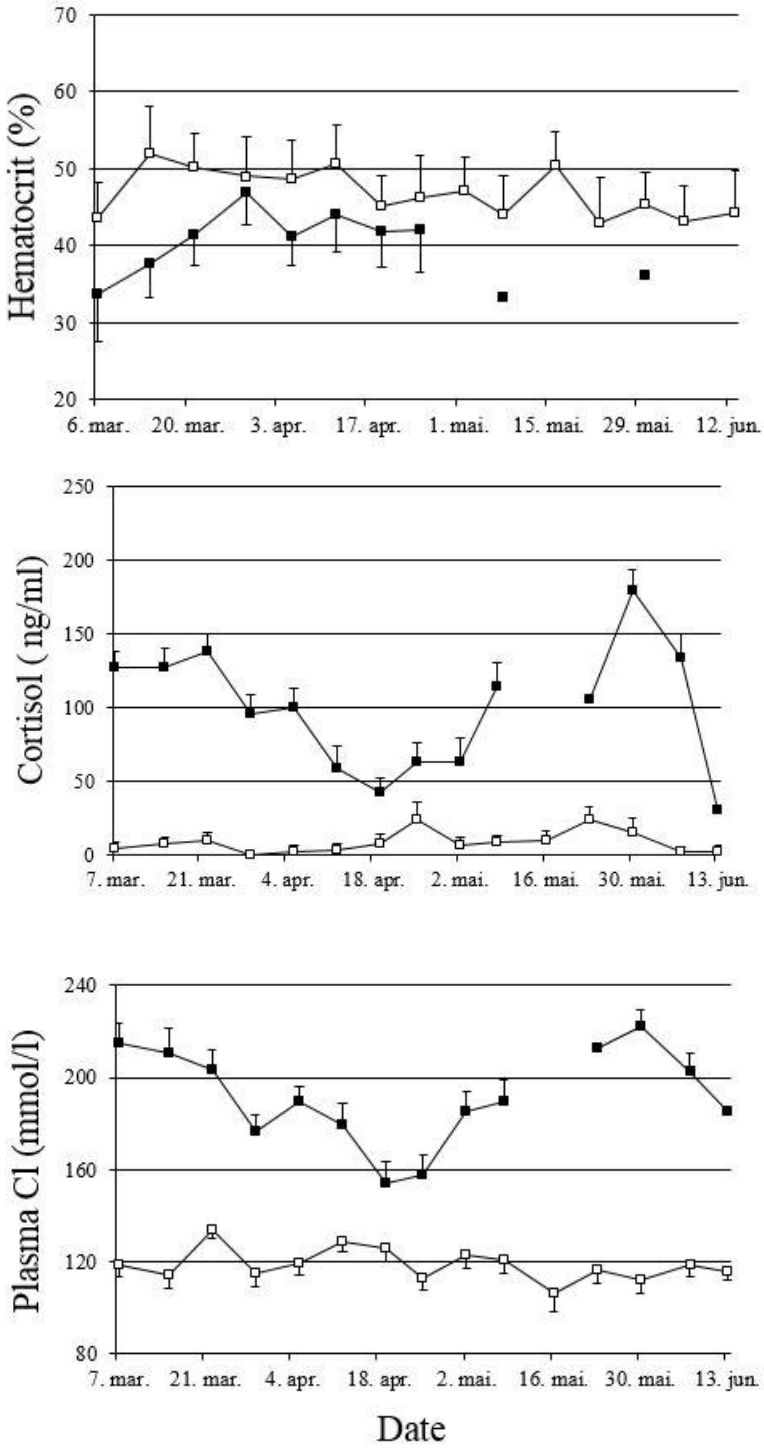

Figure 25. Physiological responses (mean ± s.e.m.) in Atlantic salmon exposed to water from Suldalslågen + Al (white) (n=10), and Suldalslågen + Al exposed salmon after 24 hours in the seawater challenge test (black) (n between 1 and 13, breakage in line due to 100% mortality).

4. Discussion

4.1 Saltwater tolerance

The results of this study suggest that hatchery-reared Atlantic salmon from the Ritland hatchery never developed a satisfying saltwater tolerance during the experiments. Throughout the period defined as the time of smolt migration (15. April – 15. June) (Kaasa et al. 1998; Saltveit 1999, 2000a; Saltveit et al. 2001; Saltveit & Bremnes 2002, 2003), clear physiological responses and high mortality was observed in salmon exposed to the seawater challenge. The physiological measurements of blood samples in surviving salmon from the early stages of the experiments, indicate similar issues with maintenance of water- and ion regulation as the samples taken from salmon in the period of smolt migration. Hence, the experimental fish cannot be characterized as smolts prior to or during the time of smolt migration. The first weeks of experiments show relatively low mortality despite altercations in physiological condition, and could possibly be explained by low temperatures or as a stress response to exposure to a novel environment (Reimers & Døving, 1992). The results of the mixed models would also argue against temperature being the sole explanation. Considering the aim of describing the development of seawater tolerance in Atlantic salmon (presmolt) exposed to different water qualities in Suldalsvassdraget, it is only partly achieved. None of the water qualities show a satisfying development of seawater tolerance in Atlantic salmon, and this presumably raises the question of the quality of the hatchery produced salmon. Interestingly, there were some differences worth mentioning.

4.2 Survival – effect of water quality

As mentioned earlier, Poléo et al. (2001b) reported in a preliminary study that salmon exposed to acidified Al-rich water showed a higher seawater tolerance than salmon exposed to limed water. A drawback with this study is the timeframe. It was conducted at the end of the period for smolt migration, and the development of seawater tolerance was not observed continuously over time. I fear this particularly source of weakness have been the case in several studies, concluding results from seawater testing to be a direct effect of poor water quality. Comparing variation in seawater tolerance in Atlantic salmon in different water qualities when fish is not developing this particular tolerance, is more than difficult.

Nevertheless, the results suggest a difference between the water qualities when comparing resistance and survival. Fish exposed to acidic aluminous water respond with a longer period of survival than fish exposed to presumed better water qualities, supporting my initial hypothesis. These findings support the results in the preliminary report of Poléo et al. (2001b). Fossåna with the overall highest concentration of both inorganic monomeric aluminium and total aluminium, in addition to lowest pH, show a definite better response to the seawater challenge. Interestingly, the mixed models revealed fish survival in Fossåna to increase with the increasing amount of the much-described toxic (Reimers & Døving, 1992) Al_i. In fact, the three presumed poorest water qualities were revealed as the best responders to the seawater challenge with higher survival rates and a longer period of survival. Although not significant, it is interesting to see Suldalslågen + Al increase in fish survival with the increasing amount of Al_i, while Suldalslågen decreases – two identical water qualities prior to the addition of aluminium. Consistent with these results is the findings in Poléo et al. (2001b) where Fossåna exposed fish show almost 90% survival, while the presumed best water quality Steinsåna (pH > 6.2) exposed fish show under 10% survival. The results from this present study indicate that fish exposed to short-term amounts of aluminium is likely to be less sensitive to changes in salinity. This supports earlier findings by Poléo et al. (2001b), where fish in Steinsåna pre-exposed to aluminium responded with much higher survival rates (80%) than fish not exposed to aluminium (15%). Considering the fact that salmon experiencing the same treatment during their whole life, with the exception of three days of additional aluminium, can respond so differently in seawater survival – is remarkable.

Steinsåna and Prestabekken with high levels of pH and relative low levels of Al_i-concentrations showed similar responses in fish survival (25 and 30%), especially during the smolt migration period, with almost no survivors after the 1. of May. Fossåna has been characterized as natural acidic (mean pH = 5.7) Al-containing (mean = 80 µg Al/l) (Blakar & Haaland 2000). Considering the results in Fossåna, I argue the unlikeliness of even low Al-concentrations to have a negative impact on the Atlantic salmon in Suldalslågen, as earlier suggested (Kroglund et al. 1998a, b; Kroglund & Finstad 2001, 2003). The question remains, why the much described (Grande et al. 1978; Poléo et al. 1997; Reimers & Døving, 1992) toxic aluminium and especially Al_i have a positive effect on fish survival in water qualities perceived as poor, and no negative effect in water qualities perceived as good. I believe that some of the answer partly lies within the fact that the aluminium concentration in

Suldalsvassdraget is too low to act as a liability. The occasional and seasonal flooding of Suldalsvassdraget accumulate acidified aluminous water from the different watersheds, but probably too briefly to act in a toxic manner (Poléo et al. 2001a). The magnitude and the duration of a flooding event, is also likely to be too small to give an unmanageable response in fish (Hytterød et al. 2001, 2003). Secondly, parts of the explanation could possibly be within the quality of the hatchery-produced fish. Considering the non-development of seawater tolerance points to a physiological disadvantage in hatchery-reared fish. When comparing wild salmon stocks and hatchery-produced salmon from the Ritland hatchery, the wild salmon has without exception been of good quality, while Ritland exposed salmon show poor seawater tolerance (Kroglund et al. 1998b; Finstad et al. 1999, 2000; Strand et al. 2000, 2001). This disadvantage however, is currently not detectable in freshwater given the blood samples in this study. The results from the physiological testing in freshwater reveal no disruptions in fish response in any water quality during the period of high mortality. The disadvantage is expressed as a poor response to changes in salinity when exposed to seawater, consistent with Kroglund et al. (1998c), Finstad et al. (1999, 2000), Strand et al. (2000, 2001) and Poléo et al. (2001b). By observing the development of seawater tolerance in Atlantic salmon exposed to different water qualities prior to and during the smolt migration period, it is clear that the compromised hatchery-produced salmon's response to changes in salinity is not associated with water quality (acidified toxic water). It is presumably the condition of the hatchery-reared fish and not the water quality itself explaining the osmoregulation related issues in Suldal.

Poléo et al. (2001b) reports an ability in fish to adapt in acidified aluminous water. A possible positive stimulating response to aluminium in physiological conditions related to osmoregulation in juveniles is not to be excluded. This possible stimulating effect presupposes the duration and magnitude of the exposures to aluminium not to be harmful for salmon (Hytterød et al. 2003). As revealed by my study, it is unlikely that small concentrations of aluminium can act harmfully in compromised fish, while high concentration act stimulating. As mentioned, I therefore argue the condition of the hatchery-produced fish to explain the non-developing seawater tolerance. In the literature, it is well documented that wild fish populations adapt better to the environment than farmed fish. Comparing wild and hatchery-produced fish in the smoltification process emphasizes these differences (Schreck et al. 1985; Virtanen & Soivio 1985; Patiño et al. 1986; Sower & Fawcett 1991; Shrimpton et al. 1994a, b; Poole et al 2003). Additionally, the smoltification

process can be affected positively when densities are low, and a more complex environment is present in the hatchery (Zydlewski et al. 2003).

4.3 Plasma cortisol and smoltification

My results indicate a general development of plasma cortisol in the control fish during the experiments, as supported by earlier findings for Atlantic salmon (Whitesel, 1992), trout (*Salmo trutta*) (Finstad & Ugedal, 1998), coho salmon (*Oncorhynchus kisutch*) (Patiño et al. 1986; Young et al. 1989; Shrimpton et al. 1994a), chinook salmon (*Oncorhynchus tshawytscha*) and sockeye salmon (*Oncorhynchus nerka*) (Franklin et al. 1992). The cortisol levels typically showed moderate increases from low levels (10 ng/ml) in Mars to 20-40 ng/ml in April-May, with a bimodal peaking. Suldalsvatnet exposed fish showed a small increase in April, but not before the end of May, the cortisol levels were high enough to indicate an ongoing smoltification (Reimers & Døving, 1992). The increased cortisol levels could not show a positive effect on ion regulation as the mortality rose to 100% in the seawater challenge during April-May. The high plasma chloride levels in the blood samples from the surviving fish also indicate unsuccessful smoltification (Reimers & Døving, 1992). The increasing level of cortisol in the blood did not stimulate for a higher gill Na^+, K^+, -ATPase activity, as opposed to several studies (Patiño et al. 1986; Franklin et al. 1992; Shrimpton et al. 1994a) where gill Na^+, K^+, -ATPase activity rose with higher levels of cortisol. Shrimpton et al. (1994a) revealed significantly lower maximum gill Na^+, K^+, -ATPase activity in hatchery-produced salmon compared to wild, with an early cortisol high in the hatchery-reared salmon. The results from my study could be of something similar as Shrimpton et al. (1994a).

Similarly to Suldalsvatnet, the water qualities Prestabekken and Steinsåna showed a moderate increase in plasma cortisol in April-May, but the survival was lower in the latter two. The ability to mobilize cortisol in Prestabekken and Steinsåna exposed fish were certain, when looking at the strong elevated cortisol levels taken from blood samples in surviving seawater tested fish. With the exception of elevated cortisol concentrations in the beginning of the period, which could remind of an ongoing smoltification, the pattern in Fossåna is similar to the other water qualities. The plasma chloride levels in the surviving seawater tested fish indicate an unsatisfactory ability to ion regulate. Consistent with the results from the other water qualities, the cortisol levels in Suldalslågen (and + Al) exposed

fish showed a similar response. An observed increase in cortisol in May, but a simultaneously mortality reaching 100%. Overall, the changes in physiological conditions suggest a stress reaction triggered by the exposure to seawater, and not by variation in water quality - supported by my second hypothesis. Consistently supported by observations done on hatchery-produced fish exposed to seawater (Avella et al. 1990; Shrimpton et al.; 1994b).

4.4 Seawater challenge test

The seawater challenge test (Clarke & Blackburn 1977) is a useful tool in predicting whether or not diadromous fish species are smoltifying. Still, the test is somewhat limited as revealed by this study. Seawater tolerance in its own, does not specify the reason for the response in fish. When inferring the seawater challenge test as a measurement for water quality criteria in diadromous fish (Kroglund & Finstad 2003), it do not exclude other factors than water quality as reason to unsuccessful development of seawater tolerance or reduced ability to endure changes in salinity. In fact, this study reveals exposure to acidified aluminous water to improve the ability to endure changes in salinity. As mentioned earlier, I fear several studies has concluded results from seawater testing to be a direct effect of poor water quality. I stress the importance of following the development of seawater tolerance continuously over time, with the additional controlling routines. I argue this necessity to be able to exclude factors like random variation and smolt quality. The results from the seawater challenge on 27. Mars highlight the importance of following the development of seawater tolerance over time. In Steinsåna exposed fish the survival was 80%, while in Fossåna it was close to 47%. Inferring results of a seawater test as a direct effect of water quality based on a shorter period of testing, could possibly give conflicting conclusions. Thus, I argue the seawater challenge test to be limited in describing the reason to non-development of seawater tolerance.

4.5 Management implications

My results suggest that hatchery reared Atlantic salmon from the Ritland hatchery never developed a satisfying saltwater tolerance during the experiments. This highlight the question of the applicability, and to what extent further production of hatchery-salmon is useful in Suldal. Studies on fish from Ritland hatchery has revealed similar results in observing a compromised fish population (Finstad et al. 1999, 2000; Strand et al. 2000,

2001). During the period 1996-2003, there has only been reported of a single successful year in smoltification (Strand et al. 2003). I recommend future studies focusing on what enables hatchery-produced fish to develop a seawater tolerance, including environmental factors as density dependence and complexity (Zydlewski et al. 2003). I also recommend studying the role of aluminium as a possible positive "stimulating" compound for hatchery-reared fish, in poorer water qualities. Identifying the mechanism explaining the reduced negative effect of seawater when exposed to aluminium, would be a considerable contribution to the scientific community. Additionally, the necessity of a long-term monitoring is needed to reveal true development of seawater tolerance. I highly recommend the use of the seawater challenge test as a tool for predicting an ongoing smoltification process, and not as a predictor of water quality. A solid design and good controlling protocols is beneficial when assessing the situation in Suldal. I therefore argue the simultaneously testing of wild salmon to be helpful in reaching further inference. As shown in this study, the results give no support to the hypothesis of Suldalsvassdraget being negatively affected by acidification (Kroglund et al. 1998a, b; Kroglund & Finstad 2001, 2003). A further important consideration for the management, is the applicability of liming in Suldalsvassdraget. The results in this study show no improved changes in physiological conditions, or improved ability in osmoregulation in salmon exposed to limed water qualities. Consistently, liming has not been shown as giving a measurable positive effect on salmon or trout in Suldalsvassdraget (Saltveit, 2000b; Saltveit & Bremnes, 2003; Jensen et al. 2001, 2002, 2003). Additionally, the acidification situation in Norwegian waters has improved for the better (Skjelekvåle et al. 2001; Tørseth et al. 2001). Assuming the results in this study as indicative to the survival and development of the hatchery-produced and released salmon, the management is faced with a huge economical expense that possibly could be used differently. As a species of socioeconomic and economic importance, the Atlantic salmon in Suldal could benefit from ecological studies accounting for these considerations.

This study has given a unique opportunity to study the development of seawater tolerance of Atlantic salmon in various water qualities in Suldalsvassdraget, and potentially illustrate the applicability of both commonly used seawater tests and quality of hatchery-produced fish in smoltifying. Additionally, exposing acidified aluminous water not to be the explanation to sensitivity in changes in salinity. Finally, giving a cautionary note for future directions in management.

5. Conclusion

This study demonstrates the limits of seawater challenge testing as a tool for predicting water quality criteria in Atlantic salmon. The methods used were successful in revealing acidified aluminous water to be overvalued as a threat to the development of seawater tolerance in Atlantic salmon in Suldalslågen. Notable was the possible stimulating role of inorganic monomeric aluminium in poorer water quality. The study further questions the applicability of the hatchery-produced fish from Ritland, and the socioeconomic and economic consequences further implementation could have on local human population. Continued monitoring of both wild and hatchery-produced salmon, as well as ecosystem monitoring and requirements for satisfactory seawater tolerance development, is highly recommended. Additionally, to establish the role of aluminium as a possible positive "stimulator" in hatchery-reared fish, and identify the mechanism explaining the reduced negative effect of seawater exposure. This combination of methods will provide management authorities and local managers with a strong scientific platform to evaluate the most appropriate actions.

6. Acknowledgement

This study was carried out as part of an earlier project funded by Statkraft SF in 2002. In parallel, a similar study was conducted on behalf of Norwegian Institute for Water Research (NIVA) and Norwegian Institute for Nature Research (NINA), funded by the Norwegian Environment Agency.

Finally, I would like to thank my supervisors professor Antonio B.S. Poléo and professor Kjartan Østbye for invaluable help during this entire process. Thank you so much for giving me the opportunity to be part of this project, for guidance and for meaningful discussion. I thank you for taking your time to help improve my thesis.

References

Avella, M., Young, G., Prunet, P. & Schreck, C.B. (1990). Plasma Prolactin and Cortisol Concentrations During Salinity Challenges of Coho Salmon (*Oncorhynchus kisutch*) at Smolt and Post-Smolt Stages. Aquaculture 91, 359-372.

Barnes, R.B. (1975). The determination of specific forms of aluminum in natural water. Chem. Geol. 15, 177-191.

Bates, D., Maechler, M., Bolker, B.M. & Walker, S. (2014) lme4: linear mixed-effects models using eigen and S4. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Berge, A.I., Berg, A., Fyhn, H.J., Barnung, T., Hansen, T., Stefansson, S.O. (1995). Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmon salar)* reared under different photoperiods. Can. J. Fish. Aquatic Sci. 52, 243–251.

Bjerknes, V., Duston, J., Knox, D., Harmon, P., (1992). Importance of body size for acclimation of underyearling Atlantic salmon parr (*Salmo salar* L.) to seawater. Aquaculture 104, 357–366.

Blackburn, J., Clarke, W.C. (1987). Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquatic Sci. 1515 35 pp.

Blakar, I.A. (1995). Vannkvaliteten i Ulla-Førre og Suldalsområdet i perioden 1990-93. LFSrapport 21, 49 pp.

Blakar, I. A. & Haaland, S. (2000). Vannkvalitet i Suldalsvassdraget i 1998 og 1999. Suldalslågen Miljørapport 6, 15 pp.

Bloom, P.R., McBride, M.B. & Weaver, R.M. (1979). Aluminium organic matter in acid soils: Buffering and solution aluminium activity. Soil Sci. Soc. Am. J. 43, 488-493.

Boeuf, G., Le Roux, A., Gaignon, J.L., Harache, Y., (1985). Gill Na –K -ATPase activity and smolting in Atlantic salmon (*Salmo salar* L.) in France. Aquaculture 45, 73–81.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution, 24, 127-135.

Burrows, W. D. (1977). Aquatic aluminium: chemistry, toxicology, and environmental prevalence. CRC Critical Reviews in Environmental Control 7, 167–216.

Clarke, W.C. & Blackburn, J. (1977). A seawater challenge test to measure smolting of juvenile salmon. Fish. Mar. Serv. Tecn. Rep. 705, 1-11.

Duston, J., Saunders, R.L., (1992). Effect of 6-, 12-, and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquatic Sci. 49, 2273–2279.

Driscoll, C. T., Baker, J. P., Bisogni, J. J. & Schofield, C. L. (1980). Effects of aluminium speciation on fish in dilute acidified waters. Nature, London 284, 161–164.

Driscoll, C.T. (1984). A Procedure for the fractionation of aqueous aluminum in dilute acidic waters. Internat. J. Environ. Analyt. Chem. 16, 267-283.

Fairchild, W.L., Swansburg, E.O., Arseenault, J.T. & Brown, S.B. (1999). Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? Environmental Health Perspectives 107: 349–357.

Finstad, B., Kroglund, F., Hartvigsen, R., Teien, H.-C., Rosseland, B.O. & Salbu, B. (1999). Suldalslågen: fisk og vannkjemisk status våren 1997. NINA-oppdragsmelding 588, 32 pp.

Finstad, B., Strand, R., Kroglund, F., Teien, H.-C. & Hartvigsen, R. (2000). Suldalslågen: fisk og vannkjemisk status våren 1998. NINA-oppdragsmelding 644, 25 pp.

Fiske, P., Thorstad, E.B., Økland, F. & Johnsen, B.O. (2001). Oppvandring hos radiomerket laks i Suldalslågen i forhold til vannføring, vannkvalitet og vanntemperatur. NINAoppdragsmelding 675, 42 pp.

Friedland, K.D. (1998). Ocean climate influences on critical Atlantic salmon (*Salmo salar*) life history events. Canadian Journal of Fisheries and Aquatic Sciences 55: 119–130.

Gensemer, R.W. & R.C. Playle. (1999). The bioavailability and toxicity of aluminum in aquatic environments. Crit. Rev. Environ. Sci. Technol., 29: 315-450.

Gibson, R.J. (1993). The Atlantic salmon in freshwater: spawning, rearing and production. Reviews in Fish Biology and Fisheries 3: 39–73.

Gibson, R.J. (2002). The effects of fluvial processes and habitat heterogeneity on distribution, growth and densities of juvenile Atlantic salmon (*Salmo salar* L.), with consequences on abundance of the adult fish. Ecology of Freshwater Fish 11: 207–222.

Grande, M., Muniz, I.P. & Andersen, S. (1978). Relative tolerance of some salmonids to acid waters. Verh. Internat. Verein. Limnol. 20, 2076-2084.

Grimnes, A., Finstad, B. & Bjørn, P.A. (2000). Registreringer av lakselus på laks, sjøørret og sjørøye i 1999. NINA oppdragsmelding 634, 34 pp.

Hartig, F. (2018) Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. Theoretical Ecology, University of Regensburg, Regensburg, Germany.

Heggberget, T.G., Blakar, I.A., Nordland, J., Saltveit, S.J. & Johnsen, B.O. (1994). UllaFørrereguleringen. Rapport fra rådgivende arbeidsgruppe for vurdering av undersøkelser og tiltak. NINA Utredning O-64, 51 pp.

Heggenes, J., Baglinie` re, J.L. & Cunjak, R.A. (1999). Spatial niche variability for young Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in heterogeneous streams. Ecology of Freshwater Fish 8: 1–21.

Henriksen, A. & Hesthagen, T. (1993). Critical load exceedance and damage to fish populations. Norwegian Institute for Water Research, Report 2900, Oslo, Norway.

Hesthagen, T. & Hansen, L. P. (1991). Estimates of the annual loss of Atlantic salmon, (*Salmo salar* L.), in Norway due to acidification. Aquaculture and Fisheries Management 22, 85–91.

Hoar, W.S., (1988). The physiology of smolting salmonids. In: Hoar, W.S., Randall, D.J. Eds. Fish Physiology, Vol. 11B. Academic Press, London, pp. 275–343.

Holst, J.C. & Jakobsen, P. (1999). Lakselus dreper. Fiskets gang 8, 25-28.

Hytterød, S., Schjolden, J. & Poléo, A.B.S. (2003). Restitueringsevne hos laks (*Salmo salar*) etter eksponering for aluminium – forsøk med parr og presmolt, gjort i Suldal 1999. Suldalslågen Miljørapport 20, 51 pp.

Jacobsen, J.A. & Hansen, L.P. (2000). Feeding habits of Atlantic salmon at different life stages at sea. In: Mills, D., ed. The Ocean Life of Atlantic Salmon: Environmental and Biological Factors Influencing Survival Oxford: Fishing News Books, Blackwell Science, pp. 170–192.

Jensen, C.S., Schjolden, J. & Poléo A.B.S. (2001). Fisketetthet og vannkvalitet i sidebekker til Suldalslågen. Årsrapport for 2000 (med data fra 1998 og 1999). Suldalslågen Miljørapport, 10, 25 pp.

Jensen, C.S., Schjolden, J. & Poléo A.B.S. (2002). Fisketetthet og vannkvalitet i sidebekker til Suldalslågen. Årsrapport for 2001 (med data fra 1998, 1999 og 2000). Suldalslågen Miljørapport, 16, 49 pp.

Jensen, C.S., Gravem, F. & Poléo A.B.S. (2003). Fisketetthet og vannkvalitet i sidebekker til Suldalslågen. Årsrapport for 2002 (med data fra 1998, 1999, 2000 og 2001). Suldalslågen Miljørapport, 24, 48 pp.

Jonsson, N., Hansen, L.P. & Jonsson, B. (1991a). Variation in age, size and repeat spawning of adult Atlantic salmon in relation to river discharge. Journal of Animal Ecology 60: 937–947.

Kaasa, H., Eie, J.A., Erlandsen, A.H., Faugli, P.E., L'Abée-Lund, J.H., Sandøy, S. & Moe, B. (1998). Sluttrapport 1990-1997. Resultater og konklusjoner. LFS-rapport 49, 82 pp.

Kaste, Ø., Hindar, A., Kroglund, F., Blakar, I.A., Holmqvist, E., Brandrud, T.E. & Johansen, S.W. (1995). Tiltak mot forsuring av Suldalslågen. Kalkingsplan. NIVA-Rapport O94236, 48 pp.

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. & Mortensen, E. (2003). Atlantic salmon (*Salmo salar* L.), brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus*) (L.): a review of aspects of their life histories. Ecology of Freshwater Fish 12, 1–59.

Kroglund, F. & Staurnes, M. (1999). Water quality requirements of smolting Atlantic salmon (*Salmo salar*) in limed acid rivers. Can. J. Fish. Aquat. Sci. 56, 2078-2086.

Kroglund, F. & Finstad, B. (2001). Effekter av ulik vannkvalitet på fysiologisk respons, vekst, vandring og marin overlevelse hos to stammer av atlantisk laks. NIVA-Rapport LNR4381-2001, 47 pp.

Kroglund, F. & Finstad, B. (2003). Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon. Aquaculture 222, 119-133.

Kroglund, F., Teien, H.C., Håvardstun, J., Rosseland, B.O., Salbu, B. & Kvellestad, A. (1998a). Varighet av ustabil og skadelig aluminiumskjemi på giftighet overfor lakseparr; renneforsøk utført i Suldalslågen, høst 1996. NIVA-Rapport LNR-3815-98, 64 pp.

Kroglund, F., Finstad, B., Rosseland, B.O., Teien, H.C., Håvarstun, J. & Salbu, B. (1998b). Fisk og vannkjemisk status i Suldalslågen våren 1996. NIVA-Rapport LNR-3863-98, 64 pp.

Kroglund, F., Teien, H.C., Rosseland, B.O., Lucassen, E., Salbu, B. & Åtland, Å. (1998c). Endring i aluminiums giftighet i en humus-fattig elv ved bruk av kjemiske tiltak. Forsøk med laksesmolt i Suldalslågen. NIVA-Rapport LNR-3970-98, 102 pp.

L'Abe´ e-Lund, J.H., Jonsson, B., Jensen, A.J., Sættem, L.M., Heggberget, T.G., Johnsen, B.O. & Næsje, T.F. (1989). Latitudinal variation in life-history characteristics of sea-run migrant brown trout (*Salmo trutta)*. Journal of Animal Ecology 58: 525–542.

Lydersen, E., Poléo, A.B.S., Nandrup-Pedersen, M., Riise, G., Salbu, B., Kroglund, F. & Rosseland, B.O. (1994). The importance of "in situ" measurements to relate toxicity and chemistry in dynamic aluminium freshwater systems. J. Ecol. Chem. 3, 357-365

Lucas, M. & Baras, E. (2001). Migration of Freshwater Fishes. Oxford, Blackwell Science.

Madsen, S.S., Naamansen, E.T., (1989). Plasma ionic regulation and gill Na^+, K^+, -ATPase changes during rapid transfer to seawater of yearling rainbow trout, (*Salmo gairdneri)*: time course and seasonal variation. J. Fish. Biol. 34, 829–840.

Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J., Tyre, A.J. & Possingham, H.P. (2005) Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecology Letters, 8, 1235-1246.

Mather, M.E., Parrish, D.L. & Folt, C.L., eds. (1998). Selected proceedings of the international workshop entitled 'Integrating across scales: predicting patterns of change in Atlantic salmon'. Canadian Journal of Fisheries and Aquatic Sciences 55 (Suppl 1): 1–323.

McCormick, S.D., Saunders, R.L., (1987). Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. American Fisheries Society Symposium 1, 211–229.

McCormick, S.D., Saunders, R.L., Henderson, E.B., Harmon, P.R., (1987). Photoperiod control of parr–smolt transformation in Atlantic salmon (*Salmo salar)* : changes in salinity tolerance, gill Na ,K -ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquatic Sci. 44, 1462–1468.

McCormick, S.D., Moyes, C.D., Ballantyne, J.S., (1989). Influence of salinity on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. 6, 243–254.

Meerburg, D.J., ed. (1986). Salmonid age at maturity. Canadian Special Publication of Fisheries and Aquatic Sciences 89. ivþ118p.

Montevecchi, W.A., Cairns, D.K. & Myers, R.A. (2002). Predation on marine-phase Atlantic salmon (Salmo salar) by gannets (Morus bassanus) in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 59: 602–612.

Muniz, I. P. (1984). The effects of acidification on Scandinavian freshwater fish fauna. Philosophical Transactions of the Royal Society of London, Series B 305, 517–528.

Olsen, Y.A., Falk, K. & Reite, O.B. (1992). Cortisol and lactate levels in Altlantic salmon (*Salmo salar)* developing infectious anaemia (ISA). Dis. Aquat. Organisms 14, 99-104.

Parrish, D.L., Behnke, R.J., Gephard, S.R., McCormick, S.D. & Reeves, G.H. (1998). Why aren't there more Atlantic salmon (*Salmo salar*)? Canadian Journal of Fisheries and Aquatic Science 55 (Suppl 1): 281–287.

Patiño, R., Schreck, C.B., Banks, J.L. & Zaugg, W.S. (1986). Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115, 828-837.

Poléo, A.B.S., Østbye, K., Øxnevad, S.A., Andersen, R.A., Heibo, E. & Vøllestad, L.A. (1997). Toxicity of acid aluminium-rich water to seven freshwater fish species: A comparative laboratory study. Environ. Pollut. 96, 129-139.

Poléo, A. B. S., Øxnevad, S.A. & Schjolden, J. (2001a). Laksens (*Salmo salar*) fysiologiske respons på endringer i vannkvalitet under vårflommer i to sidebekker til Suldalslågen. Suldalslågen Miljørapport 8, 32 pp.

Poléo, A.B.S., Schjolden, J. & Hytterød, S. (2001b). Adaptasjon hos fisk eksponert for surt Al-rikt vann. Vann 4B, 420-426.

Poléo, A. B. S., Schjolden, J. & Hytterød, S. (2002). Effekter av ulike vannkvaliteter i Suldalsvassdraget på presmolt og smolt av laks (*Salmo salar*). Suldalslågen Miljørapport 18, 47 pp.

Potter, E.C.E. & Crozier, W.W. (2000). A perspective on the marine survival of Atlantic salmon. In: Mills, D., eds. The ocean life of Atlantic salmon: environmental and biological factors influencing survival. Oxford: Fishing News Books, Blackwell Science, pp. 19–36.

Power, G. (1986). Physical influences on age at maturity of Atlantic salmon (*Salmo salar*): a synthesis of ideas and questions. In: Meerburg, D.J., ed. Salmonid age at maturity. Canadian Special Publication of Fisheries and Aquatic Sciences 89, pp. 97–101.

Randall, R.G., Thorpe, J.E., Gibson, R.J. & Reddin, D.G. (1986). Biological factors affecting age at maturity in Atlantic salmon (*Salmo salar*). In: Meerburg, D.J., ed. Salmonid age at maturity. Canadian Special Publication of Fisheries and Aquatic Sciences 89, pp. 90–96.

Randall, R.G., O'Connell, M.F. & Chadwick, E.M.P. (1989). Fish production in two large Atlantic coast rivers, Miramichi and Exploits. Canadian Special Publication of Fisheries and Aquatic Sciences 106: 292–308.

Reimers, E. & Døving, K.B. (1992) Fiskens fysiologi. John Grieg, [Bergen].

Richards, S.A. (2008) Dealing with overdispersed count data in applied ecology. Journal of Applied Ecology, 45, 218-227.

Riddell, B.E. (1986). Assessment of selective fishing on the age at maturity in Atlantic salmon (*Salmo salar*): a genetic perspective. In: Meerburg, D.J., ed. Salmonid age at maturity. Canadian Special Publication of Fisheries and Aquatic Sciences 89, pp. 102–109.

Rosseland, B.O., Skogheim, O.K. & Sevalrud, I. H. (1986). Acid deposition and effects in Nordic Europe. Damage to fish populations in Scandinavia continue apace. Water, Air, and Soil Pollution 30, 65–74.

Saltveit, S.J. (1999). Fiskeribiologiske undersøkelser i forbindelse med nytt prøvereglement. Årsrapport for 1998. Suldalslågen Miljørapport 2, 37 pp.

Saltveit, S.J. (2000a). Suldalslågen. Fiskeribiologiske undersøkelser i forbindelse med nytt prøvereglement. Årsrapport for 1999. Suldalslågen Miljørapport 4, 48 pp.

Saltveit, S.J. (2000b). Alderssammensetning, tetthet og vekst av ungfisk av laks og ørret i Suldalslågen i perioden 1976 til 1999. Suldalslågen Miljørapport 7, 28 pp.

Saltveit, S.J. & Bremnes, T. (2002). Suldalslågen. Fiskeribiologiske undersøkelser i forbindelse med nytt prøvereglement. Årsrapport for 2001. Suldalslågen Miljørapport 16, 42 pp.

Saltveit, S.J. & Bremnes, T. (2003). Suldalslågen. Fiskeribiologiske undersøkelser i forbindelse med nytt prøvereglement. Årsrapport for 2002. Suldalslågen Miljørapport 24, 47 pp.

Saltveit, S.J., Bremnes, T. & Heggenes, J. (2001). Suldalslågen. Fiskeribiologiske undersøkelser i forbindelse med nytt prøvereglement. Årsrapport for 2000. Suldalslågen Miljørapport 10, 55 pp.

Saunders, R.L., Harmon, P.R., (1990). Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter–spring. Trans. Am. Fish. Soc. 119, 689–697.

Schjolden, J., Hytterød, S. & Poléo, A.B.S. (2001). Effekten av vannkvalitetsendringer under vår- og høstflommer på fysiologien til lakseparr og smolt (Salmo salar) i en sur og en kalket sidebekk til Suldalslågen. Suldalslågen Miljørapport 12, 55 pp.

Schreck, C.B., Patiño, R., Pring, C.K., Winton, J.R. & Holway, J.E. (1985). Effects of rearing density on indices of smoltification and performance of coho salmon, (*Oncorhynchus kisutch*). Aquaculture 45, 345-358.

Shrimpton, J.M., Bernier, N.J. & Randall, D.J. (1994a). Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51, 2179-2187.

Shrimpton, J.M., Bernier, N.J., Iwama, G.K. & Randall, D.J. (1994b). Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. Can. J. Fish. Aquat. Sci. 51, 2170-2178.

Sigholt, T., Finstad, B., (1990). Effect of low temperature on seawater tolerance in Atlantic salmon (*Salmo salar*) smolts. Aquaculture 84, 167–172.

Sigholt, T., Staurnes, M., Jakobsen, H.J., Asgard, T., (1995). Effects of continuous light and short-day photoperiod on smolting, seawater survival and growth in Atlantic salmon (*Salmo salar).* Aquaculture 130, 373–388.

Simensen, E., Olson, L.D., Vanjonack, W.J. & Johnson, H.D. (1978). Determination of corticosterone concentration in plasma of turkeys using radioimmunoassay. Poultry Sci. 57, 1701-1704.

Skjelkvåle, B.S., Tørseth, K., Aas, W. & Andersen, T. (2001) Decrease in acid deposition – Recovery in Norwegian waters. Water Air Soil Pollut. 130, 1433-1438.

Solbakken, V.A., Hansen, T., Stefansson, S.O., (1994). Effects of photoperiod and temperature on growth and parr–smolt transformation in Atlantic salmon (*Salmo salar* L.) and subsequent performance in seawater. Aquaculture 121, 13–27.

Sprague, J.B. (1973). The ABC's of pollutant bioassay using fish. Spec. Tech. Publ. Am. Soc. Test. Master. 528, 6-30.

Strand, R., Finstad,B., Kroglund, F. & Teien, H.-C. (2000). Forsuringsstatus og effekter på smolt i Suldalslågen våren 1999. NINA-oppdragsmelding 672, 24 pp.

Strand, R., Finstad, B., Kroglund, F., Teien, H-C. & Mack berger, H. (2001). Forsuringsstatus og effekter på smolt i Suldalslågen våren 2000. NINA-oppdragsmelding 732, 31 pp.

Strand, R., Finstad, B., Kroglund, F. & Teien, H-C. (2003). Forsuringsstatus og effekter på smolt i Suldalslågen våren 2001. NINA-oppdragsmelding 780, 17 pp.

Sullivan, T.J., Seip, H.M. & Muniz, I.P. (1986). A comparizon of frequently used methods for the determination of aqueous aluminum. Internat. J. Environ. Analyt. Chem. 26, 61-75.

Tikhonov, V.N. (1973). Analytical chemistry of aluminium. John Wiley & Sons, New York, USA, 303 pp.

Tørseth, K., Aas, W. & Solberg, S. (2001). Trends in airborne sulphus and nitrogen compounds in Norway during 1985-1996 in relation to air mass origin. Water Air Soil Pollut. 130, 1493-1498.

Virtanen, E. & Soivio, A. (1985). The patterns of T3, T4, cortisol and Na+-K+-ATPase during smoltification of hatchery-reared Salmo salar and comparison with wild smolts. Aquaculture 45, 97-109.

Vogt, R.D., Ranneklev, S.B. & Mykkelbost, T.C. (1994). The impact of acid treatment on soilwater chemistry at the humex site. Environ. Internat. 20, 277-286.

Walters, C. & Ward, B. (1998). Is solar radiation responsible for declines in marine survival rates of anadromous salmonids that rear in small streams? Canadian Journal of Fisheries and Aquatic Sciences 55: 2533–2538.

Whitesel, T.A. (1992). Springtime Hepatosomatic Indexes, Plasma-Cortisol Levels, and Hepatic 5'-Monodeiodinase Activities in One-Year-Old Atlantic Salmon from 2 Growth Modes. Progr. Fish-Cult. 54, 174-180.

Young, G., Bjornsson, B.T., Prunet, P., Lin, R.J. & Bern, H.A. (1989). Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones, and cortisol. Gen. Comp. Endocrinol. 74, 335-345.

Zydlewski, G.B., Foott, J.S., Nichols, K., Hamelberg, S., Zydlewski, J. & Björnsson, B.T. (2003). Enhanced smolt characteristics of steelhead trout exposed to alternative hatchery conditions during the final months of rearing. Aquaculture 222, 101-117.

Øxnevad, S.A. & Poléo, A.B.S. (1998). Effekten av vannkvalitetsendringer under høstflommer på fysiologien til lakseparr (*Salmo salar*) i to sidebekker til Suldalslågen. Statkraft Engineering rapport SE 98/87, 30 pp.