The effect of high and low particle load on smoltification of Atlantic Salmon (*Salmo salar*) in RAS

Thesis for the fulfilment of the degree

Master of Science in Aquaculture

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June 2023

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Acknowledgment

I feel like I have gotten some of the best supervisors available. To my main supervisor Pablo Balseiro Vigo, you have always been available when I needed guidance, and given me great feedback and motivation to keep going. Ana Cristina Da Silva Gomes, thank for the help with the statistics and results. Sara Calabrese, it has been a joy working with you during samplings. And I would like to thank all of you for the continuous support throughout the writing of the thesis, you stood by me until the last hour. I would like to thank Sigurd Stefansson for helping me get a new masters project after my previous one had to be ended. And for helping me shaped this thesis in a good direction.

I would like to thank the Department of Biological science, and everyone that made my life easier while doing lab work. Especially Cindy Pinto Pedrosa, for teaching me about the different methods used in the lab, your patience is amazing.

I would like to thank NIVA for providing me with the master's project. I have learned allot from the whole MikroRAS project than will be useful later in me professional journey.

On a personal note, I would like to thank my family, especially my mom and dad. You have always supported me throughout my whole life and guided me to make the right decisions. Mom, you have provided me guidance and help with a lot of school assignments from the very first grade and even now at the university. Dad, thank you for introducing me to the aquaculture industry, without you I would not have been inspired to go down this path.
I would like to thank my girlfriend of 8 years for the continuous support and help, hope you also have learned something about salmon after countless of hours listening to my continuous thoughts about the thesis.

Lastly, I would like to express my gratitude towards my fellow students on Aquaculture. New friendships have made the last five years a pleasure, and hopefully some will last for the rest of my life. Thanks to the BOYS at the study hall for keeping me entertained between the countless of hours of lab work.

This thesis is a part of the FHF – Norwegian Seafood Research Fund project MikroRAS: «Mikrobiota assosiert med atlantisk laks i overgangen fra ferskvann til sjøvann i RAS: Effekt av høy/lav partikkelbelastning og konsekvenser for fiskevelferd og -helse» (Project number: 901735).

Abstract

This study explores the effect of high and low Total Suspended Solids (TSS) on smoltification, and ion regulation of Atlantic salmon (*Salmo salar*) reared in a Recirculating Aquaculture System (RAS). As the salmon aquaculture faces challenges, including sealice, disease, and environmental stressors while in open sea cages, the industry is progressively turning towards land-based RAS to reduce the time salmon smolts spend in seawater. Within these systems, TSS can accumulate if not properly managed, posing a potential threat to fish health and welfare.

Effect of TSS on smoltification and ion regulation of Atlantic salmon in RAS was studied through analysis of Na^+/K^+ ATPase (NKA) activity, gene expression of isoforms *nkaa1a nkaa1b and nkcc1a* and plasma concentration of sodium and chloride. The results indicate that a high TSS concentration within the frame of this experiment might affect the smoltification timeline and induce physiological stress. However, Atlantic salmon exhibit compensatory and adaptive mechanisms to ensure successful smoltification under the tested conditions. Despite this resilience, to optimize smoltification, growth and health for salmon in commercial RAS, results from this study suggest maintaining low TSS concentrations and avoid sudden peaks until the fish are transferred to seawater.

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Abbreviations

ADP: Adenosine 5’-diphosphate

AM: Assay Mixture

ATP: Adenosine 5’-trisphosphate

BW: Brackish Water

CC: Chloride cell

cDNA: complementary DNA

CF: Condition Factor

CFTR: Cystic Fibrosis Transmembrane Regulator

Cl: Chloride

Cq: Quantification cycle

CV: Coefficient of Variation

FW: Freshwater

GLMM: Generalized Linear Mixed Model

ISE: Ion-Selective Electrode

mOD: milli optical density unit

mRNA: messenger RNA

Na: Sodium

NADH: β-Nicotinamide Adenine Dinucleotide

NKA: Na^+/K^+ - ATPase

nkaα1a: NKA α-1a isoform

nkaα1b: NKA α-1b isoform

NKCC: $Na^+/K^+/2Cl^-$ Cotransporter

nkcc1a: NKCC 1a isoform

NTC: No-Template Control

PIT-Tag: Passive Integrated Transponder Tag

RAS: Resirculating Aquaculture System

rRNA: ribosomal RNA

RT-qPCR: Reverse Transcription Quantitative Polymerase Chain Reaction

SD: Standard Deviation

SE: Standard Error

SGR: Spesific Growth Rate

SW: Seawater

TAN: Total Ammonia Nitrogen

TSS: Total Suspended Solids

1 Introduction

1.1 Atlantic Salmon life cycle

The Atlantic salmon (*Salmo salar*) is an anadromous fish that spends the first part of its life in freshwater (FW) rivers before entering seawater (SW), where it feeds and grows. When sexually mature, the salmon migrates back to the river where it hatched to spawn (Jonsson and Jonsson, 2011). Atlantic salmon spawn in the autumn or winter. Fertilized eggs are embedded in the bottom substrate and hatch in the spring. The larvae feed on the yolk sac for the first few weeks (Jonsson and Jonsson, 2011). When the yolk is consumed, the fry emerges from beneath the gravel, mainly at night (McCormick et al., 1998), and starts to feed on other smaller vertebrates. The fry stage is highly competitive and there is a high mortality before they reach the parr stage. Salmon parr is easily recognizable because of the dark vertical bars on their side (Jonsson and Jonsson, 2011). After the parr life stage, the salmon prepares for life in SW. To do so, the parr must undergo a significant morphological, physiological, and functional transformation before going from FW to SW. This process is termed parr-smolt transformation or smoltification and is a seasonal process occurring in spring. (Stefansson et al., 2008). When the smoltification process is complete, the salmon smolts will migrate downstream from the river and out into the open ocean (McCormick et al., 1998).

1.2 Smoltification and Osmoregulation

Smoltification is a preadaptation that occurs while the fish is still in FW in preparation for a life in SW (Hansen and Mortensen, 1998). The salmon has to go through a significant morphological, physiological, and functional transformation, which includes increased salinity tolerance, size, and schooling behavior (McCormick et al., 1998). When smoltification is complete, the visual difference between parr and smolt is clear: the smolt has a silvering color and darker fins (Johnston and Eales, 1967), and reduced condition factor (CF) (Stefansson et al., 2011).

Environmental factors such as temperature and photoperiod are important triggers to activate smoltification process (Stefansson et al., 2008). To start, the parr must reach a critical size threshold of approximately 7.5-8.5 cm fork length in the autumn (Hansen and Mortensen, 1998). If a parr commits to smoltification, its specific growth rate (SGR) can increase by 4-5

times compared to a non-smoltifying parr (Stefansson et al., 2008). If the parr reaches a size of more than 12 cm in the spring, it will continue the smoltification process (McCormick, 2009). Photoperiod is considered the most important environmental factor in triggering smoltification (Hoar, 1988). In the autumn, the photoperiod decreases, which triggers the parr to start the smoltification process, if it has reached the size threshold above described (Hansen and Mortensen, 1998).

As the salmon is anadromous and travels from hypoosmotic conditions (FW) to hyperosmotic conditions (SW), the ability to absorb water and excrete ions are essential for survival in the ocean (McCormick, 2013). Before smoltification, parr has a poor SW tolerance. Therefore during smoltification, the fish develops a more robust tolerance towards SW by increasing transport proteins and the number and size of the mitochondrion-rich chloride cells (CC) in the gills (Stefansson et al., 2008). In FW, parr is hyperosmotic compared to the water and gains water passively through the gills and lose passively ions through the gills. The parr, therefore, needs mechanisms to remove water and gain ions (Hansen and Mortensen, 1998). The parr gets the monovalent ions sodium and chloride (Na^+, Cl^-) through an active uptake mechanism in the gill epithelium and from feed ingested via the gut epithelium. By excreting diluted urine containing low amounts of ions from the kidney, the parr can maintain internal homeostasis (McCormick, 2013). In contrast, when the parr has gone through smoltification and has reached SW, then the fish is hypoosmotic to SW and has a passive loss of water to the environment. To maintain internal homeostasis in SW, the salmon drink SW and excrete Na^+ and Cl^- across the gills through the CCs. In the blood plasma, Na^+ and Cl^- ions account for 90% of all the dissolved particles (Hansen and Mortensen, 1998).

In FW, the parr has a plasmatic ion concentration of Na^+ that is expected to be between 135-155 mM and between 115 – 135 mM for Cl^- (Hansen and Mortensen, 1998). In SW, the salmon Na^+ plasmatic concentration is likely between 145-165 mM and 130 – 150 mM for Cl^-. A deviation from the expected value by 20-30 % can seriously disturb the ion balance (Casanovas et al., 2021; Hansen and Mortensen, 1998). Plasma ions concentration has been used in studies as an indicator of stress in Atlantic salmon (Carey and McCormick, 1998).

At a molecular level, smoltification induces changes in transport proteins. Three transport proteins are involved in salt secretion in the gill's CC: Na^+/K^+ - ATPase (NKA), the cotransport protein $Na^+/K^+/2Cl^-$ (NKCC), and the cystic fibrosis transmembrane regulator (CFTR) (Nilsen et al., 2007). The NKA protein is located basolateral membrane in both FW CC and saltwater CC but greater numbers in the latter (Evans et al., 2005). The NKA

maintains a low Na^+ concentration and generates a negative charge in the CC cytoplasm (McCormick, 2013). The NKCC is also located in the basolateral membrane and uses the electrochemical gradient made by the NKA to transport Cl^- ions into the CC cytoplasm. The Cl^- ions exit the CC via the apical crypt, facilitated by CFTR, driven by the electrical gradient. This ultimately drives the removal of Na^+ through the paracellular pathway between the CC and accessory cells (AC) (Stefansson et al., 2008).

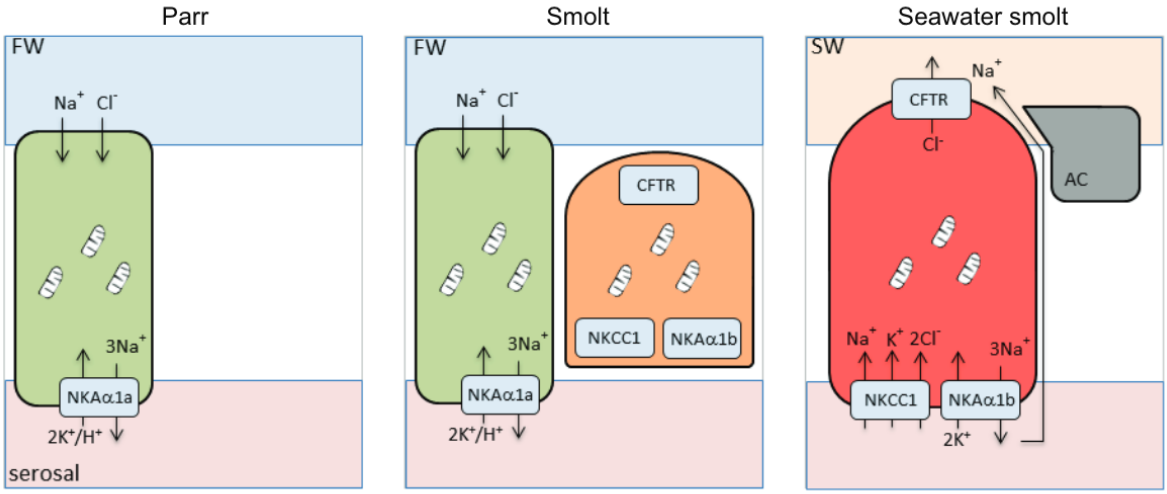

Figure 1.1: Model for the expression of nkaα1a and nkaα1b isoforms during smolt development. *In freshwater (FW), parr and smolt have a gill epithelium which is rich in ionocytes that express the nkaα1a. FW smolts also have an additional class of ionocyte here represented as an orange cell. These ionocytes express nkaα1b or both nkaα1a and nkaα1b, as well as other ion transporters (CFTR and NKCC1) that are important for salt secretion. The seawater (SW) ionocyte, which stems from the orange ionocyte, in SW smolt presented in red, expresses nkaα1b and both ion transporters. Na^+ is removed through the paracellular pathway between the SW ionocyte and accessory cell (AC) (McCormick et al., 2013).*

The NKA protein consists of three subunits: α, β and γ (Nilsen et al., 2007). In Atlantic salmon, the subunit α is important for smoltification and has two isoforms *nkaα1a* and *nkaα1b* (McCormick, 2013). Since smoltification is a preadaptation, both subunits will be present in FW CC, but as *nkaα1a* is linked to the active uptake of monovalent ions, it has a higher mRNA expression (McCormick et al., 2013). On the other hand, the *nkaα1b* isoform is linked to the excretion of monovalent ions through SW CC and, therefore, more prominent in SW smolts (McCormick, 2013). McCormick (2009) has discovered, using confocal microscopy, that *nkaα1a* is located at both the gill filament and lamella in FW, while the *nkaα1b* is only located in the gill filament in FW and both in SW.

During smoltification, *nkaα1a* mRNA expression decreases, concurrent with an increase in the mRNA abundance of *nkaα1b* and cotransporter *nkcc1a* (Nilsen et al., 2007). An increase of *nkaα1b* and *nkcc1a* is linked to a rise in NKA gill activity. An increase in activity indicates that the protein has a higher rate of excreting ions across the cell membrane (Suhail, 2010). Therefore, NKA gill activity reflects the development of hypo-osmoregulatory ability and SW tolerance and is a biomarker of a successful smoltification (Pino Martinez et al., 2021).

1.3 Aquaculture in Norway

The Norwegian salmon aquaculture has gone through a massive development since 1971. In that year (1971) the total production volume of Atlantic salmon was 531 tons. This has increased over the years, and in 2022, the production of Atlantic salmon alone was 1.26 million tons with a total export value of 105.8 billion Norwegian crown (Andersen et al., 2023; Bjørgo et al., 2011).

The main production technology in the industry is open net pens, and this has remained almost unchanged since the 1970s. However, the industry has faced an increased number of problems throughout history and challenges such as parasitic salmon lice (*Lepeophtheirus salmonis*), escapees, disease, feed residues, and other discharges still persists (Afewerki et al., 2023). To combat these challenges, one strategy has been to produce the salmon smolt in land-based facilities for a longer period. This allows to decrease the time of the salmon in SW and, thus reduces its exposure to salmon lice, diseases, and other environmental stressors (Ytrestøyl et al., 2020).

Different technologies as Flow Through Systems (FTS) and Recirculating Aquaculture Systems (RAS) have been adopted. RAS is now frequently used in the production of Atlantic salmon. The positive aspect of RAS is its ability to reuse up to 99% of the water, compared to FTS, where almost no water is reused (Bregnballe, 2022). FW exists in lakes, rivers, ice, and as groundwater; however, it is a limited resource as it only makes up about 3 % of all water on earth (Brown, 2022). Thus, RAS allows the production of aquatic species in remote locations with limited access to FW sources (Ebeling and Timmons, 2012).

Even though RAS has emerged as a popular choice for salmon production, it has its own challenges. Mechanically the biggest challenge is suspended soil removal, as they have an important impact on the performance of almost all the components in the RAS (Badiola et al., 2012). As the production has been intensified in RAS, an increase in male early maturation has occurred (Pino Martinez et al., 2021). Early maturation and smoltification are

both triggered and developed in parallel in male Atlantic salmon, but the progression of maturation impaired with hypoosmoregulation (Fjelldal et al., 2018).

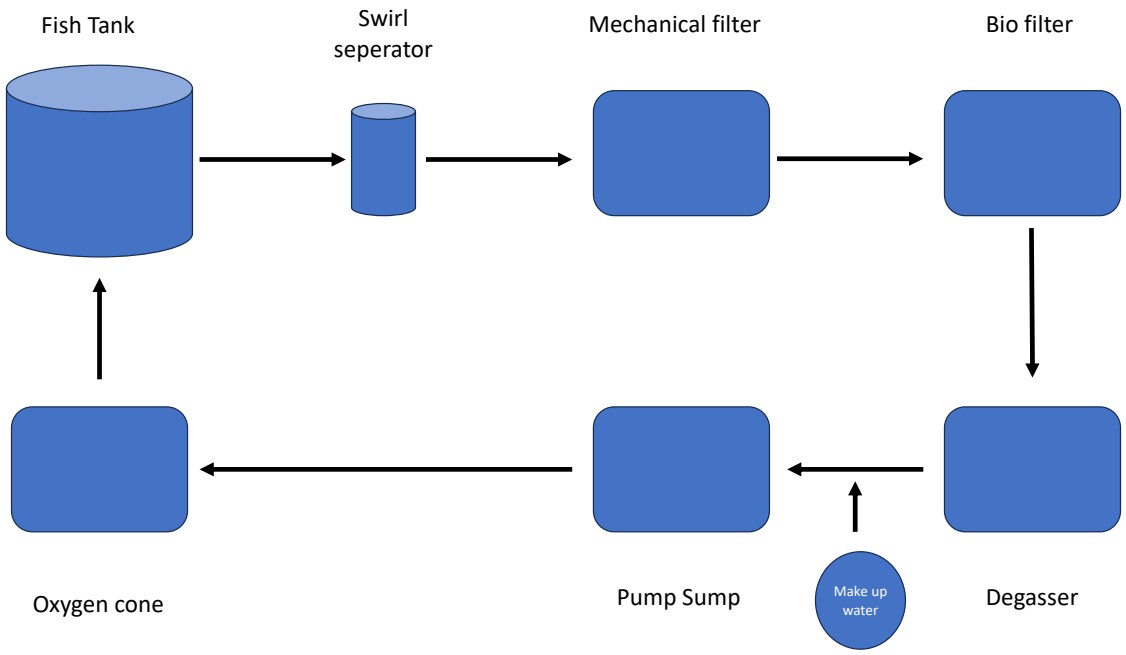

Figure 1.2: *The RAS setup at RASlab.*

The RAS consists of several different units, including the main culture tank (Figure 1.2). Because the fish waste will accumulate over time in a closed system, it is vital to treat the water continuously by adding oxygen (O_2), and removing carbon dioxide (CO_2) and ammonia (NH_3) excreted from the fish (Bregnballe, 2022). The first unit after the culture tank is often a drain that takes advantage of the sinking properties of the larger particles. This can be done with a swirl separator, mort collector, or settling basin. The larger particles sink to the bottom while the water goes through a valve at the top (Ebeling and Timmons, 2012).

The next unit is the mechanical filter, widely used to remove suspended solids in recirculating systems. The mechanical filter has a rotating microscreen filter that works as a sieve and prevents suspended particles larger than the filter pore size from passing through (Ebeling and Timmons, 2012). The microscreen filter is between 40 to 100 μm. The solids trapped in the filter are lifted to a backwash area by the rotating drum, and waterjets spray the particles into a sludge tray (Bregnballe, 2022). After the mechanical filter, water passes through UV and ozone disinfection units. UV kills bacteria and viruses, and ozone kills microorganisms and oxidizes particles increasing the water clarity (Fjellheim et al., 2016).

The main objective of the biofilter is to remove Total Ammonia Nitrogen (TAN) by nitrification. TAN is excreted by the fish through the gills, urine, and feces, and can also be

produced by bacteria decomposition waste. TAN can have two states, ammonia (NH_3) and ammonium (NH_4^+), depending on the pH level. When the pH level rises above 8, the unionized form NH_3 is more prominent, and this state is also the most toxic to the fish (Arogo et al., 2002; Fjellheim et al., 2016). NH_3 is toxic to fish as it can replace the uptake of Na^+, and induce biochemical and structural changes (Randall and Wright, 1987). However, the fish have multiple strategies to cope with the toxic effect of NH_3, including converting it into less harmful substances, increasing the excreting, and minimizing its production. Despite these adaptive mechanisms, NH_3 remains detrimental to fish at high levels (Kolarevic et al., 2012). Therefore, it is important to use nitrification to reduce the amount of TAN in the water. Nitrification is a two-step process where specialized bacteria oxidize TAN to nitrite (NO_2^-) first and then to nitrate (NO_3^-). Nitrate is less toxic to fish than NH_3^+ and NO_2^- (Ebeling and Timmons, 2012)

A degasser is used to remove carbon dioxide (CO_2) from the water in the RAS system (Figure 1.2). Both fish and heterotrophic bacteria produce CO_2 through metabolism. Increased concentrations of CO_2 in the water lower the pH (Fjellheim et al., 2016), which can have several negative health effects on salmon, where higher (>12 mg/l) doses of CO_2 significantly reduces the salmons' CF and SGR and increased the ventilating frequencies (Fivelstad et al., 2018; Hosfeld et al., 2008; Mota et al., 2019). In the degasser, air bubbles are injected at the bottom of the tank, and turbulence contact between the air bubbles and the water drives out the gases CO_2 and nitrogen (Fjellheim et al., 2016). Before the water enters the fish tank again, oxygen is added from an oxygen cone (Bregnballe, 2022).

1.4 Effect of TSS in aquaculture

Total Suspended Solids (TSS) refers to the concentration (mg/l) of inorganic and organic matter which is suspended in the water by turbulence (Bilotta and Brazier, 2008). Since RAS is a closed system, TSS will accumulate over time if the treatment process is not managed correctly (Molleda, 2008). TSS consists of fish feces, uneaten feed, bacterial matter from the biofilter, and microbiota, and deteriorates the water quality if accumulated over time (Becke et al., 2019; Noble and Summerfelt, 1996). More than 95% of TSS in RAS have a diameter of less than 20μm, and fine particles around 5-10 μm are widely regarded as harmful to fish health and welfare (Chapman et al., 1987; Chen et al., 1993).

Indeed, it has been found that TSS can cause damage to gill structures and increase stress levels in fish (Awata et al., 2011; Bilotta and Brazier, 2008). Thus, in RAS, it is recommended to maintain the concentration of TSS below 25 mg/l (Coche, 1981).

TSS can act as a growth substrate for heterotrophic bacteria and pathogenic bacteria, which can outcompete vital nitrifying bacteria within the biofilter. This could disrupt the delicate balance of the biofilters microbiome, reducing its effectiveness in maintaining water quality (Fjellheim et al., 2016; Soaudy et al., 2023). Moreover, heterotrophic bacteria use oxygen during the degrading of organic matter, which may lead to low dissolved oxygen concentration (Ryan, 1991). If the fish experience a decline in dissolved oxygen, the appetite will gradually decrease, and it will experience respiratory stress and, finally, mortality (Remen, 2012).

Increased TSS is linked to increased turbidity. Turbidity is a measurement of the optical property of the water, where higher turbidity is correlated with a higher concentration of TSS (Bash et al., 2001). The presence of higher turbidity and TSS increases the absorption and scattering of light. Since the Atlantic salmon is a visually oriented predator, an increase in turbidity can suppress the visual cues and reduce rate and efficiency of feeding, impairing the growth of the salmon (Ali, 1959; Becke et al., 2019; Berg and Northcote, 1985; Schumann and Brinker, 2020).

Since the negative effect of suspended solids was recognized, there have been multiple studies on the TSS impact on the health of salmonids and other fish. Hughes and Morgan (1973) discovered that exposure to high concentrations of suspended solids thickened the gill epithelium of rainbow trout (*Oncorhynchus mykiss*) and caused fusion of the adjacent lamella. Chapman et al. (1987) looked at the effect of high sludge concentrations (1000 mg/l) on rainbow trout and reported 100% mortality because of respiratory failure and gill filaments breaking down. Becke has multiple studies on the effect of TSS on rainbow trout in the RAS (Becke et al., 2019, 2018, 2017). The first study focused on the short-term effect of High TSS exposure on rainbow trout, showing no significant differences in the fish health but a reduced appetite was observed in the High TSS group (Becke et al., 2017). Further, studies on the long-term effect of High TSS exposure on rainbow trout have shown similar results (Becke et al., 2019, 2018). There are no studies on the TSS effect on NKA activity, mRNA abundance, or plasma ions in salmonids. However, Au et al. (2004) looked at the TSS effect on osmoregulation in Green grouper (*Epinephelus coioides)*, and reported a significant decrease in NKA activity and increase in CC density at a TSS concentration of 2000 mg/l. (Lin et al., 1992) investigated the effect of increased suspended solids on gill NKA activity in shrimp

(*Penaus japonicus*) and discovered that the NKA activity increased when exposed to higher turbidity as a compensating mechanism to the disruption of osmotic and ionic balance.

1.5 Motivation and Aim of the Study

A noticeable knowledge gap exists on the effect of total suspended solids on the fish gills and its impact on smoltification, growth, stress, and behavior in Atlantic salmon reared in RAS. Thus, this thesis aims to investigate the effect of High TSS and Low TSS in RAS on the smoltification process and ion regulation of Atlantic salmon by analyzing NKA activity, gene expression of isoforms *nkaα1a nkaα1b and nkcc1a,* and plasmatic concentration of sodium and chloride. This will contribute with new knowledge to the aquaculture industry to optimize rearing conditions and water quality in RAS.

The study was based on the following hypotheses

$H0_{1A}$: High TSS treatment in RAS has no significant effect on Condition Factor (CF) in Atlantic salmon.

HA_{1A}: High TSS treatment in RAS has a significant effect on Condition Factor (CF) in Atlantic salmon.

$H0_{2A}$: High TSS treatment in RAS has no significant effect on Specific Growth Rate (SGR) in Atlantic salmon.

HA_{2A}: High TSS treatment in RAS has a significant effect on Specific Growth Rate (SGR) in Atlantic salmon.

$H0_{3A}$: High TSS treatment in RAS has no significant effect on NKA activity in Atlantic salmon gills.

HA_{3A}: High TSS treatment in RAS has a significant effect on NKA activity in Atlantic salmon gills.

$H0_{4A}$: High TSS treatment in RAS has no significant effect on *nkaα1a* mRNA abundance in Atlantic salmon gills.

HA_{4A}: High TSS treatment in RAS has a significant effect on *nkaα1a* mRNA abundance in Atlantic salmon gills.

$H0_{5A}$: High TSS treatment in RAS has no significant effect on *nkaα1b* mRNA abundance in Atlantic salmon gills.

HA_{5A}: High TSS treatment in RAS has a significant effect on *nkaα1b* mRNA abundance in Atlantic salmon gills.

$H0_{6A}$: High TSS treatment in RAS has no significant effect on *nkcc1a* mRNA abundance in Atlantic salmon gills.

HA_{6A}: High TSS treatment in RAS has a significant effect on *nkcc1a* mRNA abundance in Atlantic salmon gills.

$H0_{7A}$: High TSS treatment in RAS has no significant effect on plasmatic sodium concentration in Atlantic salmon.

HA_{7A}: High TSS treatment in RAS has a significant effect on plasmatic sodium concentration in Atlantic salmon.

$H0_{8A}$: High TSS treatment in RAS has no significant effect on plasmatic chloride concentration in Atlantic salmon.

HA_{8A}: High TSS treatment in RAS has a significant effect on plasmatic chloride concentration in Atlantic salmon.

$H0_{9A}$: High TSS treatment in RAS has no significant effect on plasmatic sodium concentration in Atlantic salmon after simulated transport.

HA_{9A}: High TSS treatment in RAS has a significant effect on plasmatic sodium concentration in Atlantic salmon after simulated transport.

$H0_{10A}$: High TSS treatment in RAS has no significant effect on plasmatic chloride concentration in Atlantic salmon after simulated transport.

HA_{10A}: High TSS treatment in RAS has a significant effect on plasmatic chloride concentration in Atlantic salmon after simulated transport.

2 Material and Methods

2.1 Ethical statement

This experiment was conducted in accordance with the Guidelines of the Norwegian regulation on Animal Experimentation, and the experimental protocol was reviewed and approved by the Norwegian Animal Research Authority (FOTS ID 29601, approved 04.07.2022).

2.2 Experimental facility

Figure 2.1: Picture of RASlab: *Picture showing three separate RAS modules, with the rearing tanks in the forefront of the picture. Picture: (GC Rieber Eiendom, 2022).*

The first stage of the experiment, which lasted until the fish was transferred into SW, was conducted at the RASlab (Figure 2.1), which is located at Marineholmen in Bergen, Norway, and it is a research and innovation company focusing on Recirculating Aquaculture System (RAS) technology. The facility consists of 12 independent 2.5 m^3 RAS systems. Each system comprised a 1 m^3 fish tank, followed by a 464 mm diameter swirl separator, a 40 µm drum filter, a moving bed biofilter of 0.58 m^3 stocked to 65 % with 625 m^2/m^3 (A_{media} = 377

m^2) mature bio media (KSK Saddle Chips 1.0, KSK Aqua Aps, Skive, Denmark) and a trickling degassing chamber.

The hatching of the fish eggs and the last part of the experiment, the SW stage, was conducted at the Industrilabratoriet i Bergen (ILAB). ILAB is a foundation that facilitates public and privately funded research within aquaculture and sustainable land-based fish farming. ILABs Post-smolt Hall was used in this experiment and is a facility comprising ten 1800 l tanks with a flow-through seawater system.

2.3 Experimental design

Fry were tagged with a Passive Integrated Transponder (PIT) on the 26th of April 2022, and transferred from ILAB to the RASlab experimental tanks on the 20th of July 2022. The experiment was carried out between the 8th of August 2022 and the 31st of January 2023, with the following three different stages: Recirculating aquaculture system (RAS), simulated transport, and ending in a flow-through aquaculture system. To establish the high particle-load treatment at the start of the project, the waste was collected in the swirl tank, and the fecal matter was separated from the feed waste before being introduced back into the system daily.

The small-scale RAS stage experiment took place between the 8th of August 2022 and the 14th of December 2022. The trial was conducted in six replicated RAS. Before fish stocking, each of the six RAS was randomly assigned to one of two treatments: High or Low total suspended solids (TSS) load. On day 1, all fish were weighed, and measured for fork length and each system was stocked with an initial density of 7.7 kg/m^3 of Atlantic salmon (mean weight of 39.5 ± 4.3 g). The fish were fed with Skretting Nutra RC 2- or 3-mm pellets daily and on-demand with 15% in excess of expected feed intake based on RASlab feeding tables. Tanks 5, 7, and 8 were kept at a low organic load (mean of 1.13 ± 0.83 mg/l TSS in freshwater (FW) and 2.06 ± 1.51 mg/l TSS in brackish water (BW)), and tanks 4, 6, and 9 were kept at a high organic load (mean of 6.77 ± 6.86 mg/l TSS in FW and 7.31 ± 6.66 mg/l TSS in BW). Each system automatically controlled salinity, dissolved oxygen, temperature, and pH (Georg Fischer AS, Rud, Norway). The fish were maintained at 12.3 ± 0.8 °C, under 93.0 ± 5.6 % O_2saturation, and salinity of 1.4 ± 0.1‰. From the start, 8th of August, until the 14th of September, the fish received a winter signal photoperiod of (12L:12D). From the 14th of September until SW transfer (14th of December), the fish were

under continuous lighting (24L:0D). On the 13th of October, the salinity was increased to 15 ± 0.9‰ to establish brackish conditions.

On the 14th of December, the fish underwent a six-hour simulated transport. Four tanks of 600 l of seawater were used. Each tank was distributed with an oxygen supply. All the fish from Low TSS were transferred and equally distributed between transport tanks 1 and 2, and the fish from High TSS to transport tanks 3 and 4. Each tank had a fish density of 90 ± 10 kg/m^3. After the simulated transport, all the fish were mixed and randomly dispersed in three different 2.5 m^3 tanks at ILAB, with flow-through seawater, simulating open sea cage production. In this period, the salinity was 33.6 ± 0.7 ‰ and fish were reared under a continues light photoperiod (24L:0D). The temperature was 8.9 ± 0.2 °C, and O_2 saturation of 88.7 ± 3.4 %. The last sample point in this MSc project was the 31st of January (16 days after transfer). However, the project, in which this thesis is a part of ended in late March, and the growth data is included until then. The sampling dates are summarized in Table 2.1.

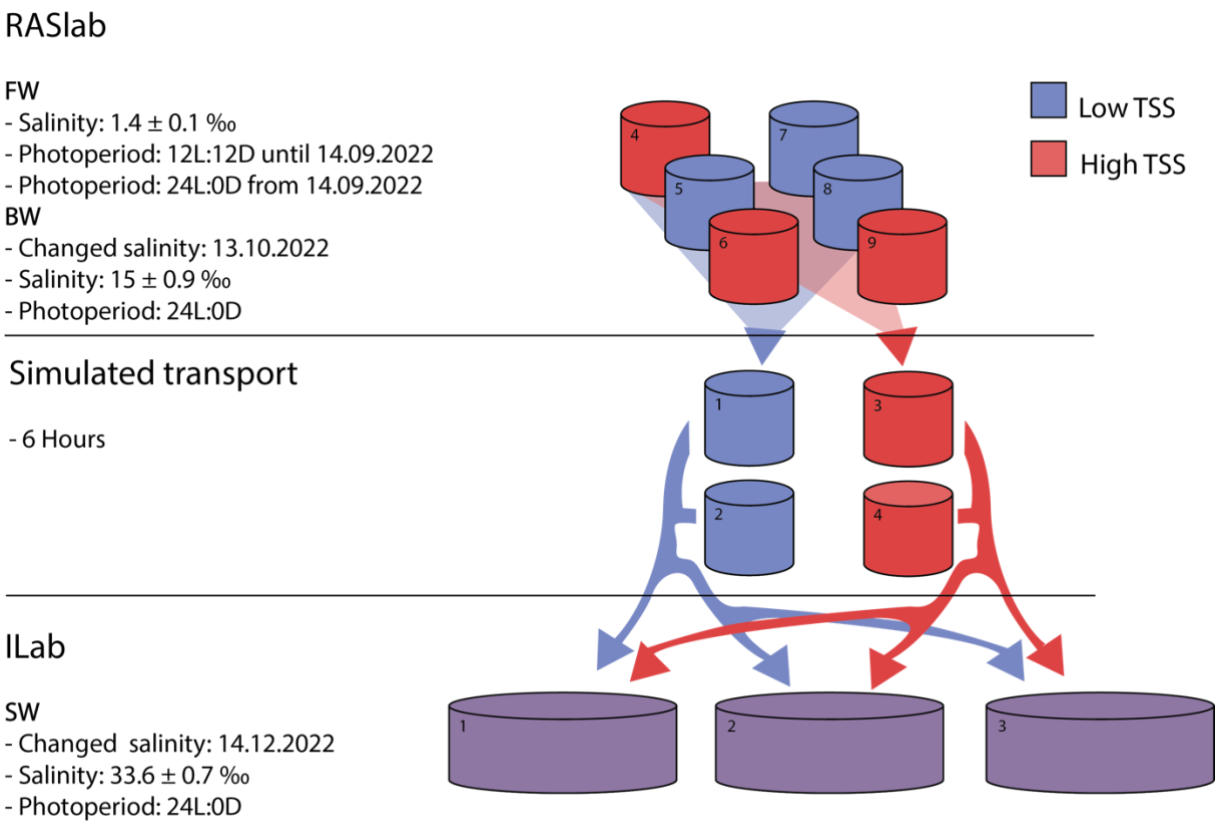

Figure 2.2: Scheme of the experimental design: *Top section showing experimental design at RASlab, where red tanks are High TSS treatment and blue tanks Low TSS treatment. The second section shows the simulated transport experimental design, and which tanks contain each treatment. The last section shows experimental design at ILab, where arrows illustrate fish being randomly put in different tanks.*

2.4 Sampling protocol

In total, 12 sampling points were conducted (and used) in this study, the last two was only for biometric data. The initial sampling point T0 was before experimental conditions on the 8th of August 2022. The next four sampling points (T1 to T4) occurred during the freshwater stage, followed by three sampling points (T5 to T7) in the brackish water stage. The final sampling point (T8) was taken during the seawater stage. A simulated transport was conducted between the T7 and T8 sampling points. For the transport stimulation experiment, blood samples were collected from 24 fish randomly at three different times: before transport, immediately after transport, and three hours after transport. T9 and T10 was only for biometric data.

Table 2.1: *Dates, photoperiod, salinity, and the analyses corresponding to the different sampling points. ST: Simulated transport.*

Samplings	**T0**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**ST**	**T8**	**T9**	**T10**
Date	08.08.22	23.08.22	13.09.22	20.09.22	18.10.22	25.10.22	15.11.22	06.12.22	14.12.22	15.12.22	31.01.23	21.03.23
Photoperiod	12L:12D	12L:12D	12L:12D	24L:0D	24L:0D	24L:0D	24L:0D	24L:0D		24L:0D	24L:0D	24L:0D
Salinity	FW	FW	FW	FW	BW	BW	BW	BW	BW/SW	SW	SW	SW
Biometry	X		X		X			X			X	X
Plasma ions	X	X	X	X	X	X	X	X	X	X		
NKA act	X	X	X	X	X	X	X	X		X		
Gene exp	X	X	X	X	X	X		X		X		

At each sampling point, three fish were randomly netted from each tank at two separate intervals (n=6 per each tank), and then humanly euthanized with a lethal dose of 150 mg/l MS222 before sampling. Blood was collected within the first 1-3 minutes after netting. Blood was sampled from the caudal vein using 1-3 ml (depending on fish size) heparinized syringes with 23G needles to prevent blood coagulation. The samples were centrifuged for 8 min at 5000 rpm to separate the plasma from the blood. At least 200 ml of plasma was transferred into 0.5 ml Pentra sample tubes and frozen on dry ice. After sampling, the samples were transported to Høyteknologisentert, Bergen, and stored at -80 °C until further analysis. After the blood was sampled, the fish PIT tag was read, and the biometric data was collected by measuring weight and length. At the end of each sampling, to determine the gonadosomatic index, the gonad was removed and weighed and the gender recorded.

The third-gill arch on the left side of the fish was collected and put in Eppendorf 1.5 ml tubes containing SEI buffer (250 mM Sucrose, 10 mM Na_2EDTA, 50 mM Imidazole) to analyze NKA activity. The samples were immediately frozen on dry ice, before transported to

Høyteknologisentert, Bergen, and stored at -80 °C until further analysis. The third-gill arch on the right side was collected and put in Eppendorf 1.5 ml tubes containing RNAlater (Sigma-Aldrich, USA) for mRNA transcription analysis. The samples were transported to Høyteknologisentert, and stored overnight at 4 °C to allow the solution to penetrate the tissue before storing at -80 °C until further analysis.

2.5 Analytical analysis

2.5.1 Na^+/K^+ - ATPase protein activity gills

Na^+/K^+ - ATPase protein activity in gills (NKA) was determined using McCormick´s method (McCormick, 1993). Assay mixture (5 U/ml Pyruvate Kinase, 4 U/ml Lactic Dehydrogenase, 0.22 mM b-Nicotinamide Adenine Dinucleotide, 0.7 mM Adenosine Triphosphate, 2.8 mM Phosphoenolpyruvate, 50 mM Imidazole buffer) was prepared. For validation analyses of the assay mixture, an Adenosine diphosphate (ADP) (Sigma-Aldrich, USA) standard was conducted with a concentration range of (0, 25, 50, and 100 µl ADP stock, and 200, 175, 150, and 100 µl Imidazole buffer, Total volume 200 µl), with the objective of obtaining at least 16 mOD nmol ADP per well (mOD = milli optical density unit).

The NKA activity was determined from gill samples that were thawed just before collecting 2 to 6 gill filaments (depending on size) into a 0.5ml Eppendorf vial containing 100 ml of SEI buffer (250 mM Sucrose, 10 mM Na_2EDTA, 50 mM Imidazole) and 25 ml of 0.5 % SEID buffer Then, the tissue was homogenized with a motor pestle (VWR, USA) for 10 s. The homogenized tissue was centrifuged for 1 min at 5000 g at 4 °C using a centrifuge 5424 R (Eppendorf, Hamburg, Germany). From the sample, 10 ml of supernatant was added in each well as quadruplicates to a 96-well Nunc microplate (VWR™ Microwell™, ThermoFisher Scientific Inc., USA) to measure NKA activity. To determine protein content, a triplicate of each sample was added to the Sigma Costar protein plate (Sigma-Aldrich, USA). The assay mixture was divided into two: one containing only the assay mixture (AMA) and one containing 0.5 mM ouabain (AMB). Both AMA and AMB had a salt solution (50 mM Imidazole, 189 mM NaCl, 10.5 mM $MgCl_2.6H_2O$, 42 mM KCl) added before the analyses of the samples. 200 ml of AMA and AMB was each added to each half of the quadruplicate sample on the 96-well Nunc microplate. Then absorbance was measured at the Tecan Spark reader (Tecan Group, Switzerland) at 340 nm at a temperature of 25 °C. The program consists of 60 cycles lasting 10 s over 10 minutes. The Tecan Sparks measures the linear rate of NADH disappearance, which is quantified as mOD/10ml/min. To calculate the specific NKA

activity, the measurement taken in the presence of ouabain (AMB) is subtracted from the measurement without ouabain (AMA) using the following formula:

$$Na^+/K^+ - ATPase(\text{mOD}/10\mu\text{l}/\text{min} = \text{AMA mOD}/10\text{ ml}/\text{min} - \text{AMB mOD}/10\text{ ml}/\text{min}$$

The activity measurement was divided by the standard curve to yield a measure of nanomoles ADP per minute:

$$\frac{Na^+/K^+ - ATPase(\text{mOD}/10\mu\text{l}/\text{min})}{ADP\ Standard\ curve\ (mOD(nanomoles\ ADP))} = nanomoles\ \text{mOD}/10\mu\text{l}/\text{min}$$

The Pierce BCA Protein assay kit (ThermoFisher Scientific Inc., USA) was used for colorimetric detection and quantification of the total amount of protein in the samples. A Bovine Serum Albumin (BSA) standard mixture was created using 2 mg/ml BSA standard and MQ water, then 10 µl was pipetted to the protein plate as triplicates. A working reagent was created by mixing Reagent A and B from the kit with a ratio of 50:1 (v/v). 200 µl of the working reagent was pipetted in the wells containing the samples. A protecting film covered the protein plate to prevent evaporation before shaking for 30 s. Then it was incubated for 30 minutes at 35 °C. The protein plate was left at room temperature for two minutes before the absorbance was measured in the Tecan Spark multimode microplate reader (VWR™ Microwell™, ThermoFisher Scientific Inc., USA) at 562 nm (end-point). The output of this analysis is expressed µg protein/10 µl and is used to calculate the NKA activity. The following formula is used for this calculation:

$$\frac{\text{nanomoles ADP}/10\mu\text{l}/\text{min}}{\mu\text{g}/10\ \mu\text{l protein}} = \mu moles\ ADP/mg\ protein/min$$

2.5.2 RNA isolation, quantification, and qPCR

2.5.2.1 Total RNA isolation

Total RNA from gill samples were isolated using the following procedure (Chomczynski, 1993). Approximately 60 mg of gill tissue was added into tubes containing 1 ml TRI Reagent Reagent (Thermo Fisher Scientific Inc., USA) and one 5 mm stainless steel bead (Quiagen, Germany), and kept in an ice block for 5 minutes. The tubes were then transferred into a Precellys 24 homogenizer (Bertin Technologies, France) and tissue homogenized at a speed of 5000 rpm for 15 s. After, the samples were kept at room temperature for 5 min and then 200 µl of chloroform (Sigma-Aldrich, USA) was added, vortexed for 30 s, and centrifuged for 15 min at 4 °C and 13200 g (Centrifuge 5424 R, Eppendorf). The aqueous phase containing RNA was transferred into new tubes and 500 µl isopropanol (Sigma-Aldrich, USA) was added. The tubes were inverted five times and left at room temperature for 10 minutes before centrifuged for 10 minutes at 4 °C and 13200 g. The supernatant was removed, and the pellet was left in the tube. The pellet was washed by adding 1 ml of 80 % ethanol and centrifuged for 10 min at 4 °C and 10000 g. The supernatant was removed, and the pellet was left to dry on the ice block for 5-10 min. The pellet was resuspended with 70-135 µl of UltraPure™ distilled water (Invitrogen™, ThermoFisher Scientific Inc., USA), depending on the pellet size. After, the samples were incubated on a heatblock (VWR, USA) for 10-15 min at 57 °C to allow the pellet to be completely dissolved. RNA purity was assessed with the NanoDrop One (ThermoFisher Scientific Inc., USA). The purity was confirmed with 260/280 nm and 260/230 nm absorbance above 1.7.

2.5.2.2 DNase treatment

The TURBO DNA-free kit (ThermoFisher Scientific Inc., USA) was used to remove any possible genomic DNA contamination from the samples. RNA was added to a 0.2 ml tube (PCR tube). In a final reaction of 30 µl, 10 µg of total RNA, UltraPure distilled water (Invitrogen, ThermoFisher Scientific Inc., USA), 0.1 volume (3µl) of 10X TURBO DNase buffer and 1 µl of TURBO DNase enzyme were combined and mixed gently before a 30-minute incubation at 37 ºC in a GeneAmp® PCR System 2700 (Applied Biosystems, USA). Then, 0.1 volume (3 µl) of DNase inactivation reagent was added, and the reaction was incubated at room temperature for 5 minutes. The tubes were placed in a centrifuge for 1.5 minutes at 10000 g and the aqueous phase containing the total RNA was transferred to new 25 µl tubes. The RNA purity was determined with the NanoDrop One, and the total RNA

concentration in the gill samples was measured using the Qubit® 4 fluorometer (Invitrogen; ThermoFisher Scientific Inc., USA). The manufactures protocol was used on the Qubit™ RNA BR assay kit (Invitrogen™, ThermoFisher Scientific Inc., USA). The measurement was set to Broad Range. The samples were then stored at -80 °C.

2.5.2.3 Complementary DNA (cDNA) synthesis

Total RNA (1000 ng) was reverse transcribed to cDNA using SuperScript™ III Reverse Transcriptase kit (ThermoFisher Scientific Inc., USA). Samples were normalized in UltraPure™ distilled water (Invitrogen™, ThermoFisher Scientific Inc., USA) to a total volume of 11 µl in order to get a total amount of 1000 ng of RNA. A mix 1# was made with 1 µl of the reagent Oligo $(dt)_{20}$ (50µM) and 1 µl 10 mM dNTP Mix. 2 µl of the mix was added to each sample tube, creating a total volume of 13 µl. The tubes were incubated in the T100 Thermal Cycler (Bio-Rad Laboratories, USA) at 65 °C for 5 minutes. Mix 2# was made using 4 µl 5X First-Strand Buffer and 1 µl of 0.1 M DTT, 1 µl 40 U RNaseOUT™ Recombinant RNase inhibitor, and 1µl 200U Superscript™ III-RT. 7 µl of mix 2# was added to the sample tubes resulting in a final volume of 20 µl. The sample tubes were incubated at 50 °C for 50 minutes before the reaction was inactivated by heating them at 70 °C for 15 minutes. Reverse transcribed samples were stored at -20 °C until further analysis.

2.5.2.4 Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Real-time quantitative PCR (RT-qPCR) was carried out to measure the mRNA transcription of *nkaα1a*, *nkaα1b*, and *nkcc1a* in the gills, and the reference genes *ef1α* and *rps20* using the specific primers listed in the Table 2.2. To determine the efficiency of the primers and determine the optimal dilution for the cDNA to use for all the samples, a pool of 32 random samples was used in a triplicate two-fold dilutions (1:5, 1:10, 1:20, 1:40, 1:80, and 1:160) were used to create a dilution curve. The regression line of the dilution curve was used to determine the efficiency of the primers using the following formula (Pfaffl, 2001):

$$E = 10^{\left(-\frac{1}{Slope}\right)}$$

For a complete overview of the dilution curves, primer efficiency, and melting curves, see appendix 1.

Table 2.2: Primers used for gill qPCR: *List of the qPCR primer sequences used to measure mRNA transcription of targeted nkaα1a, nkaα1b, nkcc1a, and reference genes ef1α and rps20.*

Gene	Primer sequence (5'>3')	Reference
nkaα1a	F: CCAGGATCACTCAATGTCACTCT R: CAAAGGCAAATGAGTTTAATATC	(Nilsen et al., 2007) / Modified after Nilsen et al., 2007
nkaα1b	F: GCTACATCTCAACCAACAACATTACAC R: TGCAGCTGAGTGCACCAT	(Nilsen et al., 2007)
nkcc1a	F: GATGATCTGCGGCCATGTTC R: TCTGGTCATTGGACAGCTCTTTG	(Nilsen et al., 2007) / (Esbaugh et al., 2014)
ef1α	F: CCCCTCCAGGACGTTTACAAA R: CACACGGCCCACAGGTACA	(Olsvik et al., 2005)
rps20	F: GCAGACCTTATCCGTGGAGCTA R: TGGTGATGCGCAGAGTCTTG	(Olsvik et al., 2005)

The qPCR reaction was performed using a total volume of 12.5 ml that included 6.25ml of iTaq^{TM} Universal SYBR (Bio-Rad Laboratories, USA), 3.25 ml of nuclease-free water, 0.5 ml of reverse and forward Primer (10 µM), and 2.5 ml of 1:40 diluted cDNA (1.25 ng/µl) for a total volume of 12.5 ml. A 96-well plate (Bio-Rad Laboratories, USA) was used for the analyses, and samples were run in duplicate, two wells with non-template control (NTC), and two wells with a cDNA pool were used for intercalibration among the plates. The qPCR was conducted using a C1000^{TM} Thermal Cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA) and CFX Manager Software version 3.1 (Bio-Rad Laboratories, USA). The following cycle program was used:

1. 95 °C for 3 minutes
2. 35 cycles of 95 °C for 15 seconds and 60 °C for 1 minute
3. 95 °C for 10 seconds
4. Followed by a melting curve analysis over a 65 – 95 °C temperature range, the temperature was incremented by 0.5 °C in each cycle and ran for 5 seconds.

A common sample (the pool) was used to obtain the intercalibration factor, which is the relationship between the measurement obtained for the common sample in each plate and the mean of the measurements for that sample in all the plates. To get the CV (coefficient of variation) value, which is used to determine the quality of the measurment, the quantification cycle (Cq) SD is divided by the Cq mean and multiplied by 100. Only the samples with

CV<1.25 were selected for further analysis. To get the relative quantification (RQ) for all the mRNA transcriptions, the calculation method from Pfaffl was used (Pfaffl, 2001):

$$RQ = \frac{E_{Target\ Sample}^{-Cq}}{2\sqrt{E_{Ref1\ Sample}^{-Cq} * E_{Ref2\ Sample}^{-Cq}}}$$

E = Efficiency for each particular primer pair (for target gene or any of the reference genes Ref1, Ref 2).

-Cq = Mean (negative) Cq value for particular sample (for target gene or any of the reference genes Ref1, Ref 2).

2.5.3 Plasma Ions

The concentration of chloride, potassium, and sodium ions in the plasma samples were determined using the potentiometry method with the Ion-Selective Electrode (ISE) module of the Pentra c400 clinical chemistry analyzer (HORIBA, Japan). The calibration of the ISE module was performed using ABX Pentra Standard 1, ABX Pentra Standard 2, and ABX Pentra Reference solutions. Specific electrodes corresponding to each ion were used for the measurement. The potential difference across the electrode membrane was altered by the ions present in the sample, and it was then compared with the reference potential generated by a known ion concentration (Buck, 1981). The samples had a plasma volume of more than 20 µl.

2.6 Biometric calculations

The condition factor (CF) for each measured fish was calculated using Fulton's equation for the condition factor.

$$CF = \frac{Weight(g)}{(Length(cm))^3} * 100$$

The weight-specific growth rate was calculated using the following formula:

$$SGR = \frac{log(Weight_2(g)) - log(Weight_1(g))}{T_2 - T_1} * 100$$

where, $Weight_1$ is the average starting weight, and $Weight_2$ is the average ending weight between two sampling times T.

The Gonadosomatic index (GSI) was calculated using the following formula:

$$GSI = \frac{Gonad\ weight(g)}{Fish\ weight\ (g)} * 100$$

2.7 Statistical analysis

The data was structured using Microsoft Excel (version 16.73). Statistical analyses were performed using R Studio (version 1.4.1717). The following packages were used: tidyverse, lubridate, glmmTMB (Brooks et al., 2023), mgcv, splines, DHARMa (Hartig and Lohse, 2022), and emmeans (Lenth et al., 2023). Mature males (GSI>0.06) were removed before the statistical analyses were performed.

Data exploration, including density plots and boxplots, were performed to analyze the data distribution and identify possible outliers. A generalized linear mixed model (GLMM) was used to analyze the relationship between the response variable and the explanatory variables (Sampling and Treatment), and tank was added as a random effect. The most suitable distribution family, Gaussian for the ion plasma model, Tweedie for the NKA activity and gene expression models, and Student-t distribution for the growth models, were used after validation. Residuals are the difference between the observed and predicted values of the dependent variable, and the GLMM is a complex model to calculate the residuals. Therefore, the DHARMa package is used to create simulation-based scaled residuals. For each model, different diagnostics were run to validate the models graphically. Normality is checked with the QQ plot residuals, where the residuals should be evenly distributed. And residuals vs

predicted, which checks for homoscedasticity, where the residuals should be randomly scattered around the horizontal axis. A Tukey's HSD posthoc test was applied to analyze pairwise differences between the treatments and sampling points. The statistical significance was set to $p < 0.05$ and marked with asterisks where * is $p < 0.05$, ** is $p < 0.01$, and *** is $p < 0.001$.

3 Results

3.1 Morphological values

3.1.1 Condition factor

From the GLMM analyses, a weak individual fish and negligible tank effect was observed for CF (Appendix 3 Figure A3.1), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish CF differences (Appendix 3 Table A3.1). The fish CF increased significantly ($p < 0.001$) from T0 to T2 (FW) for High and Low TSS treatments. From T2 (FW) to T4 (BW), a significant decrease in CF ($p < 0.001$) was observed for both treatments. From T4 to T7 (BW), a significant decrease in fish CF ($p < 0.001$) was observed for High TSS treatment, but no significant differences were observed for the Low TSS treatment fish between these time points. From T7 (BW) to T9 (SW) and T9 to T10 (SW), a significant increase in the fish CF ($p < 0.001$) was observed for both High and Low TSS treatments (Figure 3.1, Appendix 3 Table A3.2).

There were no significant differences in the fish CF between High TSS and Low TSS treatments at any of the FW sampling points. However, during BW phase (sampling points T4 and T7) a significant difference in fish CF ($p < 0.001$) was observed between the two TSS treatments, where Low TSS had a significantly higher CF. In the SW phase, a significant difference in CF (T9: $p < 0.01$, T10: $p < 0.05$) was observed between treatments, where in both T9 and T10 fish reared under Low TSS treatment had a significantly higher CF than fish at High TSS (Figure 3.1, Appendix 3 Table A3.3).

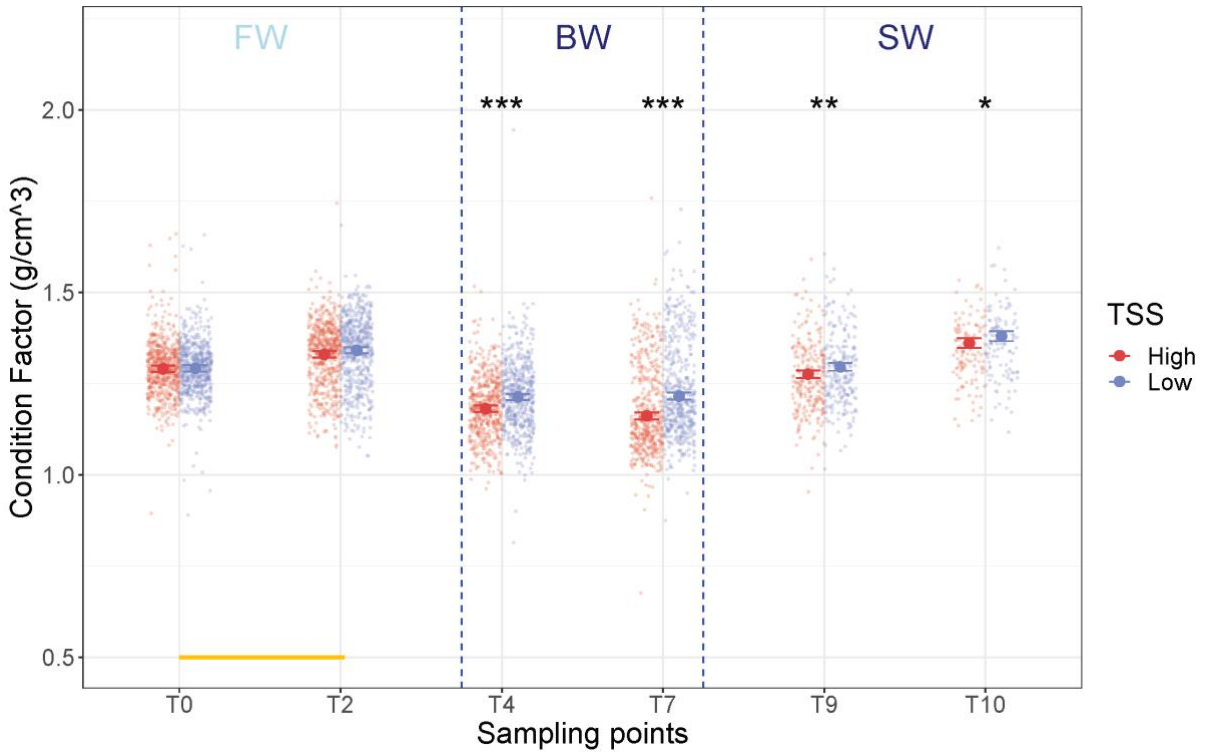

Figure 3.1: Atlantic salmon condition factor by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.2 and Table A3.3.*

3.1.2 Specific Growth Rate

From the GLMM analyses, a weak individual fish and negligible tank effect was observed for SGR (Appendix 3 Figure A3.2), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish SGR differences (Appendix 3 Table A3.4) The Fish SGR decreased significantly ($p < 0.001$) for High and Low TSS treatments from T2 (FW) to T4 (BW). From T4 to T7 (BW), a significant increase in SGR ($p < 0.001$) was observed for High and Low TSS treatments. From T7 BW to T9 (SW), a significant decrease in SGR ($p < 0.001$) was observed for High and Low TSS treatments (Figure 3.2, Appendix 3 Table A3.6).

There were no significant differences in the fish SGR during the winter signal (T0-T2) for High and Low TSS treatments. At (T2-T4) (FW) and (T4-T7) (BW), Low TSS treatment had a significantly higher SGR ($p<0.001$). In SW, at (T7-T9) and (T9-T10), High TSS treatment had a significantly higher SGR ($p < 0.001$: $p < 0.05$) (Figure 3.2, Appendix 3 Table A3.6).

There was a significant decrease in SGR ($p < 0.001$) from the RAS stage (T0-T7) to the SW phase (T7-T10). During the RAS stage (T0-T7), Low TSS treatment had a significantly higher SGR ($p < 0.001$). For the SW phase, high treatment had a significantly higher SGR ($p < 0.01$) (Figure 3.3).

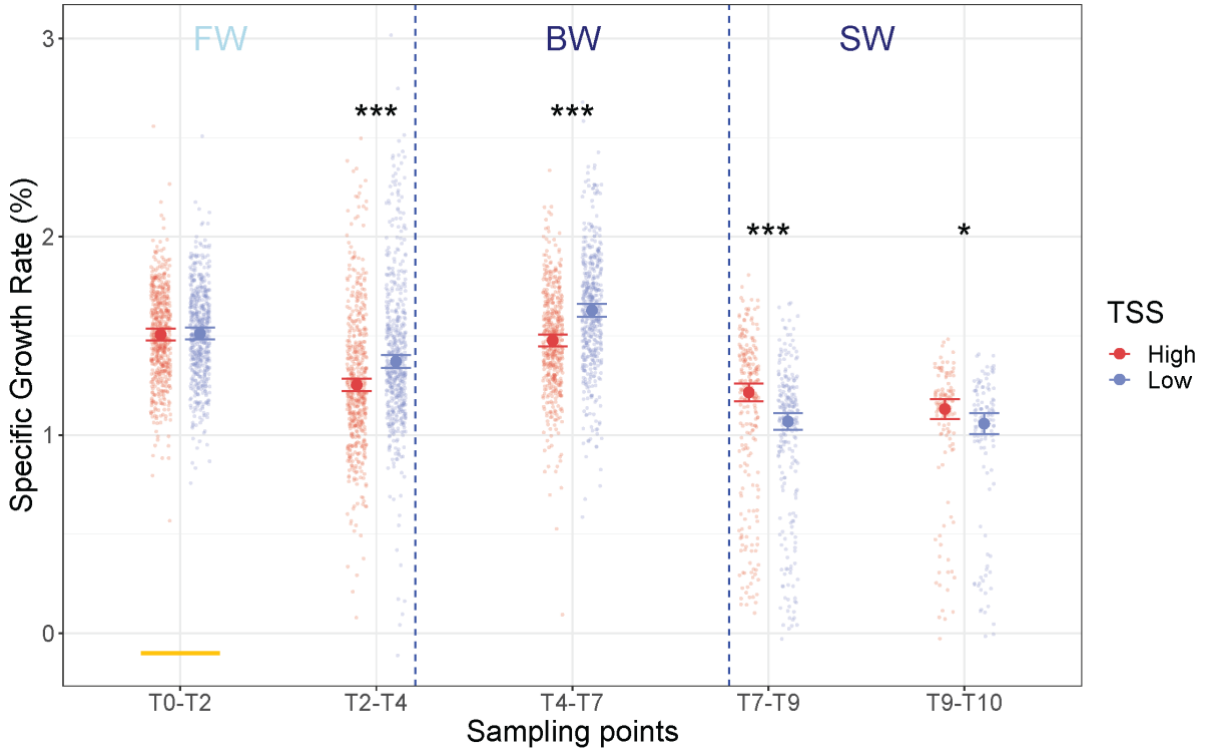

Figure 3.2: Atlantic salmon SGR by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.5 and Table A3.6.*

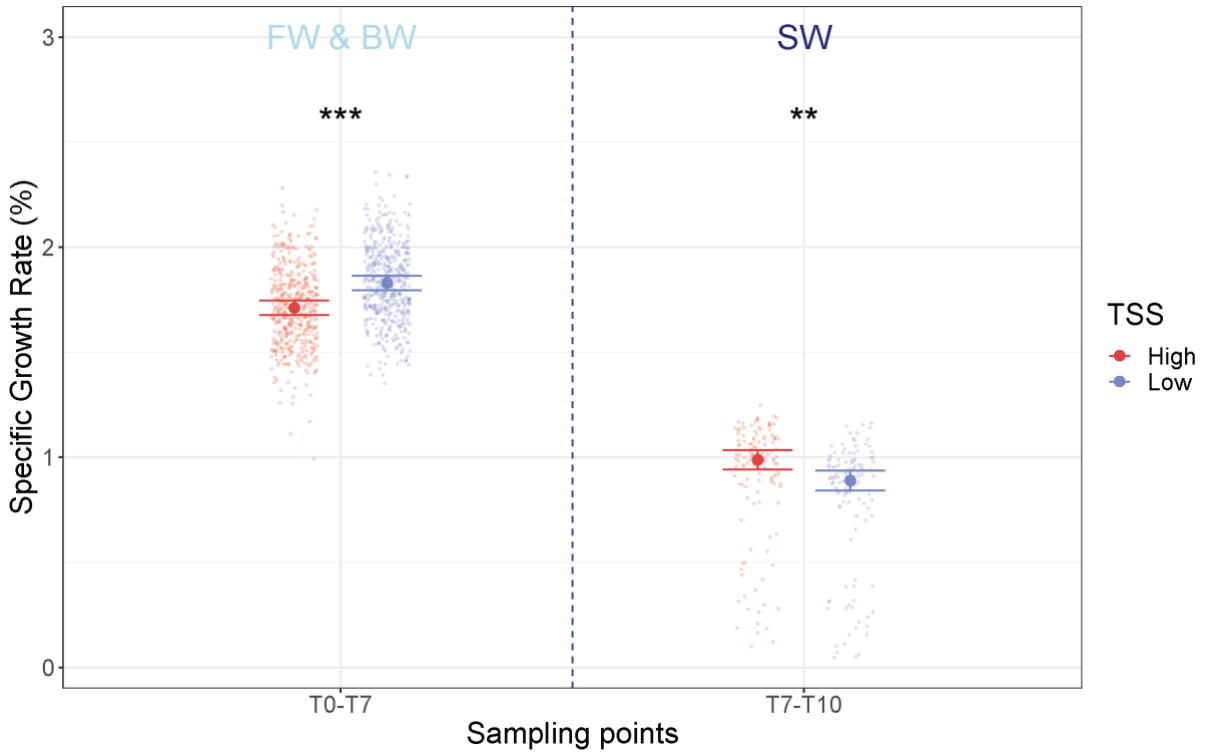

Figure 3.3: Atlantic salmon SGR by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points can be seen in Appendix 3 Table A3.5.*

3.2 Osmoregulation

3.2.1 NKA activity

From the GLMM analyses, a weak individual fish and negligible tank effect was observed for NKA activity (Appendix 3 Figure A3.3), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish NKA activity differences (Appendix 3 Table A3.7). In the FW phase, from T0 to T3, no significant differences were observed in NKA activity for the High and Low TSS treatments. From T0 (FW) to T4 (BW), a significant increase in NKA activity (High: $p < 0.05$, Low: $p < 0.01$) was observed for the High and Low TSS treatments. From T6 to T7 (BW), a significant increase in NKA activity ($p < 0.05$) was observed for the High TSS treatment. From T7 in (BW) to T8 (SW), a significant decrease in NKA activity ($p < 0.01$) was observed for the High TSS treatment (Figure 3.4, Appendix 3 Table A3.8).

High TSS treatment had a significantly higher NKA activity at T7 ($p < 0.01$) (Appendix 3 Table A3.9).

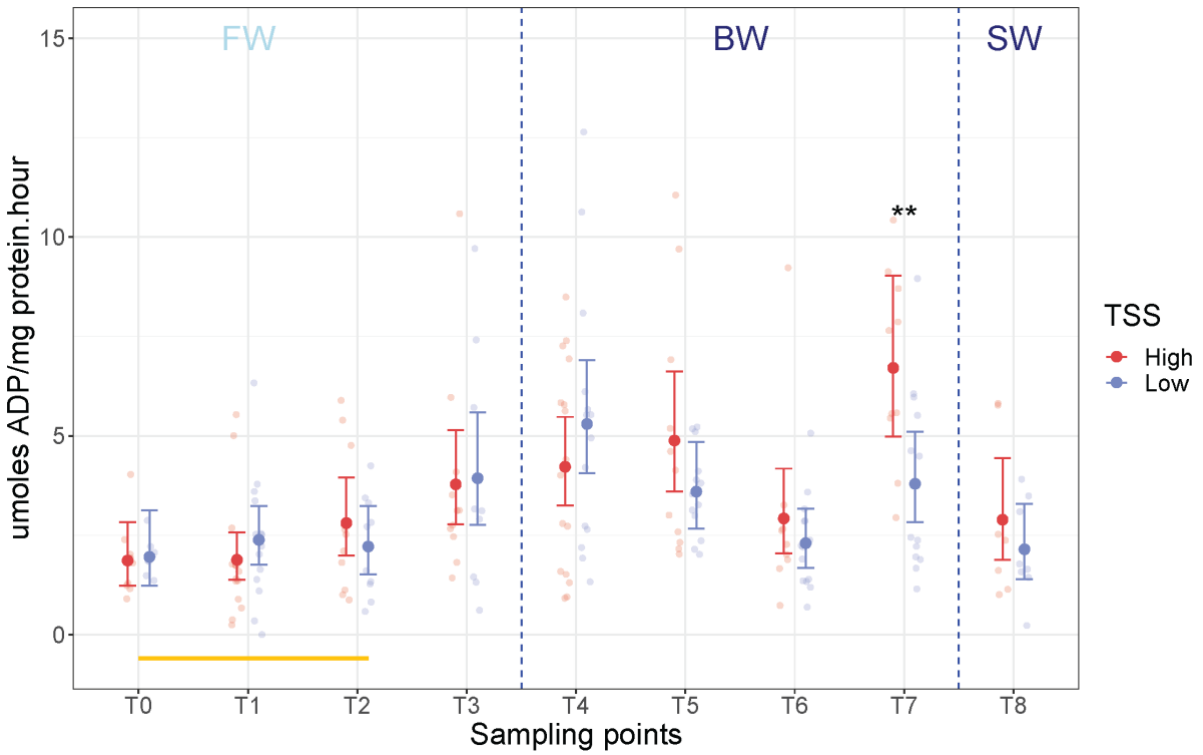

Figure 3.4: Atlantic salmon NKA protein activity levels (µmolesADP/mg Protein/hour) by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.8 and Table A3.9.*

3.2.2 Gene expression

3.2.2.1 nkaα1a

From the GLMM analyses, a negligible tank effect was observed for *nkaα1a* (Appendix 3 Figure A3.4), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish *nkaα1a* mRNA abundance differences (Appendix 3 Table A3.10). In the FW phase, from T0 to T3, no significant differences in *nkaα1a* mRNA abundance was observed over time for the High and Low TSS treatments. From T3 (FW) to T4 (BW), a significant decrease in *nkaα1a* mRNA abundance ($p < 0.001$) was observed for the High and Low TSS treatments. From T7 (BW) to T8 (SW), a significant increase in *nkaα1a* mRNA abundance ($p < 0.01$) was observed for the High TSS treatment (Figure 3.5, Appendix 3 Table A3.11).

No significant difference in *nkaα1a* mRNA abundance was observed between the High and Low TSS treatments at any sampling point (Figure 3.5, Appendix 3 Table A3.12)

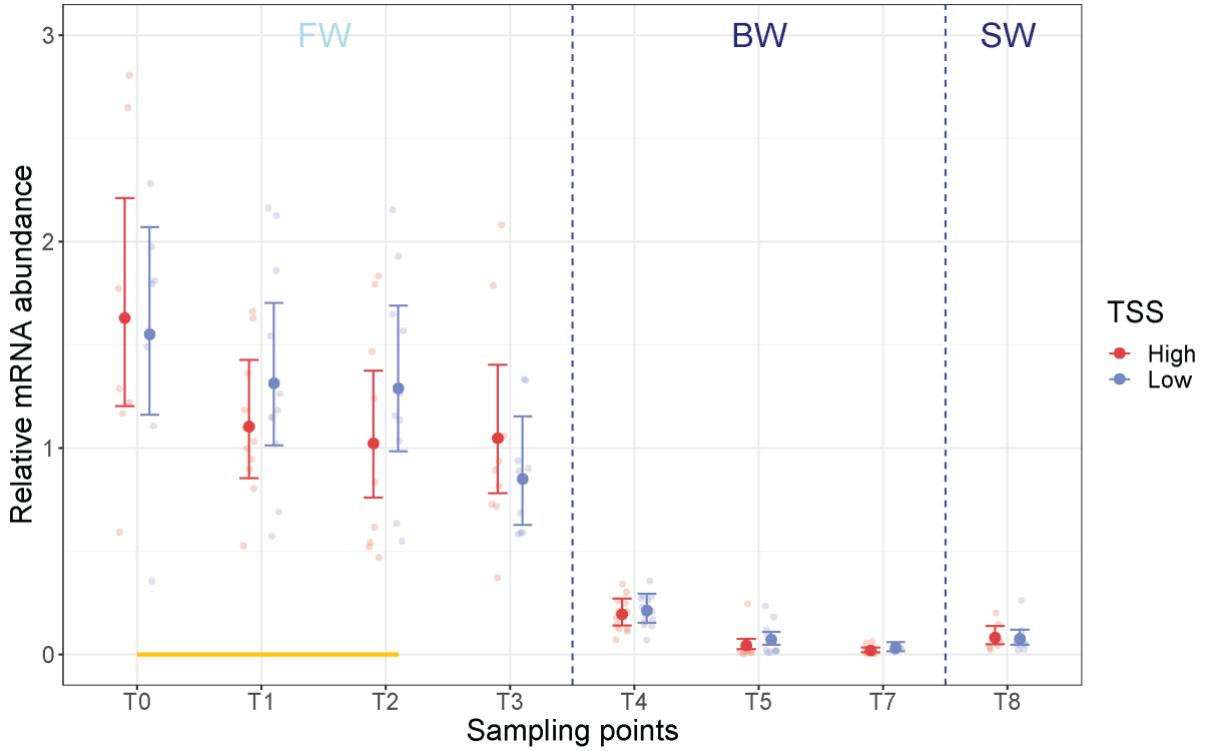

Figure 3.5: Atlantic salmon nkaα1a mRNA abundance by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.11 and Table A3.12.*

3.2.2.2 nkaα1b

From the GLMM analyses, no tank effect was observed for *nkaα1b* (Appendix 3 Figure A3.5), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish *nkaα1b* mRNA abundance differences (Appendix 3 Table A3.13). In the FW phase, from T0 to T3, a significant increase in *nkaα1b* mRNA abundance ($p < 0.001$) was observed over time for the High and Low TSS treatments. From T3 (FW) to T5 (BW), a significant decrease in *nkaα1b* mRNA abundance (High: $p < 0.05$, Low: $p < 0.001$) was observed for the High and Low TSS treatments. From T7 (BW) to T8 (SW), no significant difference was observed over time for the High and Low TSS treatments (Figure 3.6, Appendix 3 Table A3.14).

Low TSS treatment had a significantly higher *nkaα1b* mRNA abundance at T1 ($p < 0.05$), T2 ($p < 0.05$), and T7 ($p < 0.01$) (Figure 3.6, Appendix 3 Table A3.15).

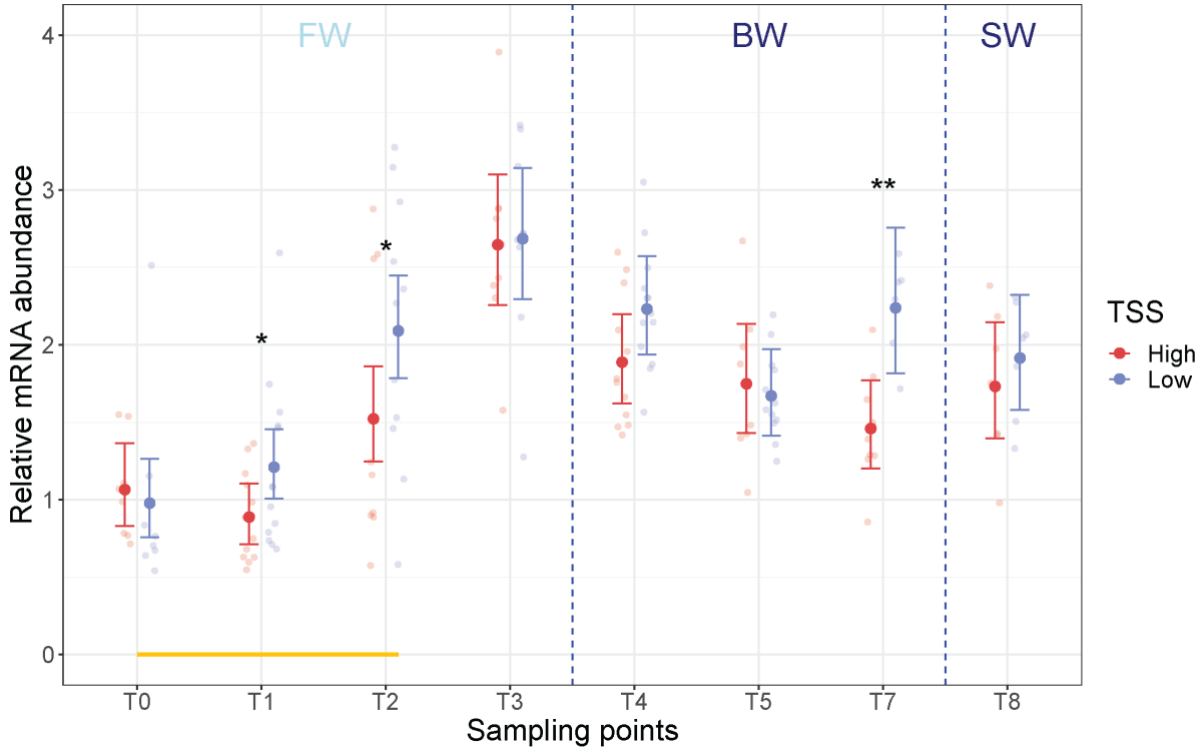

Figure 3.6: Atlantic salmon nkaα1b mRNA abundance by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.14 and Table A3.15.*

3.2.2.3 nkcc1a

From the GLMM analyses, no tank effect was observed for *nkcc1a* (Appendix 3 Figure A3.6), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish *nkcc1a* mRNA abundance differences (Appendix 3 Table A3.16). In the FW phase, from T0 (FW) to T3 (FW) a significant increase in *nkcc1a* mRNA abundance ($p < 0.001$) was observed over time for the High and Low TSS treatments. From T3 (FW) to T5 (BW), a significant decrease in *nkcc1a* mRNA abundance (High: $p < 0.01$, Low: $p < 0.05$) was observed for the High and Low TSS treatments. From T7 (BW) to T8 (SW), a significant increase in *nkcc1a* mRNA abundance ($p < 0.01$) was observed for the High TSS treatment (Figure 3.7, Appendix 3 Table A3.17).

No significant difference in *nkcc1a* mRNA abundance was observed between the High and Low TSS treatments at any sampling point (Figure 3.7, Appendix 3 Table A3.18).

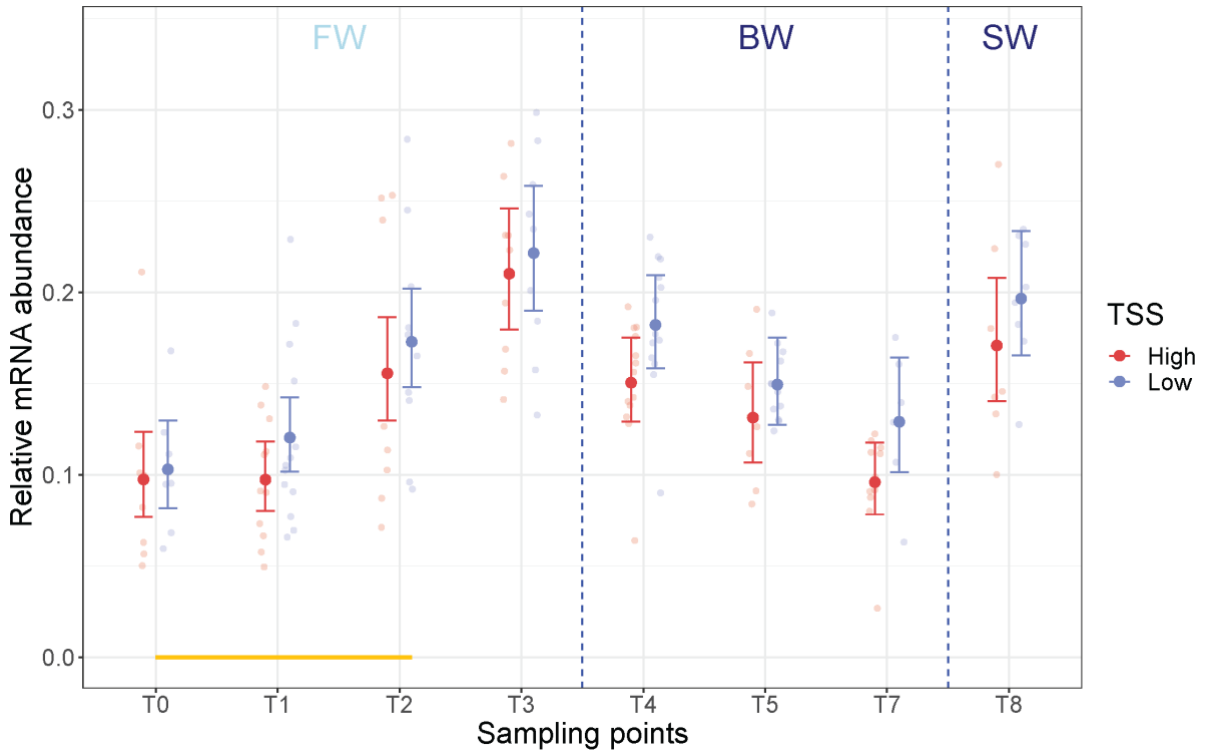

Figure 3.7: Atlantic salmon nkcc1a mRNA abundance by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.17 and Table A3.18.*

3.2.3 Plasma Ion's

3.2.3.1 Sodium

From the GLMM analyses, no tank effect was observed for sodium (Appendix 3 Figure A3.7), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish sodium concentration differences (Appendix 3 Table A3.19). In the FW phase, from T0 to T3, no significant differences in plasmatic sodium concentration was observed over time for the High and Low TSS treatments. From T4 to T5 (BW), a significant decrease in sodium concentration ($p < 0.001$) was observed for High TSS treatment. From T5 to T6 (BW), a significantly increased in sodium concentration ($p < 0.05$) was observed for High TSS treatment (Figure 3.8, Appendix 3 Table A3.20).

Low TSS treatment had a significantly higher plasmatic sodium concentration at T5 ($p < 0.01$) (Figure 3.8, Appendix 3 Table A3.21).

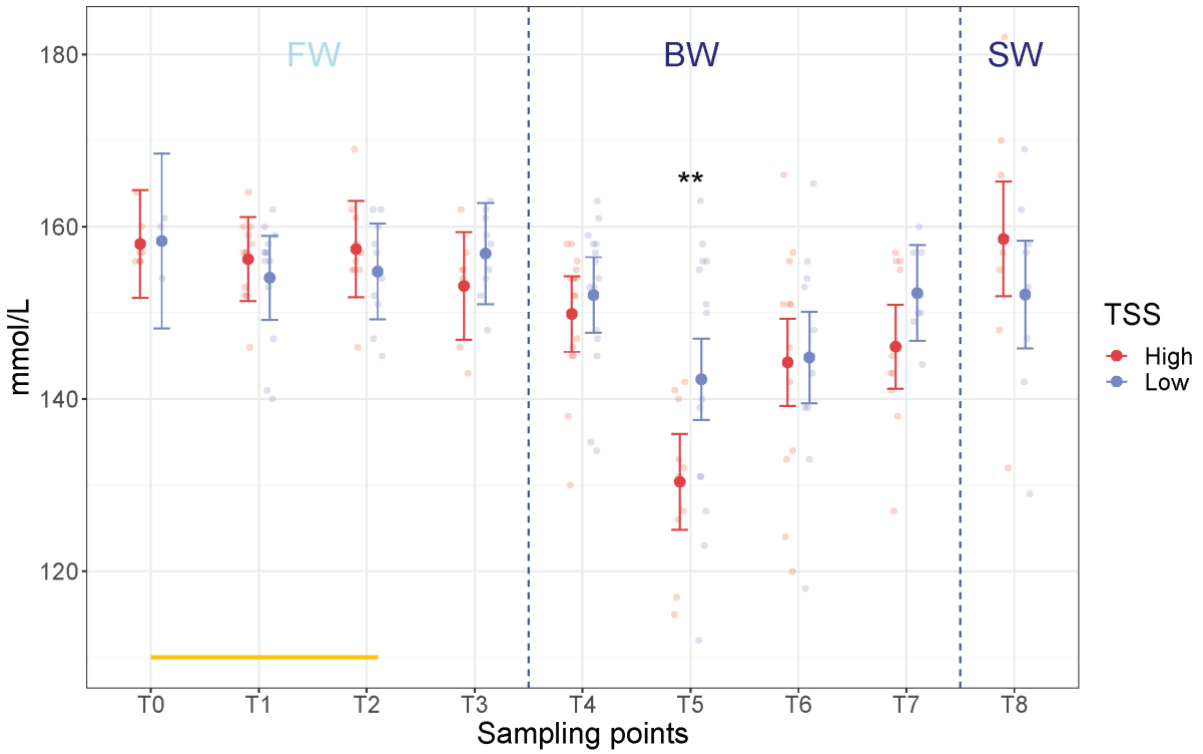

Figure 3.8: Atlantic salmon plasmatic sodium concentration (mmol/L) by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.20 and Table A3.21.*

3.2.3.2 Chloride

From the GLMM analyses, no tank effect was observed for chloride (Appendix 3 Figure A3.8), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish chloride concentration differences (Appendix 3 Table A3.22). From T4 (BW) to T8 (SW), a significant difference in plasmatic sodium concentration (High: $p < 0.001$, Low: $p < 0.01$) was observed over time for the High and Low TSS treatments. From T5 (BW) to T8 (SW), a significant difference in plasmatic sodium concentration ($p < 0.001$) was observed over time for the High TSS treatment. From T7 (BW) to T8 (SW), a significant difference in plasmatic sodium concentration ($p < 0.05$) was observed over time for the High TSS treatment (Figure 3.9, Appendix 3 Table A3.23).

Low TSS treatment had a significantly higher plasmatic sodium concentration at T3 ($p < 0.05$) and T5 ($p < 0.01$) (Figure 3.9, Appendix 3 Table A3.24).

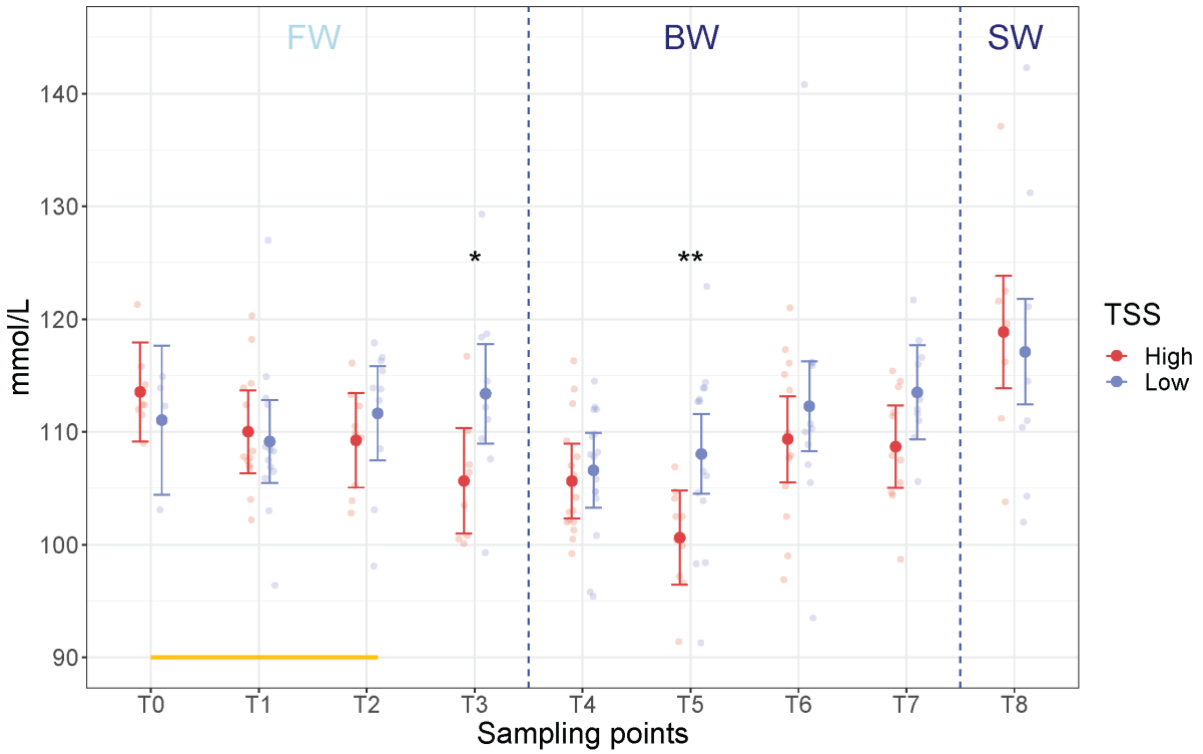

Figure 3.9: Atlantic salmon plasmatic chloride concentration (mmol/L) by sampling in freshwater (FW), brackish water (BW) and after seawater (SW) transfer reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.23 and Table A3.24.*

3.2.4 Simulated Transport

3.2.4.1 Sodium

From the GLMM analyses, no tank effect was observed for sodium (Appendix 3 Figure A3.9), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish sodium concentration differences (Appendix 3 Table A3.25). During simulated transport, from pre-transport (BW) to post-transport (SW) a significant increase in plasmatic sodium concentration ($p < 0.001$) was observed for High TSS treatment. From post-transport (SW) to 3hpost-transport (SW) a significant decrease in plasmatic sodium concentration ($p < 0.01$) was observed for High TSS treatment. From pre-transport (BW) to 3hpost-transport (SW) a significant increase in plasmatic sodium concentration ($p < 0.05$) was observed for Low TSS treatment (Figure 3.10, Appendix 3 Table A3.26).

High TSS treatment had a significantly higher plasmatic sodium concentration at post-transport ($p < 0.001$) (Figure 3.10, Appendix 3 Table A3.27).

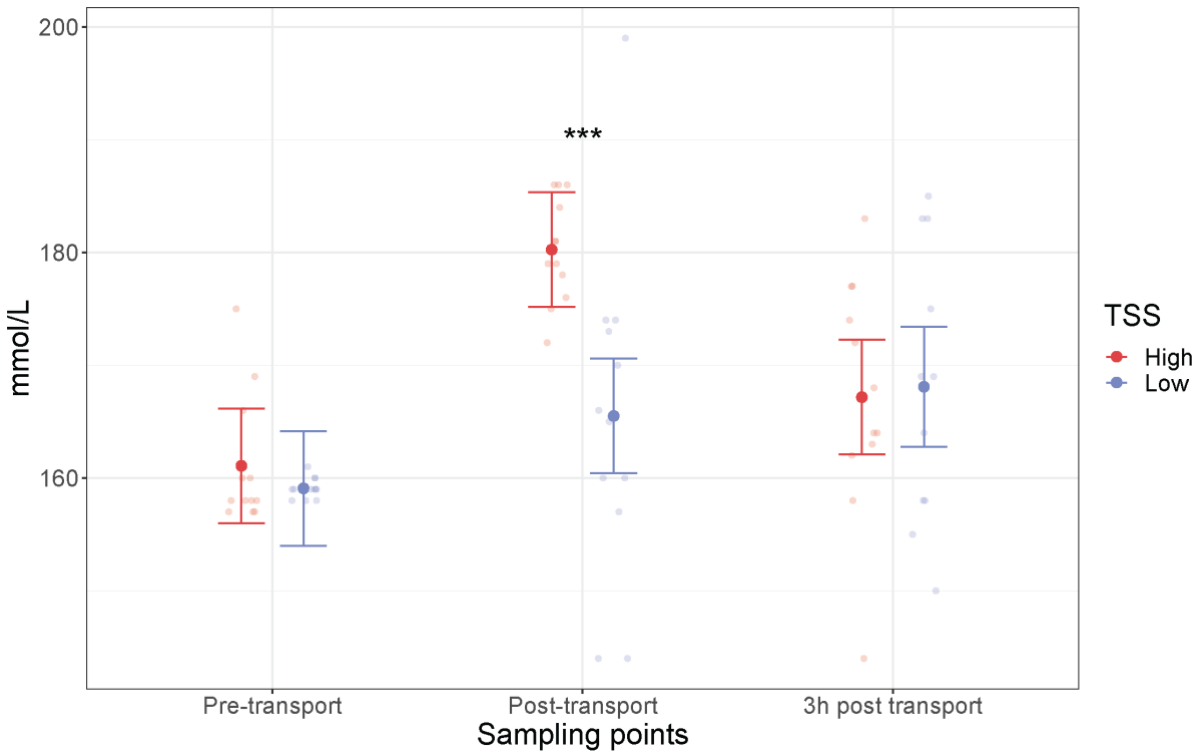

Figure 3.10: Atlantic salmon plasmatic sodium concentration (mmol/L) by sampling during simulated transport reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.26 and Table A3.27.*

3.2.4.2 Chloride

From the GLMM analyses, no tank effect was observed for chloride (Appendix 3 Figure A3.10), and the fixed variables sampling and interaction between sampling and treatment are important to explain the fish chloride concentration differences (Appendix 3 Table A3.28 During simulated transport, from pre-transport (BW) to post-transport (SW) a significant increase in plasmatic chloride concentration ($p < 0.001$) was observed for High TSS treatment. From post-transport (SW) to 3hpost-transport (SW) a significant decrease in plasmatic chloride concentration ($p < 0.001$) was observed for High TSS treatment. (Figure 3.11, Appendix 3 Table A3.29).

High TSS treatment had a significantly higher plasmatic chloride concentration at post-transport ($p < 0.001$) (Figure 3.11, Appendix 3 Table A3.30).

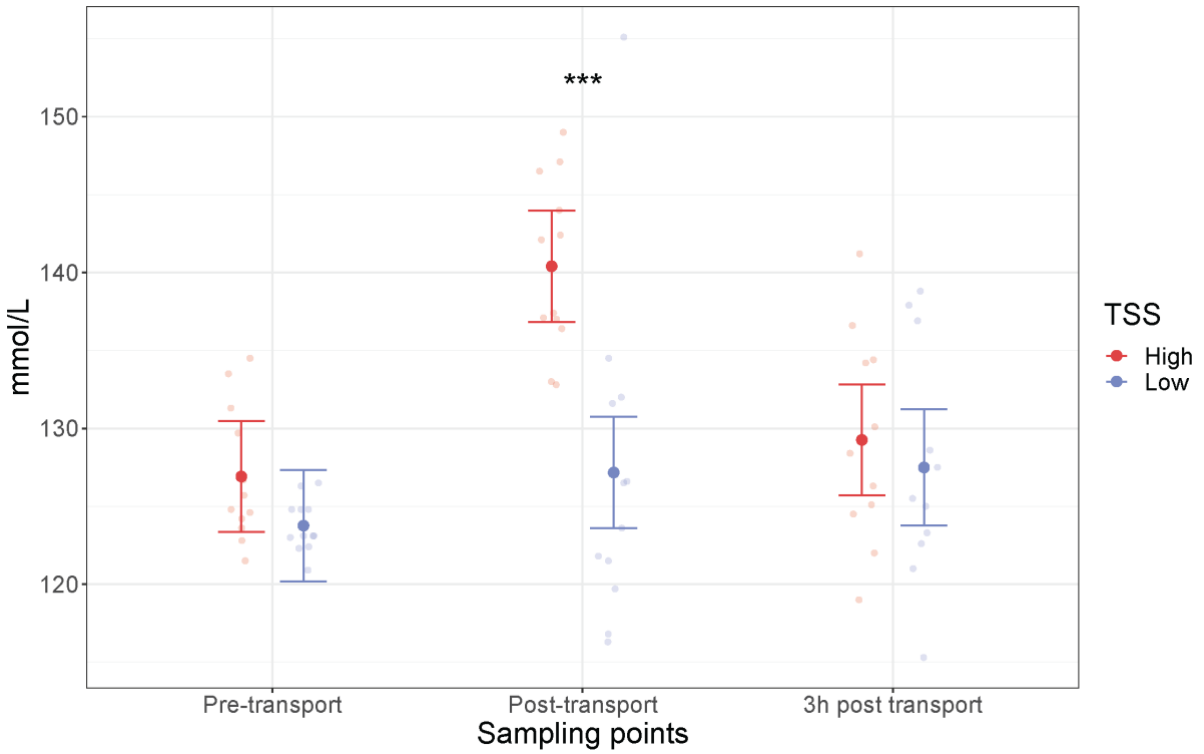

Figure 3.11: Atlantic salmon plasmatic sodium concentration (mmol/L) by sampling during simulated transport reared in RAS under High TSS (in red) and Low TSS (in blue) conditions. *Each sampling point is presented as estimated mean ± 95 % confidence interval, and the raw data of each fish. Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Significant difference between sampling points and treatments can be seen in Appendix 3 Table A3.29 and Table A3.29.*

4 Discussion

4.1 Discussion of Methods

The study was conducted in a small-scale RAS testing facility. The water parameters such as O_2, CO_2, and temperature were controlled for each experimental tank, and were kept stable throughout the study. The two experimental groups, High and Low TSS, were treated with different concentrations of TSS: Low TSS group had a mean of 1.13 mg/l of TSS in freshwater (FW) and 2.06 mg/l TSS in brackish water (BW), while the High TSS group had a mean 6.77 mg/l of TSS in FW and 7.31 mg/l TSS in BW (Appendix 6, Table A6.1). At the end of FW, High TSS group had a TSS peak of 26.5 ± 12.1 mg/l, and at the end of BW, a TSS peak of 19.2 ± 9.6 mg/l (Appendix 6, Figure A6.1), after each of these peaks, the high TSS RAS modules had to be flushed to not get a further increase of TSS. The desirable TSS mean concentration of 12-15 mg/l in the High TSS group was never achieved. There are several reasons for this; at the start of the study, the fish were very small and the biomass was low and therefore not enough waste was produced to increase the TSS. The RAS was small compared to commercial facilities, and when the TSS finally increased, the systems were clogged with "snot" like slime and had to be flushed, restarting the production of accumulated TSS in the system. Even though the desired TSS concentration was not achieved, the concentrations used were highly relevant to what is observed in commercial facilities.

The nitrogenous parameters TAN (NH_3 and NH_4^+), NO_3^-, and NO_2^- were continuously measured. At the start of the study, the High TSS group had a high peak in the amount of TAN and NO_2^- concentration compared to the Low TSS treatment group (Appendix 6, Figure A6.2 and A6.3). This is believed to be because of the reduced biofilter maturation. High TSS treatment also had a peak in TAN at the end of the FW stage, believed to be because of the peak in TSS levels that occurred at the same time. TAN tended to be higher in the High TSS treatment group throughout the study. This is because the bacterial degrading of organic matter produces NH_4^+ and may impair the nitrification rate (Hastuti et al., 2019). It is important to note that despite the observed peaks, the concentration of nitrogenous parameters, especially TAN, in the rearing tanks remained below the levels that are expected to affect welfare and smoltification (Kolarevic et al., 2013). NO_3^- had the same concentration for both treatments and was stable throughout the study (Appendix 6, Figure A6.4). The method of increasing TSS concentration by reintroducing waste into the system was new to

RASlab, and the outcome for each system was uncertain. Therefore, triplicated RAS systems were applied to each treatment to reduce the possibility of a tank effect.

The sampling was conducted at RASlab, where a long workbench was set up with different sampling stations. The fish was netted from the tank and put directly in a bucket with a lethal dose of anesthesia. After the fish was killed, the fish was quickly processed, starting with the blood samples to reduce post-mortem changes (Railo et al., 1985). The fish were identified by PIT-tag scanning before weighing and taking length measurements. The gills of the fish were then sampled. The first-gill arch on either side was removed and excluded because of the risk of being damaged during gill lid removal, and the second-gill arch on either side was used for a different study. For my study, the third-gill arch on each side was used. The third-gill arch was consistently smaller than the first and second-gill arches. To ensure a good tissue quality when performing the analyses, gills going to gene expression were stored in RNAlater immediately after dissection, as RNAlater rapidly permeates the tissue and stabilizes and protects RNA (Trösse et al., 2010). Gills for NKA activity were stored in SEI buffer and frozen immediately using dry ice (McCormick, 1993).

In the scope of this thesis, four samplings were conducted during the freshwater stage (T0-T3), four during the brackish water stage (T4-T7), and one during the seawater stage (T8). The project experiment also included T9 and T10. While it was desired to also include these sampling points in my study, as it could show the long-term effect of High TSS concentration for salmon adapting to seawater, it was unfortunately not possible due to the limited time to process the samples and perform data analyses.

4.1.1 NKA protein activity measurement

Na^+/K^+ - ATPase protein activity in gills (NKA) was determined using McCormick´s method (McCormick, 1993). To measure the NKA activity, the conversion of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and phosphate (Pi) is analysed. The ouabain sensitive hydrolysis of ATP is enzymatically coupled to the oxidation of nicotinamide adenine dinucleotide (NADH) with the use of controlled temperature (25 °C). The standard curve was used to determine the assay mixture quality , and it should range between 19 – 20 mMOD. In this study, however, the standard curve ranged from15 – 17 mMOD. There could be multiple reasons for that. The most common cause is inaccurate measurements of the reagents. Still, this is not the case for this study as the weight was calibrated and multiple weighing repetitions was performed to ensure correct measurement

regarding the protocol. The most likely explanation for the low standard curve could be attributed to the hydration of the ADP product or impurities (Balseiro, per communication). This would alter the result even if the measurement of the reagents was done correctly.

4.1.2 Gene expression

When performing RNA isolation of the gills, the tissue was manually cut from the cartilage in a standardized manner and weighed. Optimally, 50 to 100 mg of tissue should be used per reaction to secure a high RNA yield for the method described in (Chomczynski, 1993). As the fish was small during T0 – T1 (< 60 g), having enough gill tissue for the extraction was a challenge, thus, only approximately ~ 30 mg of gill tissue was used for these two time points. There was no problem securing enough gill tissue (~ 60 mg) for the other samplings points (T2 – T8). The tissue was transferred to TRI-reagent and homogenized, and RNA was isolated.

The RNA purity were assessed using a NanoDrop One (ThermoFisher Scientific Inc., USA). The purity was confirmed with 260/280 nm absorbance above 1.95 and 260/230 nm absorbance above 1.7. Samples with a value < 1.7 went through a precipitation step and were remeasured to increase the purity. The RNA is accepted as pure if the 260/230 nm is between 1.8 – 2.2. A value significantly different from this can indicate the presence of protein, phenol, and other contaminants which absorbs 230 nm . But 1.8 can be challenging to achieve, and a ratio of above 1.7 still indicates sufficient RNA purity for this protocol (Gomes A.S, per communication). Normally it is wishful to do an RNA integrity check on at least ~ 20 - 25 % of the samples. Gills are a perfect tissue to work with for gene expression, and there are rarely any problems. Therefore, it was determined not to do an RNA integrity check based on experience (Balseiro, per communication).

RNA preparations can be contaminated with small amounts of DNA. Because of the amplification power of kinetic PCR, even a small amount of DNA contaminant will interfere with the desired specific amplification. RNase-free DNase removes unwanted DNA contaminants (Pfaffl, 2004). All the samples go through DNase treatment before the cDNA synthesis step. DNase treatment can result in RNA degradation if not inactivated (Pfaffl, 2004). Therefore, a DNase inactivator reagent was added before the cDNA synthesis.
The NanoDrop One spectrophotometer measures not only the quality but also the concentration of DNA/RNA in the sample through absorption at 260 nm (Kapp et al., 2015). Before the cDNA synthesis, it is essential to determine the specific concentration of RNA

alone. To accomplish this, the Qubit® 4 fluorometer (Invitrogen; ThermoFisher Scientific Inc., USA) was used, which detects fluorescence from a dye that specifically binds to the target RNA, thus allowing for a more precise concentration determination (Kapp et al., 2015). The final step before performing RT-qPCR involves producing single-stranded (ss) complementary DNA (cDNA) by performing reverse transcriptase on the RNA (Pfaffl, 2004).. Oligo-dT primers were selected due to their ability to selectively target the poly-A tail of mRNA. The cDNA synthesis using oligo-dT is a more specific for mRNA than random priming because it is less likely to transcribe rRNA or any RNA lacking a poly-A tail. However, it should be noted that this method is not 100 % specific, and some rRNA or non-poly-A-tailed RNA may also be reversed transcribed. Random primers, which could transcribe all types of RNA, including those without a poly-A tail, were not used in this instance. Target-specific primers were not used because they require a separate priming reaction for each target, making it impossible to return to the same preparation at a later stage (Bustin and Nolan, 2004).

Gene expression is a multistep process that involves transcription, translation, and turnovers of mRNAs and proteins and is a key process in the functioning of any cell (Schwanhäusser et al., 2011). Though crucial for cellular function, it does not always directly correlate with final protein abundance due to various post-transcriptional regulatory mechanisms (Liu et al., 2016). In our study, the mRNA levels observed might not directly match the protein levels, and the post-transcriptional variations could limit the conclusions drawn from our gene expression data.

Regardless of what method is used to prime cDNA synthesis, the qPCR step requires target-specific genes. To ensure an accurate comparison of gene expression levels across different samples, reference genes, commonly known as housekeeping genes, are used. Reference genes are genes that have stable expression across different experimental conditions. Several known reference gene primers were ten-fold diluted from the original stock concentration of 100 μm and tested as duplicates on a 96-well plate (Bio-Rad Laboratories, USA). *ef1α* and *rps* had the smallest variation in Cq value and were chosen. Accurate quantification relies on gene primer sets that enable maximum amplification efficiency (Bustin et al., 2005). To determine the efficiency of the primers, a triplicate ten-fold dilutions series of cDNA were used to make a slope for each primer gene. A duplicate NTC (water instead of cDNA in the well) was also present on the plate to detect possible contaminants. From the slope, the efficiency of each primer was determined and can be seen

in Appendix 1, Table A1.1 . High-quality results have been achieved using these assays and protocols in previous studies (Nilsen et al., 2007; Olsvik et al., 2005).

For this study SYBRgreen (Applied Biosystems, USA) was chosen as the fluorescent dye. The dye binds to the double-stranded DNA, and when monitored in real-time, it emits detectable fluences that will increase proportionally with the quantity of amplified DNA (Bustin, 2000). The qPCR reaction has four phases: First, the denaturation, where the temperature (95 °C) is so high that the double-stranded DNA is separated into two single-stranded DNA strands, and here the SYBRgreen exhibits little fluorescence. The second step is annealing, where the temperature (60 °C) is lowered, allowing for the primers to bind to their complementary sequence on the single-stranded sequence resulting in some light emission. The third step is polymerization, where more and more molecules bind to the newly synthesized DNA, further increasing the fluorescence. These steps were repeated 35 times before a final denaturation step, where the dye molecules are realized, and the fluorescence signal returns to the background (Bustin, 2000). The principle of calculation quantification is based on a simple concept: the more target genes there are at the beginning of the assay, the fewer amplification cycles are required for the fluorescence to reach the target fitted line threshold (Bustin et al., 2005).

4.1.3 Plasma ions

The concentration of chloride, potassium, and sodium ions in the plasma samples were determined using the potentiometry method with the Ion-Selective Electrode (ISE) module of the Pentra c400 clinical chemistry analyzer (HORIBA). Specific electrodes corresponding to each ion were used for the measurement. The potential difference across the electrode membrane was altered by the ions present in the sample, and it was then compared with the reference potential generated by a known ion concentration (Buck, 1981).

Potassium concentration was supposed to be included in this thesis, but 65 % of the samples were under the lower limit of detection of the Pentra c400 of 1.35 mmol/l. At the start of the study, the fish was small, which in turn, made the extraction of blood challenging. From eleven fish (T0 = 4, T1 = 5, T2 = 2), the plasma volume extracted was less than 20 µl, which was too low of plasma for the Pentra c400 to analyze. A dilution was performed, but the concentration were under low limits of detection, and they were excluded.

4.1.4 Statistical analyses

Which distribution model best fitted for each data type was tested using the DHARMa package, as explained in the methods section. The plasma ions were fitted with a Gaussian distribution, this is since the data is most likely symmetrically distributed around the mean concentration. The data followed a normal distribution, which is consistent with the Gaussian distribution. For NKA activity and gene expression, the Tweedie distribution was the best fit, since both the NKA activity and gene expression contained zeroes, which the Tweedie distribution can handle. It is worth noting that in gene expression *nkaα1b* and *nkcc1a* both were a great fit for Gaussian distributions because they did not contain any zeroes, but the *nkaα1a* contained a lot of zeroes in the SW stage and therefore only Tweedie distribution fit. To have consistency in the data, all the genes were then fitted with the same distribution. For performance values such as CF and SGR, the best fit was t_family (T-Distribution). This is most likely because of the large sample size and the fact that fish was removed over time, creating a more complex dataset with more variability and extreme values, which the t_family works great with.

It was decided to exclude some samples for two different reasons. One reason was the gene expression data, which is a time-consuming and expensive analysis. Therefore it was decided to exclude one fish from each tank (n = 6) which resulted in a total sample size of n = 30 for each sampling point. This is still 15 samples per treatment and can still give great statistical power. Sampling point T6 was also excluded from the gene expression analysis to save time and resources.

After all the samples were analyzed, it was decided to exclude the mature male salmon (GSI > 0.06). This is because they commit to mature instead of smoltifying, giving a wrong impression of the effect of treatment on smoltification (Stefansson, Per communication). This was clear in the gene expression result, where all the mature males had an increase in *nkaα1a* mRNA abundance right before the SW phase while all other fish were close to zero. The downside of this decision is the reduction of sample size and statistical power. This was only a downside for the gene expression data, as the sample size already was reduced. To reduce the impact of removing mature males from gene expression data, the choice of removing them should have been taken before the lab analyses, then, six matured males could be removed instead of six potentially not matured.

4.2 Discussion of Results

4.2.1 Performance values

The fish weight and length of the Atlantic salmon steadily increased from start to finish of the study, which is consistent with other studies of smoltification in RAS (Lorgen-Ritchie et al., 2021; Mota et al., 2019; Pino Martinez et al., 2021). The fish experienced a high frequency of samplings, and this could affect the growth, but as the SGR was at its highest point during the most frequent sampling period T4-T7 this can be rejected. At T7 and T9 samplings, the fish from the Low TSS group had a significantly higher weight and length than the High TSS group. However, at T10 this significant difference was not observed anymore (Appendix 2, Figure A2.1 and A2.2). The condition factor (CF) significantly increased during the parr stage T0 – T1 for both experimental groups, before decreasing when smoltifying from T2 -T4. This decrease in the CF is a result of changes in critical metabolic aspects during smoltification, such as depletion of lipids in different tissue and the lengthening of the caudal fin (Stefansson et al., 2011). After seawater transfer, both groups had a significant increase in the CF, most likely because the salmon had gone through seawater adaptation and could now focus on growth (S. O. Handeland et al., 2003). The High TSS group also experienced optimal conditions because both TSS groups experienced the same rearing conditions, which further stimulated growth.

After the winter signal (T2), the High TSS group always had a significantly lower CF. The specific growth rate (SGR) significantly decreased in both TSS groups after the winter signal, probably due to the same reason as above mentioned for the CF. While the CF had no significant difference from T4 to T7, SGR significantly increased for both groups during this period, meaning the fish grew both in length and weight. After SW transfer, both groups had an increase in CF, but a decrease in SGR, meaning the fish grew relatively more in weight than in length. After the winter signal and for the rest of the RAS stage, the Low TSS group had a significantly higher SGR than the High TSS group indicating that the High TSS group had a not-so-optimal condition during the RAS stage.

At RASlab, it was observed that more food waste accumulated from the High TSS group compared to the Low TSS, indicating lesser appetite. This changed when the fish was transferred to SW, where the High TSS group had a significantly higher SGR than the Low TSS group. (Becke et al., 2019, 2018, 2017) observed in all these studies that fish in the High TSS group had a reduced appetite. Since the Atlantic salmon is a visual-oriented predator, an increase in TSS/turbidity can suppress the visual cues and reduce appetite (Ali, 1959; Berg

and Northcote, 1985; Schumann and Brinker, 2020). This can be the scenario in the present study, indicating that High TSS affects the growth of the fish during smoltification. Another possibility is that they are using more energy to find the feed due to reduced visibility, which could divert resources away from growth. During smoltification, ion regulation is a highly important process and requires additional energy. This could lead to a physiological compensatory mechanism where energy is allocated to survival-related functions like ion regulation over the growth (Calabrese et al., 2023; Pichavant et al., 2001). A further reason for the two possibilities mentioned above is the fact that the High TSS group changed growth patterns when transferred to SW, where the rearing condition was the same for both groups. The SGR for the High TSS group was significantly higher than the Low TSS group during this time, and at the final sampling point, there were no significant differences between weight and length for both TSS groups. The ability of the fish in the High TSS group to overtake the SGR of the fish in the Low TSS once in optimal conditions is known as compensatory growth and is a well-known and well-studied mechanism in Atlantic salmon (Hvas et al., 2021; Nicieza and Metcalfe, 1997; Pino Martinez et al., 2021).

4.2.2 Smoltification and Osmoregulation

4.2.2.1 NKA activity

An increase in activity indicates that the protein has an increased rate of excreting ions across the cell membrane (Suhail, 2010). Therefore, NKA gill activity reflects the development of hypo-osmoregulatory ability and SW tolerance and is a sign of a successful smoltification (Pino Martinez et al., 2021). In this study, it was measured during smoltification to see if the protein activity was affected by different particle loads.

The gill NKA activity was low and stable (~ 2.5 μmolesADP/mg protein.h) from the start of the study and during the winter signal (T0-T2) which is expected from salmon parrs before smoltification (Pino Martinez et al., 2021). After the winter signal, the Low TSS group reached its peak at T4 (~ 5.1 μmolesADP/mg protein.h), 5 weeks after the winter signal, and the High TSS group at T5 (~ 4.9 μmolesADP/mg protein.h), 6 weeks after the winter signal excluding the T7 peak that will be discussed later. The increase in NKA activity after the winter signal is on par with other studies (Sigurd O. Handeland et al., 2003; McCormick et al., 1987; Pino Martinez et al., 2021; Stefansson et al., 2011). Although, it seems like the NKA activity has been decreasing in studies over the years (Pino Martinez et al., 2021), the low NKA activity peak measured in this study could be the result of relatively high water

temperature and low pH/high aluminum present in the water (McCormick et al., 1999; Nilsen et al., 2010). This could reduce the post-smolts ability to fully prepare for the SW transfer (Pino Martinez et al., 2021).

Both the TSS groups had a significant decrease in NKA activity from their peak activity to T6, where it was back at parr level activity (~ 2.5 μmolesADP/mg protein.h). From T6 to T7, both TSS groups had a significant increase in NKA activity, where the High TSS group where significantly higher (~ 6.5 μmolesADP/mg protein.h) than the Low TSS group (~ 3 μmolesADP/mg protein.h). The increase in NKA activity for the High TSS group could be because the TSS concentration at this point of measuring was as high as ~ 17.5 mg/l (Appendix 6, Figure A6.1), which is more than double the concentration of the average TSS concentration (7.31 mg/l). (Lin et al., 1992) discovered in shrimp (*Penaus japonicus*) that the NKA activity increased when exposed to higher turbidity as a compensating reaction to the disruption of osmotic and ionic balance. Although shrimp is not related to Salmonids, the comparison can be still relevant as they use gills to breathe, and ions excretion through gills share the same metabolic pathway (Ituarte et al., 2008).

Mature male salmons were decided to be excluded from the result section of this thesis. Figure A4.1 in appendix 4 shows the NKA activity result, which includes the mature males. The result shows that the NKA activity follows the same trends as without the mature males, but the High TSS treatment had a significantly higher NKA activity in both T6 and T7. Figure A5.1 in appendix 5 shows that there is no correlation between GSI and NKA activity.

4.2.2.2 Gene expression

In Atlantic salmon CCs, the NKA protein subunit α is important for smoltification and has two isoforms *nkaα1a* and *nkaα1b* (McCormick, 2013). *nkaα1a* is linked to the active uptake of monovalent ions in FW CC, whereas the *nkaα1b* isoform is linked to the excretion of monovalent ions through SW CC and, therefore, more prominent in SW smolts (McCormick, 2013). During smoltification, *nkaα1a* mRNA expression will decrease, concurrent with an increase in the mRNA abundance of *nkaα1b*, and cotransporter *nkcc1a* (Nilsen et al., 2007). In this study, isoforms *nkaα1a, nkaα1b, and nkcc1a* were measured during smoltification to investigate if different particle loads will effected their mRNA abundance.

Before smoltification, the *nkaα1a* isoform is expected to be present in the CC as it is linked to the uptake of monovalent ions through the FW CC in the gill, and during

smoltification, it is expected to decrease (Handeland et al., 2014, 2013; McCormick et al., 2009; Pino Martinez et al., 2021). This could also be observed during this study. From T0 to T3 in FW, the isoform was expressed for salmon in both TSS groups before having a significant decrease from T3 in FW to T4 in BW,and it remains with very low levels until the end of the study. There was no significant difference between the two groups at any time, but the Low TSS group had a significant decrease in *nkaα1a* mRNA abundance from T2 to T3, while the High TSS group had stable *nkaα1a* mRNA abundance at this point. This could be interpreted as the Low TSS group starting the smoltification process earlier.

Before smoltification in FW, *nkaα1b* is expressed in small amounts in the CCs in the gills (McCormick et al., 2009). And during smoltification, the mRNA abundance is expected to increase and reach its peak before decreasing when in SW conditions (Handeland et al., 2014; Nilsen et al., 2007). This was observed during this study, where the *nkaα1b* mRNA abundance was low but present during the start of the winter signal for both TSS groups. From T1 to T2 and T2 to T3, both TSS groups had a significant increase in *nkaα1b* mRNA abundance and reached their peak at T3 in FW conditions. The Low TSS group had a significantly higher *nkaα1b* mRNA abundance at both T1 and T2, indicating a similar profile as for the *nkaα1a*, that the smoltification process starts earlier for the Low TSS group.

The *nkcc1a* cotransporter is expected to increase at the same time as the *nkaα1b* because of the increase in the total amount of CCs (Nilsen et al., 2007). In this study, the *nkcc1a* mRNA abundance followed the expression of *nkaα1b* and had a significant increase from T1 to T2 and T2 to T3, where the peak was. When the salinity increased from FW conditions to brackish conditions, the expression then decreased before having a significant increase after SW transfer, this was true for both the TSS groups. There was no observed significant difference between the two TSS groups, but the Low TSS group had a constant higher *nkcc1a* mRNA abundance (though not a significantly higher) *nkcc1a* mRNA abundance at any of the sampling points.

Mature male salmons were decided to be excluded from the result section. However, figure A4.2 for *nkaα1a*, figure A4.3 for *nkaα1b*, and figure A4.4 for *nkcc1a* in Appendix 4 shows the results, including mature male salmons. The results for *nkcc1a* and *nkaα1b* follow the same trends with mature males as without. *nkaα1a* results, including mature males, had a significant difference in T7, where all the mature males in the Low TSS treatment had a significantly higher *nkaα1a* mRNA abundance compared to the non-mature fish. This is consistent with the findings of Elgen (2011), which also had an increase in *nkaα1a* mRNA

abundance in mature males. Elgen paper discussed that this could be consider as a preparation to reenter FW rivers to mate. Figure A5.2 and A5.3 in Appendix 5 shows that there is no correlation between GSI and mRNA abundance for either of *nkaα1a* or *nkaα1b*. But there is a negative correlation between GSI and mRNA abundance for *nkcc1a* in Appendix 5 Figure A5.4.

4.2.2.3 Plasma ions

In FW, parr has a plasmatic ion concentration of Na^+ that is expected to be between 135-155 mM and 115 – 135 mM for Cl^- (Hansen and Mortensen, 1998). In SW, the salmon Na^+ plasmatic concentration is likely between 145-165 mM and 130 – 150 mM for Cl^-. A deviation from the expected value by 20-30 % would seriously disturb the ion balance (Casanovas et al., 2021; Hansen and Mortensen, 1998). Plasma ion concentration has been used in studies as an indicator of stress in Atlantic salmon (Carey and McCormick, 1998).

The plasmatic sodium concentration was held at a constant concentration with little deviation from expected levels during the FW stage for both TSS groups. When the fish was experiencing BW conditions, a larger dispersion of plasmatic sodium concentration between the fish was observed for both TSS groups. A large dispersion in the same group of fish indicates that some of the fish are experiencing issues with the ion regulation (Hansen and Mortensen, 1998). From T4 to T5, both TSS groups had a significant reduction in plasmatic sodium concentration, and between the two treatments, the High TSS treatment had a significantly lower plasmatic sodium concentration, while Low TSS group was still within expected values, but the High TSS group was below the limit. A dip below the expected values in plasmatic sodium concentration is an indication of osmoregulatory stress, as also observed for smolts and pre-smolts in the study of (Carey and McCormick, 1998). The mentioned study also observed that the smolts managed to get back to normal osmoregulatory balance after 24 hours, which was consistent with the results in this thesis, where the plasmatic sodium concentration was back within normal values at T6. before High TSS group had a significant increase in plasmatic sodium concentration when entering SW.

The plasmatic chloride concentration was mostly stable during the whole experiment. However, a significant difference in plasmatic chloride concentration between the two treatments was observed at T3 where Low TSS treatment had a significantly higher plasmatic chloride concentration which is a result of the smoltification itself (Carey and McCormick, 1998). When in BW, the plasmatic chloride concentration had no significant differences

between treatments, but in T5 High TSS treatment had a significantly lower plasmatic chloride concentration compared to Low TSS, same result as observed for sodium.

During this study, T5 is the sampling point with the most intresting results for plasmatic ion concentration. At this point, plasmatic ion concentration was significantly lower in the High TSS group compared to the Low TSS group, even though the Low TSS group also experienced a decrease in overall plasmatic ion concentration. As mentioned in discussion of methods, High TSS RAS modules experienced a peak of TSS (26.5 mg/l ± 12.1 mg/l) two weeks prior, before having an approximate TSS concentration of ~ 4 mg/l at T5 (Appendix 6 Figure A6.1). This fluctuation in TSS could induce osmoregulatory stress.

Mature male salmons were decided to exclude from the analyses and thus, from the result section. Figure A4.5 for sodium, and figure A4.6 for chloride in the Appendix 4 show the data, including mature male salmons. The results for both ions concentration follow the same trend with mature males as without mature males. The only difference is that by including mature males, there is no significant difference in ion concentration between the two TSS treatments at any point during the RAS stage, while without mature males, at the sampling point T5, a significant difference in both sodium and chloride ion concentrations was observed between the TSS treatments. Additionally, figure A5.5, and A5.6 in appendix 5 shows that there is a positive correlation between GSI and ion concentration for both sodium and chloride.

4.2.2.4 Simulated transport

Simulated transport was conducted to mimic the stressful conditions fish goes through when transported from a land-based facility to a sea cage. Handling and transport can initiate severe stress responses in Atlantic salmon, where cortisol release in the fish can suppress immunological capacity and affect SW tolerance, growth, and survival (Iversen et al., 2005, 1998). Before the simulated transport, both High TSS and Low TSS treatments had no significant difference in plasmatic sodium or chloride concentration. When the simulated transport was finished, the fish experienced full-strength SW (33.6 ± 0.7 ‰). The High TSS group had a significant increase in plasmatic sodium and chloride concentrations at the end of the simulated transport. The mean plasmatic sodium concentration in the High TSS fish was 180 mM, which is well above the expected values (145-165 mM) for salmon smolts in SW, and this indicates a disruption in the ionic balance (Hansen and Mortensen, 1998). 3 hours after simulated transport the High TSS group had a significant decrease in both plasmatic ion

concentrations, which is the same as (Carey and McCormick, 1998) observed, where the fish managed to re-balance after a stressful situation, indicating that the High TSS groups ion concentration was within their scope for physiological adaptation. Even under a stressful situation and transfer to a new environment, the fish from the Low TSS managed to maintain ion homeostasis throughout the whole process. It is also worth noting that the results from simulated transport most probably include mature males salmon. However, since the GSI could not be measured, it was not impossible to exclude them from the analyses.

5 Conclusion

The overall effect of treatment was not an important factor in explaining the difference in the results, except for *nkaα1b* mRNA abundance. Whereas time was an important factor for all the results. The results show that the salmon, regardless of TSS treatment within the frame of this experiment, will stick to the expected morphological and physiological changes associated with smoltification.

The salmon in both groups demonstrated a distinctive growth pattern. The High TSS group had a reduced growth during the RAS period compared to the Low TSS group. A reduced feeding behavior and potentially reduced visibility do to a higher turbidity could affect the growth during smoltification. However, a significant shift in SGR was observed after SW transfer, indicating a compensatory growth mechanism under optimal conditions.

The NKA activity, which is an indicator of smoltification, showed a consistent trend regardless of TSS treatment. A significant peak was observed for High TSS treatment in T7, but the TSS levels were over the double concentration of the mean TSS treatment at this time. This indicates that overall, the salmon can show some resilience towards having a higher TSS but will react if there is a sudden significant increase in concentration.

From a gene expression perspective, *nkaα1a, nkaα1b, and nkcc1a* isoforms followed the expected expression patterns during smoltification. There were subtle differences between the two TSS treatments, with a delayed response in the High TSS group indicating a possible interference with normal smoltification progression.

Lastly, the plasma ions, sodium, and chloride, remained within normal concentration ranges during the study. However, some significant differences in concentration were observed during the BW stage, especially in the High TSS group, suggesting osmoregulatory stress during this period. This is further demonstrated right after the simulated transport, where the High TSS group had a significant increase in both ion concentrations to a point where it was significantly higher than the expected values for the plasmatic concentration of sodium in salmon in seawater.

In summary, while high TSS appeared to influence the timeline of smoltification and induce physiological stress, compensatory and adaptive mechanisms ensured the successful completion of the smoltification process. This study provides valuable insights into the resilience and adaptation an Atlantic salmon provides during smoltification under sub-optimal rearing conditions. To optimize the smoltification process and timing, and to ensure optimal growth and health in Atlantic salmon, large-scale commercial Recirculating Aquaculture

Systems it is suggested to keep total suspended solids as low as possible until the fish are transferred to seawater.

Following the statistical analyses, the following conclusions about the formulated hypothesis were obtained.

$H0_{1A}$: High TSS treatment in RAS has no significant effect on Condition Factor (CF) in Atlantic salmon, **is rejected.** There was a significant difference in Condition Factor between the two treatments at T4, T7, T9, and T10, and thereby **HA_{1A} is not rejected:** High TSS treatment in RAS has a significant effect on Condition Factor (CF) in Atlantic salmon.

$H0_{2A}$: High TSS treatment in RAS has no significant effect on Specific Growth Rate (SGR) in Atlantic salmon, **is rejected.** There was a significant difference in SGR between the two treatments at T2-T4, T4-T7, T7-T9, and T9-T10, and thereby **HA_{2A} is not rejected:** High TSS treatment in RAS has a significant effect on Specific Growth Rate (SGR) in Atlantic salmon.

$H0_{3A}$: High TSS treatment in RAS has no significant effect on NKA activity in Atlantic salmon gills, **is rejected.** There was a significant difference in NKA activity between the two treatments at T7 and thereby **HA_{3A} is not rejected:** High TSS treatment in RAS has a significant effect on NKA activity in Atlantic salmon gills.

$H0_{4A}$: High TSS treatment in RAS has no significant effect on *nkaα1a* mRNA abundance in Atlantic salmon gills, **is not rejected.** There was no significant difference in *nkaα1a* mRNA abundance between the two treatments at any of the sampling points.

$H0_{5A}$: High TSS treatment in RAS has no significant effect on *nkaα1b* mRNA abundance in Atlantic salmon gills, **is rejected.** There was a significant difference in *nkaα1b* mRNA abundance between the two treatments at T1, T2, and T7 and thereby **HA_{5A} is not rejected:** High TSS treatment in RAS has a significant effect on *nkaα1b* mRNA abundance in Atlantic salmon gills.

$H0_{6A}$: High TSS treatment in RAS has no significant effect on *nkcc1a* mRNA abundance in Atlantic salmon gills, **is not rejected.** There was no significant difference in *nkcc1a* mRNA abundance between the two treatments at any of the sampling points

$H0_{7A}$: High TSS treatment in RAS has no significant effect on plasmatic sodium concentration in Atlantic salmon, **is rejected.** There was a significant difference in plasmatic sodium concentration between the two treatments at T5 and thereby **HA_{7A} is not rejected:** High TSS treatment in RAS has a significant effect on plasmatic sodium concentration in Atlantic salmon.

$H0_{8A}$: High TSS treatment in RAS has no significant effect on plasmatic chloride concentration in Atlantic salmon, **is rejected.** There was a significant difference in plasmatic chloride concentration between the two treatments at T3 and T5 and thereby **HA_{8A} is not rejected:** High TSS treatment in RAS has a significant effect on plasmatic chloride concentration in Atlantic salmon.

$H0_{9A}$: High TSS treatment in RAS has no significant effect on plasmatic sodium concentration in Atlantic salmon after simulated transport, **is rejected.** There was a significant difference in plasmatic sodium concentration between the two treatments at post-transport and thereby **HA_{9A} is not rejected:** High TSS treatment in RAS has a significant effect on plasmatic sodium concentration in Atlantic salmon after simulated transport.

$H0_{10A}$: High TSS treatment in RAS has no significant effect on plasmatic chloride concentration in Atlantic salmon after simulated transport, **is rejected.** There was a significant difference in plasmatic chloride concentration between the two treatments at post-transport and thereby **HA_{10A} is not rejected:** High TSS treatment in RAS has a significant effect on plasmatic chloride concentration in Atlantic salmon after simulated transport.

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7 Appendix

1 Melting curve, dilution curve, and primer efficiency for RT-qPCR

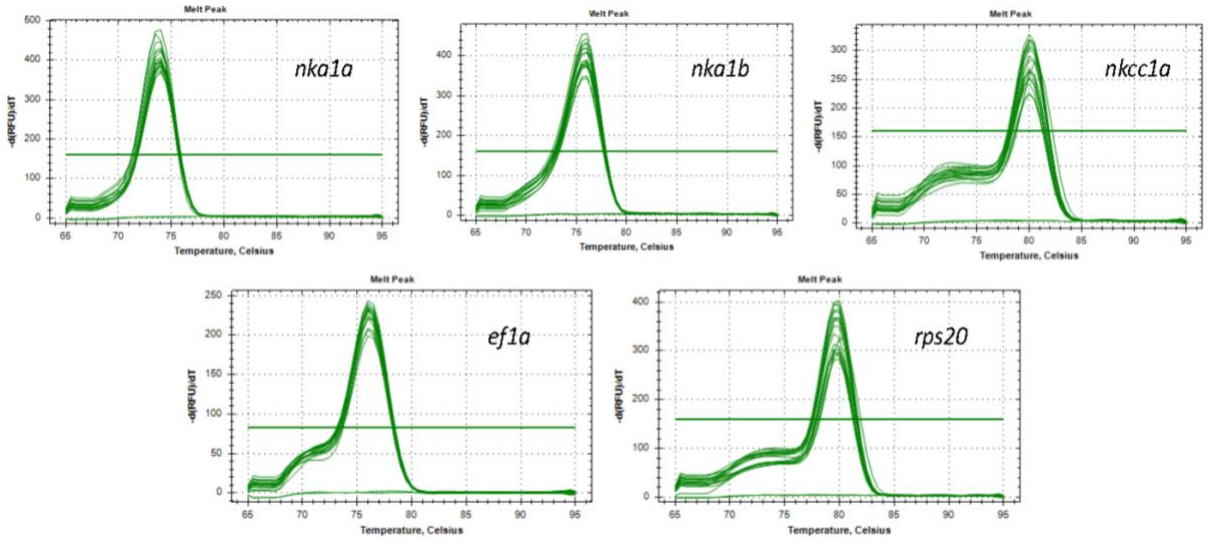

Figure A1.1: Melting curve profile of *nka*a*1a* , *nka*a*1b*, *nkcc1a, ef1a and rps20* from the qPCR analysis.

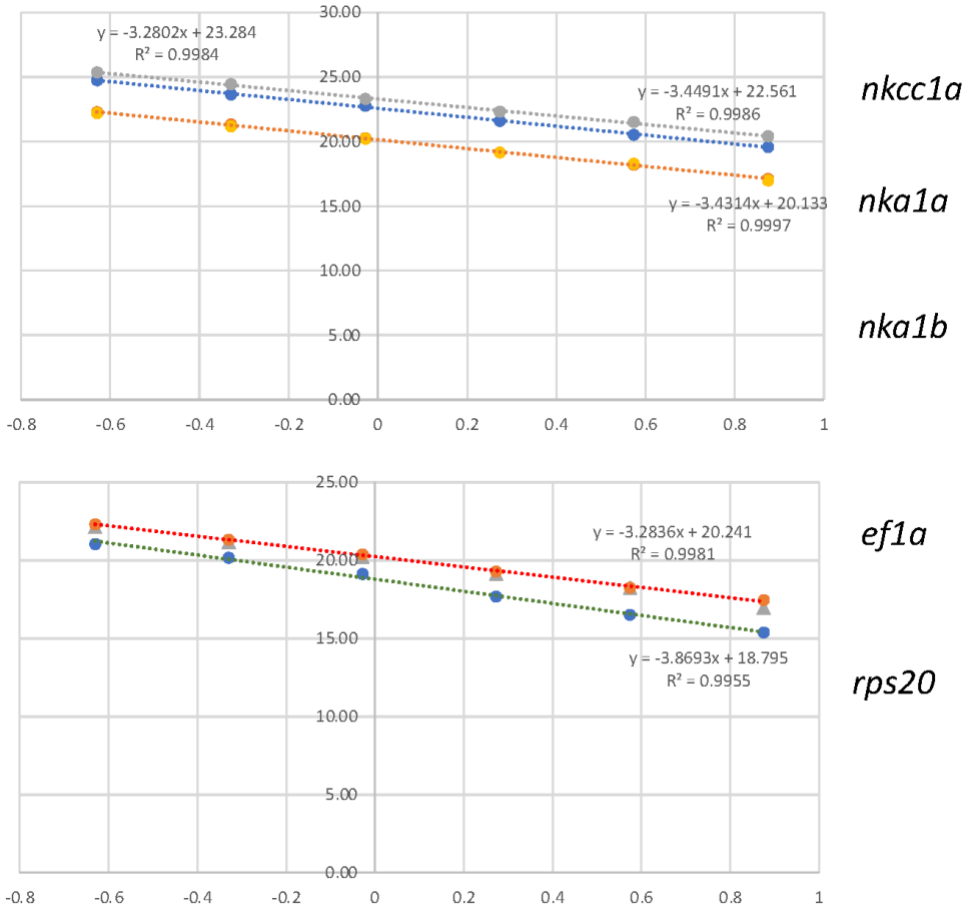

Figure A1.2: Dilution curve profile of *nkaa1a* , *nkaa1b*, *nkcc1a*, *ef1a and rps20* from the qPCR analysis.

Table A1.1: Primer efficiency of *nkaa1a* , *nkaa1b*, *nkcc1a*, *ef1a and rps20* from the qPCR analysis.

Primer	**Slope**	**Efficiency**
nkaα1a	-3.4491	95%
nkaα1b	-3.4314	96%
nkcc1a	-3.2802	102%
ef1α	-3.2836	102%
rps20	-3.408	97%

2 Result graphs weight and length

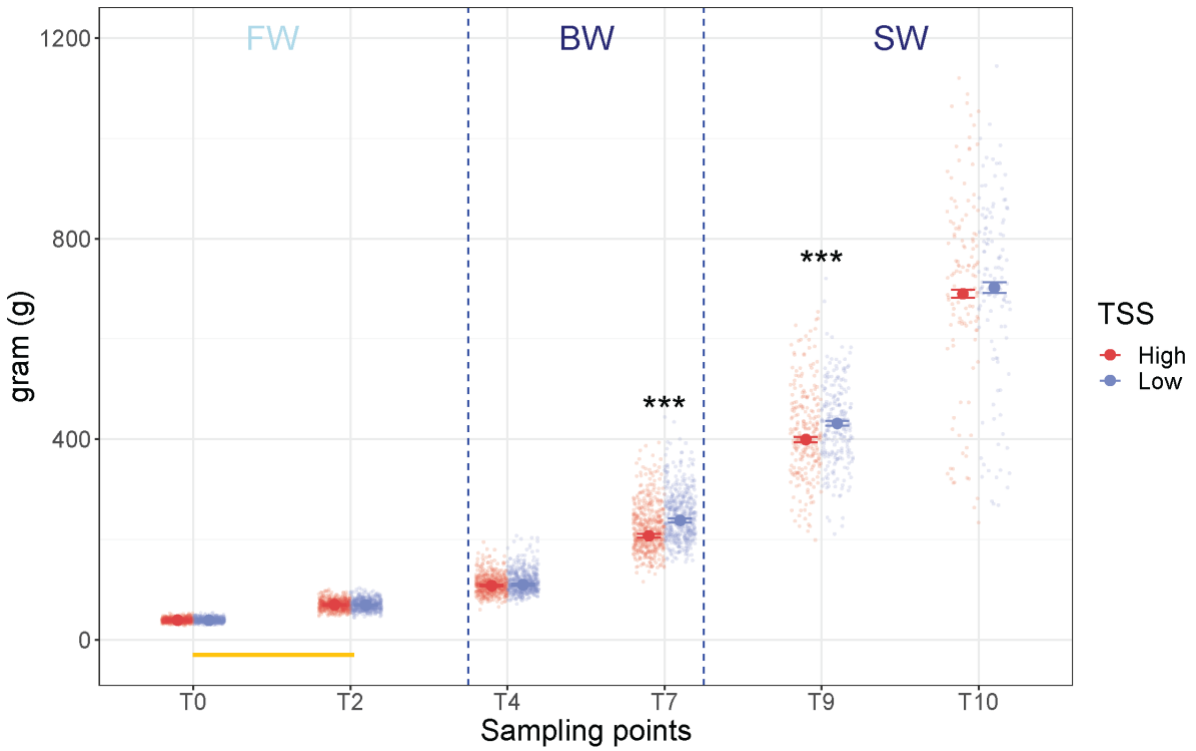

Figure A2.1: Difference in weight between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between High TSS and Low TSS treatment; *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates High TSS treatment and blue colour Low TSS treatment.

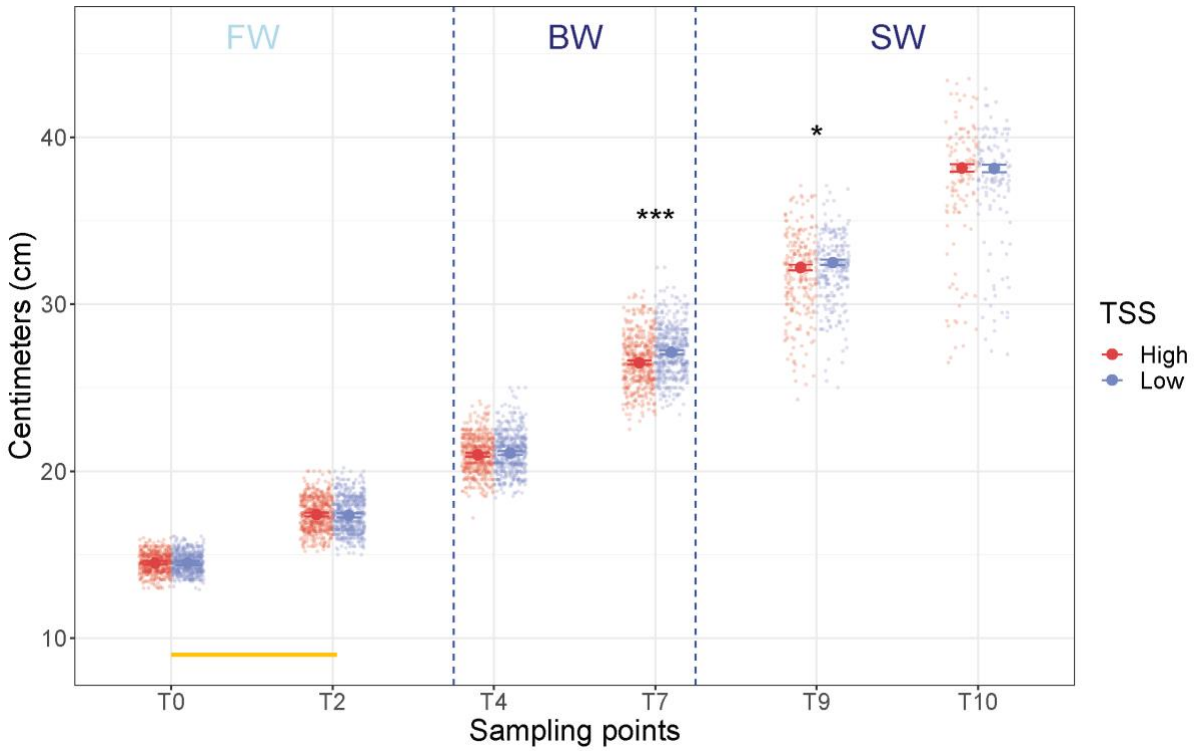

Figure A2.2: Difference in length between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

3 Statistics of Atlantic salmon under High TSS and Low TSS treatment

Condition factor

Table A3.1: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon condition factor (CF) data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	1.291	0.004	308.860	<.0001
TreatmentLow	1.293	0.004	308.920	<.0001
SamplingT2	0.040	0.003	11.710	<.0001
SamplingT4	-0.109	0.003	-32.490	<.0001
SamplingT7	-0.129	0.004	-31.350	<.0001
SamplingT9	-0.015	0.005	-3.200	0.001
SamplingT10	0.071	0.006	11.820	<.0001
TreatmentLow:SamplingT2	0.010	0.005	2.040	0.041
TreatmentLow:SamplingT4	0.030	0.005	6.380	<.0001
TreatmentLow:SamplingT7	0.052	0.006	9.140	<.0001
TreatmentLow:SamplingT9	0.019	0.007	2.850	0.004
TreatmentLow:SamplingT10	0.018	0.009	2.000	0.046

Table A3.2: Post-hoc Tukey HSD analysis between samplings for each treatment in the condition factor of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
T0-T2	High	-0.040	0.003	-11.705	<.0001
T0-T4	High	0.109	0.003	32.49	<.0001
T0-T7	High	0.129	0.004	31.349	<.0001
T0-T9	High	0.015	0.005	3.202	0.0172
T0-T10	High	-0.071	0.006	-11.817	<.0001
T2-T4	High	0.149	0.003	42.698	<.0001
T2-T7	High	0.168	0.004	39.552	<.0001
T2-T9	High	0.054	0.005	11.502	<.0001
T2-T10	High	-0.031	0.006	-5.112	<.0001
T4-T7	High	0.020	0.004	4.831	<.0001
T4-T9	High	-0.094	0.005	-20.255	<.0001
T4-T10	High	-0.180	0.006	-29.933	<.0001
T7-T9	High	-0.114	0.005	-22.164	<.0001
T7-T10	High	-0.199	0.006	-31.286	<.0001
T9-T10	High	-0.085	0.006	-13.151	<.0001
T0-T2	Low	-0.049	0.003	-14.494	<.0001

T0-T4	Low	0.079	0.003	23.324	<.0001
T0-T7	Low	0.077	0.004	18.305	<.0001
T0-T9	Low	-0.004	0.005	-0.828	0.9624
T0-T10	Low	-0.088	0.006	-13.689	<.0001
T2-T4	Low	0.128	0.004	36.036	<.0001
T2-T7	Low	0.126	0.004	28.695	<.0001
T2-T9	Low	0.046	0.005	9.511	<.0001
T2-T10	Low	-0.039	0.007	-5.926	<.0001
T4-T7	Low	-0.002	0.004	-0.517	0.9955
T4-T9	Low	-0.083	0.005	-17.599	<.0001
T4-T10	Low	-0.167	0.006	-25.743	<.0001
T7-T9	Low	-0.080	0.005	-15.623	<.0001
T7-T10	Low	-0.165	0.007	-23.98	<.0001
T9-T10	Low	-0.084	0.007	-12.17	<.0001

Table A3.3: Post-hoc Tukey HSD analysis between treatments for each sampling point in the condition factor of Atlantic salmon data.

PARAMETERS	CONTRAST	ESTIMATE	SE	Z-VALUE	P-VALUE
Sampling: T0	High-Low	-0.002	0.006	-0.263	0.793
Sampling: T2	High-Low	-0.011	0.006	-1.855	0.064
Sampling: T4	High-Low	-0.032	0.006	-5.257	<.0001
Sampling: T7	High-Low	-0.054	0.007	-7.844	<.0001
Sampling: T9	High-Low	-0.020	0.008	-2.669	0.008
Sampling: T10	High-Low	-0.019	0.010	-1.995	0.046

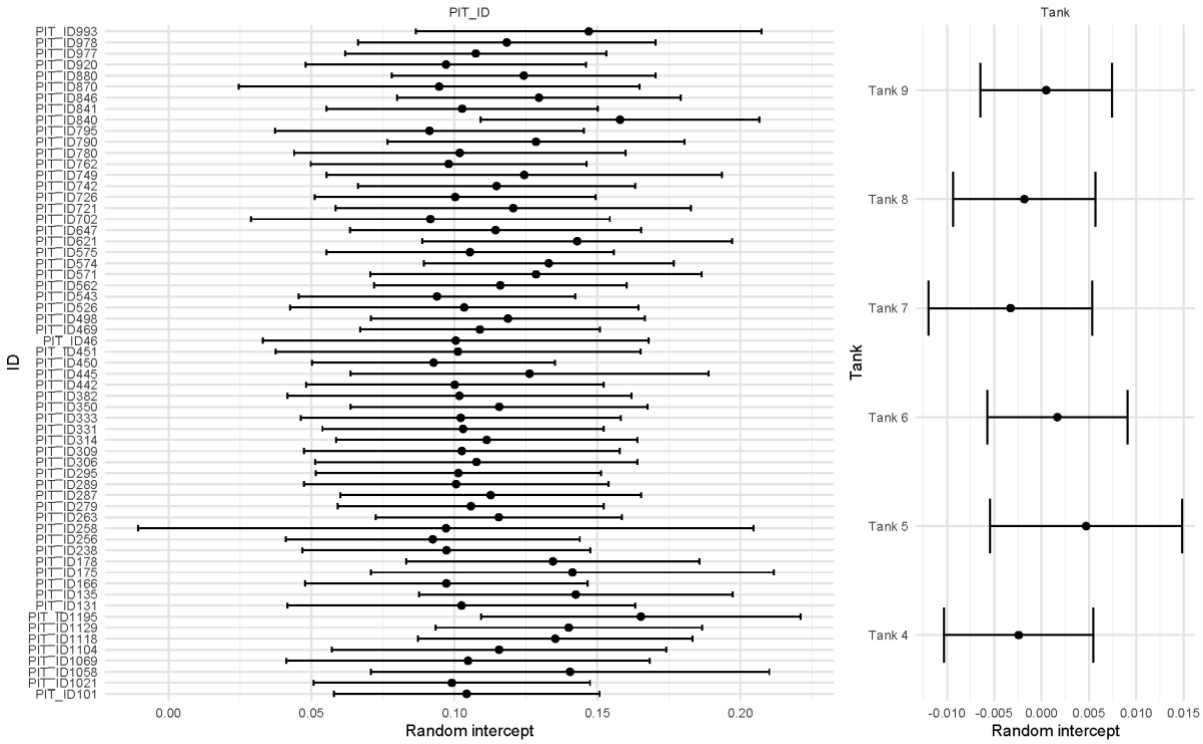

Figure A3.1: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the condition factor of Atlantic salmon under high TSS and low TSS treatment. Tank SD= 0.004176 and Fish ID SD= 0.062161.

Specific Growth Rate

Table A3.4: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon Specific Growth rate (SGR) data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	1.506	0.015	101.360	<.0001
TreatmentLow	1.512	0.015	101.770	<.0001
SamplingT2-T4	-0.254	0.017	-15.050	<.0001
SamplingT4-T7	-0.030	0.016	-1.810	0.071
SamplingT7-T9	-0.291	0.023	-12.500	<.0001
SamplingT9-T10	-0.376	0.026	-14.350	<.0001
fTreatmentLow:SamplingT2-T4	0.113	0.024	4.750	<.0001
fTreatmentLow:SamplingT4-T7	0.146	0.024	6.220	<.0001
fTreatmentLow:SamplingT7-T9	-0.152	0.032	-4.800	<.0001
fTreatmentLow:SamplingT9-T10	-0.079	0.038	-2.090	0.036

Table A3.5: Post-hoc Tukey HSD analysis between samplings for each treatment in the Specific Growth rate of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
(T0-T2)-(T2-T4)	High	0.254	0.017	15.045	<.0001
(T0-T2)-(T4-T7)	High	0.030	0.017	1.809	0.3683
(T0-T2)-(T7-T9)	High	0.292	0.023	12.497	<.0001
(T0-T2)-(T9-T10)	High	0.376	0.026	14.347	<.0001
(T2-T4)-(T4-T7)	High	-0.224	0.018	-12.626	<.0001
(T2-T4)-(T7-T9)	High	0.038	0.025	1.537	0.5383
(T2-T4)-(T9-T10)	High	0.122	0.027	4.48	0.0001
(T4-T7)-(T7-T9)	High	0.262	0.024	10.889	<.0001
(T4-T7)-(T9-T10)	High	0.346	0.027	12.905	<.0001
(T7-T9)-(T9-T10)	High	0.084	0.031	2.716	0.0516
(T0-T2)-(T2-T4)	Low	0.141	0.017	8.366	<.0001
(T0-T2)-(T4-T7)	Low	-0.117	0.017	-6.928	<.0001
(T0-T2)-(T7-T9)	Low	0.443	0.022	20.008	<.0001
(T0-T2)-(T9-T10)	Low	0.455	0.028	16.364	<.0001
(T2-T4)-(T4-T7)	Low	-0.258	0.018	-14.238	<.0001
(T2-T4)-(T7-T9)	Low	0.302	0.024	12.868	<.0001
(T2-T4)-(T9-T10)	Low	0.314	0.029	10.85	<.0001
(T4-T7)-(T7-T9)	Low	0.560	0.023	24.221	<.0001
(T4-T7)-(T9-T10)	Low	0.571	0.029	20.005	<.0001
(T7-T9)-(T9-T10)	Low	0.012	0.032	0.369	0.9961

Table A3.6: Post-hoc Tukey HSD analysis between treatments for each sampling point in the Specific Growth rate of Atlantic salmon data.

PARAMETERS	CONTRAST	RATIO	SE	Z-VALUE	P-VALUE
From: T0-T2	High-Low	-0.005	0.021	-0.246	0.8055
From: T2-T4	High-Low	-0.118	0.023	-5.147	<.0001
From: T4-T7	High-Low	-0.152	0.023	-6.677	<.0001
From: T7-T9	High-Low	0.146	0.031	4.725	<.0001
From: T9-T10	High-Low	0.074	0.037	1.985	0.0472

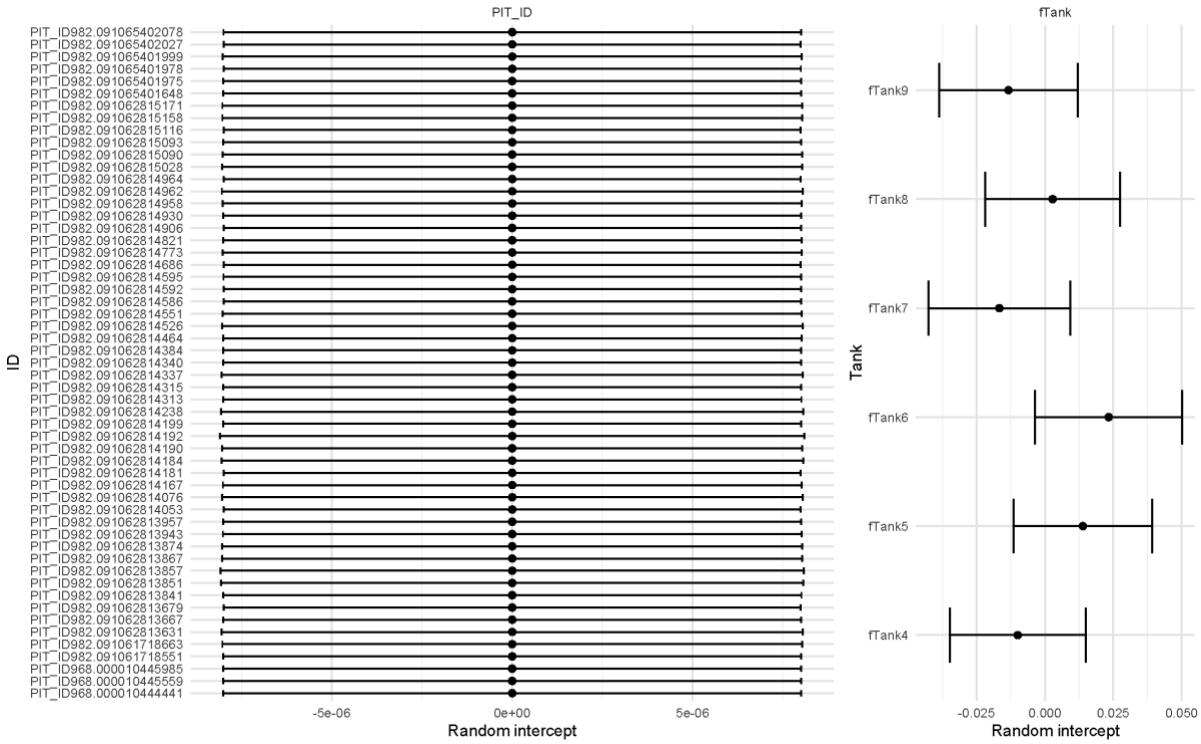

Figure A3.2: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for Specific Growth Rate of Atlantic salmon under high TSS and low TSS treatment. Tank SD= 1.737e-02 and Fish ID SD= 2.394e-06.

NKA activity

Table A3.7: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon NKA activity data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	0.621	0.212	2.924	0.003
TreatmentLow	0.671	0.238	2.817	0.005
SamplingT1	0.011	0.265	0.040	0.968
SamplingT2	0.410	0.276	1.487	0.137
SamplingT3	0.708	0.264	2.677	0.007
SamplingT4	0.818	0.251	3.265	0.001
SamplingT5	0.964	0.263	3.662	<.0001
SamplingT6	0.450	0.280	1.608	0.108
SamplingT7	1.282	0.261	4.910	<.0001
SamplingT8	0.404	0.306	1.319	0.187
TreatmentLow:SamplingT1	0.186	0.389	0.479	0.632
TreatmentLow:SamplingT2	-0.286	0.413	-0.694	0.488
TreatmentLow:SamplingT3	-0.010	0.399	-0.025	0.980
TreatmentLow:SamplingT4	0.177	0.371	0.478	0.633

TreatmentLow:SamplingT5	-0.355	0.387	-0.919	0.358
TreatmentLow:SamplingT6	-0.289	0.402	-0.719	0.472
TreatmentLow:SamplingT7	-0.620	0.384	-1.613	0.107
TreatmentLow:SamplingT8	-0.271	0.444	-0.610	0.542

Table A3.8: Post-hoc Tukey HSD analysis between samplings for each treatment in the NKA activity of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
T0-T1	High	0.989	0.2626	-0.04	1
T0-T2	High	0.664	0.1829	-1.487	0.8619
T0-T3	High	0.493	0.1303	-2.677	0.1559
T0-T4	High	0.441	0.1106	-3.265	0.0302
T0-T5	High	0.381	0.1004	-3.662	0.0077
T0-T6	High	0.637	0.1785	-1.608	0.8006
T0-T7	High	0.277	0.0724	-4.91	<.0001
T0-T8	High	0.668	0.2044	-1.319	0.9259
T1-T2	High	0.671	0.1589	-1.686	0.7556
T1-T3	High	0.498	0.1114	-3.117	0.0478
T1-T4	High	0.446	0.0924	-3.897	0.0031
T1-T5	High	0.386	0.0857	-4.289	0.0006
T1-T6	High	0.644	0.1559	-1.817	0.6707
T1-T7	High	0.28	0.0616	-5.785	<.0001
T1-T8	High	0.675	0.1834	-1.447	0.8796
T2-T3	High	0.742	0.175	-1.264	0.9417
T2-T4	High	0.665	0.1464	-1.855	0.6448
T2-T5	High	0.575	0.1347	-2.364	0.3041
T2-T6	High	0.96	0.2431	-0.16	1
T2-T7	High	0.418	0.097	-3.759	0.0054
T2-T8	High	1.006	0.2834	0.022	1
T3-T4	High	0.895	0.1844	-0.537	0.9998
T3-T5	High	0.774	0.1711	-1.158	0.9649
T3-T6	High	1.293	0.3116	1.068	0.9787
T3-T7	High	0.563	0.1231	-2.627	0.1751
T3-T8	High	1.355	0.3669	1.123	0.971
T4-T5	High	0.865	0.1767	-0.711	0.9987
T4-T6	High	1.445	0.3262	1.63	0.7885
T4-T7	High	0.629	0.1269	-2.298	0.3429
T4-T8	High	1.514	0.3896	1.611	0.7991
T5-T6	High	1.671	0.4003	2.143	0.4438
T5-T7	High	0.727	0.1579	-1.466	0.8714
T5-T8	High	1.751	0.4719	2.078	0.4887

T6-T7	High	0.435	0.1033	-3.504	0.0136
T6-T8	High	1.048	0.2997	0.163	1
T7-T8	High	2.407	0.644	3.283	0.0285
T0-T1	Low	0.821	0.2338	-0.692	0.9989
T0-T2	Low	0.884	0.2716	-0.402	1
T0-T3	Low	0.498	0.1486	-2.337	0.3197
T0-T4	Low	0.369	0.1012	-3.635	0.0085
T0-T5	Low	0.544	0.154	-2.15	0.4389
T0-T6	Low	0.851	0.2454	-0.56	0.9998
T0-T7	Low	0.516	0.1455	-2.347	0.314
T0-T8	Low	0.876	0.2819	-0.412	1
T1-T2	Low	1.076	0.2678	0.295	1
T1-T3	Low	0.606	0.1443	-2.104	0.4705
T1-T4	Low	0.45	0.0928	-3.872	0.0035
T1-T5	Low	0.663	0.1446	-1.886	0.6234
T1-T6	Low	1.036	0.2333	0.157	1
T1-T7	Low	0.628	0.1363	-2.143	0.4437
T1-T8	Low	1.066	0.2844	0.241	1
T2-T3	Low	0.563	0.149	-2.171	0.4251
T2-T4	Low	0.418	0.0988	-3.689	0.007
T2-T5	Low	0.616	0.152	-1.965	0.5683
T2-T6	Low	0.963	0.2437	-0.15	1
T2-T7	Low	0.584	0.1435	-2.191	0.4117
T2-T8	Low	0.991	0.288	-0.032	1
T3-T4	Low	0.742	0.1672	-1.323	0.9249
T3-T5	Low	1.093	0.2582	0.378	1
T3-T6	Low	1.71	0.4148	2.211	0.3981
T3-T7	Low	1.037	0.2436	0.153	1
T3-T8	Low	1.76	0.4955	2.008	0.5379
T4-T5	Low	1.473	0.3005	1.898	0.6154
T4-T6	Low	2.303	0.4869	3.946	0.0026
T4-T7	Low	1.396	0.283	1.647	0.7788
T4-T8	Low	2.37	0.6049	3.382	0.0206
T5-T6	Low	1.564	0.3489	2.004	0.5406
T5-T7	Low	0.948	0.2037	-0.248	1
T5-T8	Low	1.609	0.4264	1.796	0.6849
T6-T7	Low	0.606	0.1345	-2.255	0.3696
T6-T8	Low	1.029	0.2786	0.106	1
T7-T8	Low	1.698	0.4481	2.005	0.5394

Table A3.9: Post-hoc Tukey HSD analysis between treatments for each sampling point in the NKA activity of Atlantic salmon data.

PARAMETERS	CONTRAST	RATIO	SE	Z-VALUE	P-VALUE
Sampling: T0	High-Low	0.951	0.304	-0.156	0.8757
Sampling: T1	High-Low	0.79	0.176	-1.061	0.2887
Sampling: T2	High-Low	1.267	0.331	0.904	0.3661
Sampling: T3	High-Low	0.961	0.23	-0.168	0.8667
Sampling: T4	High-Low	0.797	0.151	-1.199	0.2306
Sampling: T5	High-Low	1.357	0.296	1.401	0.1611
Sampling: T6	High-Low	1.27	0.31	0.978	0.3281
Sampling: T7	High-Low	1.769	0.379	2.663	0.0077
Sampling: T8	High-Low	1.247	0.385	0.716	0.474

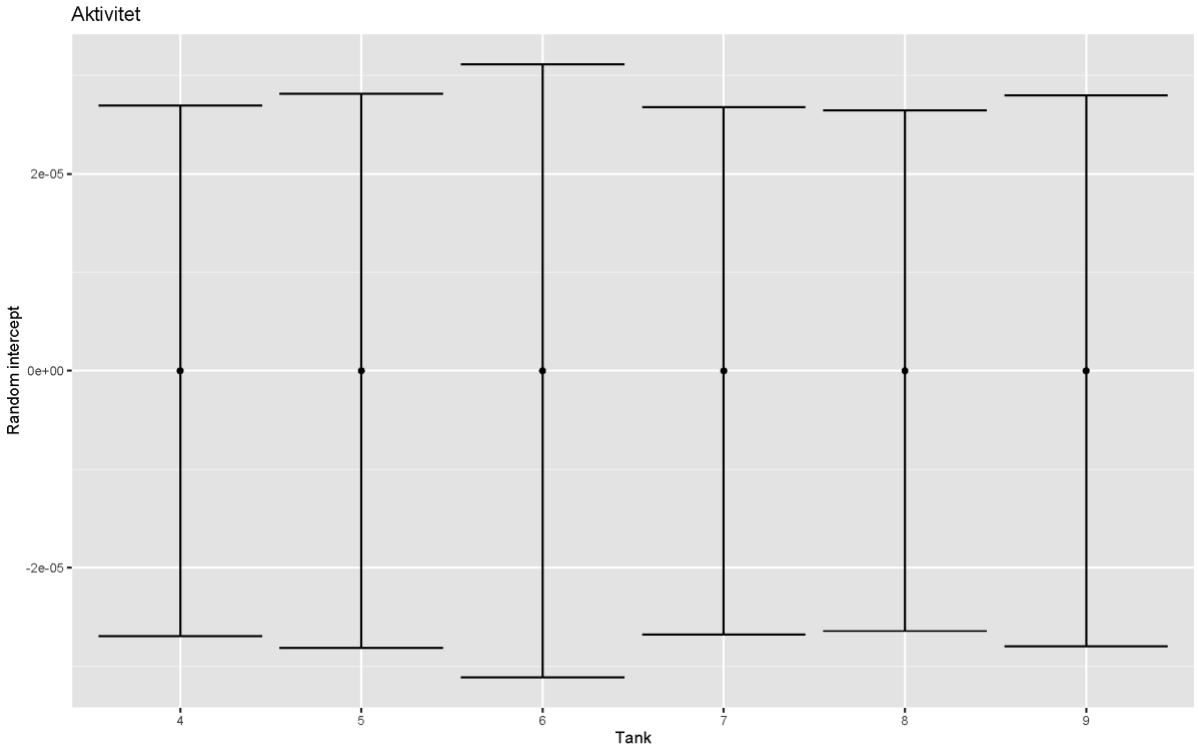

Figure A3.3: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the NKA activity of Atlantic salmon under high TSS and low TSS treatment. SD= 1.33e-05.

nkaα1a

Table A3.10: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon *nkaα1a* data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	0.489	0.155	3.146	0.002
TreatmentLow	0.439	0.147	2.978	0.003
SamplingT1	-0.389	0.188	-2.072	0.038
SamplingT2	-0.466	0.201	-2.320	0.020
SamplingT3	-0.442	0.202	-2.191	0.028
SamplingT4	-2.125	0.216	-9.857	<.0001
SamplingT5	-3.615	0.305	-11.853	<.0001
SamplingT7	-4.406	0.303	-14.544	<.0001
SamplingT8	-2.985	0.303	-9.840	<.0001
TreatmentLow:SamplingT1	0.223	0.263	0.848	0.396
TreatmentLow:SamplingT2	0.281	0.275	1.024	0.306
TreatmentLow:SamplingT3	-0.159	0.285	-0.559	0.576
TreatmentLow:SamplingT4	0.142	0.299	0.473	0.636
TreatmentLow:SamplingT5	0.549	0.394	1.392	0.164
TreatmentLow:SamplingT7	0.514	0.469	1.094	0.274
TreatmentLow:SamplingT8	-0.023	0.402	-0.057	0.955

Table A3.11: Post-hoc Tukey HSD analysis between samplings for each treatment in the *nkaα1a* of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
T0-T1	High	1.476	0.277	2.072	0.433
T0-T2	High	1.594	0.321	2.320	0.2826
T0-T3	High	1.556	0.314	2.191	0.3571
T0-T4	High	8.372	1.805	9.857	<.0001
T0-T5	High	37.136	11.325	11.853	<.0001
T0-T7	High	81.980	24.838	14.544	<.0001
T0-T8	High	19.790	6.004	9.840	<.0001
T1-T2	High	1.080	0.200	0.416	0.9999
T1-T3	High	1.054	0.193	0.287	1
T1-T4	High	5.672	1.124	8.755	<.0001
T1-T5	High	25.158	7.370	11.009	<.0001
T1-T7	High	55.538	16.129	13.832	<.0001
T1-T8	High	13.407	3.910	8.902	<.0001
T2-T3	High	0.976	0.195	-0.122	1
T2-T4	High	5.252	1.119	7.787	<.0001
T2-T5	High	23.296	7.053	10.399	<.0001

T2-T7	High	51.427	15.504	13.069	<.0001
T2-T8	High	12.414	3.741	8.358	<.0001
T3-T4	High	5.381	1.135	7.977	<.0001
T3-T5	High	23.870	7.216	10.495	<.0001
T3-T7	High	52.694	15.756	13.258	<.0001
T3-T8	High	12.720	3.823	8.463	<.0001
T4-T5	High	4.436	1.379	4.793	<.0001
T4-T7	High	9.792	3.020	7.397	<.0001
T4-T8	High	2.364	0.732	2.777	0.1009
T5-T7	High	2.208	0.831	2.104	0.4121
T5-T8	High	0.533	0.201	-1.668	0.7083
T7-T8	High	0.241	0.091	-3.784	0.0038
T0-T1	Low	1.181	0.217	0.908	0.9854
T0-T2	Low	1.203	0.226	0.986	0.9766
T0-T3	Low	1.824	0.365	3.006	0.0538
T0-T4	Low	7.266	1.509	9.550	<.0001
T0-T5	Low	21.457	5.356	12.283	<.0001
T0-T7	Low	49.053	17.626	10.834	<.0001
T0-T8	Low	20.247	5.594	10.888	<.0001
T1-T2	Low	1.019	0.178	0.106	1
T1-T3	Low	1.545	0.290	2.318	0.2833
T1-T4	Low	6.152	1.216	9.189	<.0001
T1-T5	Low	18.167	4.388	12.004	<.0001
T1-T7	Low	41.531	14.777	10.473	<.0001
T1-T8	Low	17.142	4.601	10.588	<.0001
T2-T3	Low	1.516	0.292	2.165	0.3732
T2-T4	Low	6.040	1.216	8.929	<.0001
T2-T5	Low	17.834	4.359	11.787	<.0001
T2-T7	Low	40.772	14.594	10.359	<.0001
T2-T8	Low	16.829	4.564	10.410	<.0001
T3-T4	Low	3.983	0.851	6.469	<.0001
T3-T5	Low	11.761	2.998	9.670	<.0001
T3-T7	Low	26.888	9.812	9.021	<.0001
T3-T8	Low	11.098	3.110	8.589	<.0001
T4-T5	Low	2.953	0.769	4.160	0.0008
T4-T7	Low	6.751	2.480	5.199	<.0001
T4-T8	Low	2.786	0.797	3.583	0.0082
T5-T7	Low	2.286	0.897	2.107	0.4099
T5-T8	Low	0.944	0.300	-0.183	1
T7-T8	Low	0.413	0.169	-2.156	0.3791

Table A3.12: Post-hoc Tukey HSD analysis between treatments for each sampling point in the *nkaα1a* of Atlantic salmon data.

PARAMETERS	CONTRAST	RATIO	SE	Z-VALUE	P-VALUE
Sampling: T0	High-Low	1.051	0.225	0.231	0.8173
Sampling: T1	High-Low	0.841	0.156	-0.932	0.3512
Sampling: T2	High-Low	0.793	0.162	-1.134	0.2568
Sampling: T3	High-Low	1.232	0.266	0.968	0.3332
Sampling: T4	High-Low	0.912	0.214	-0.392	0.6949
Sampling: T5	High-Low	0.607	0.211	-1.436	0.1511
Sampling: T7	High-Low	0.629	0.271	-1.075	0.2825
Sampling: T8	High-Low	1.075	0.366	0.212	0.8319

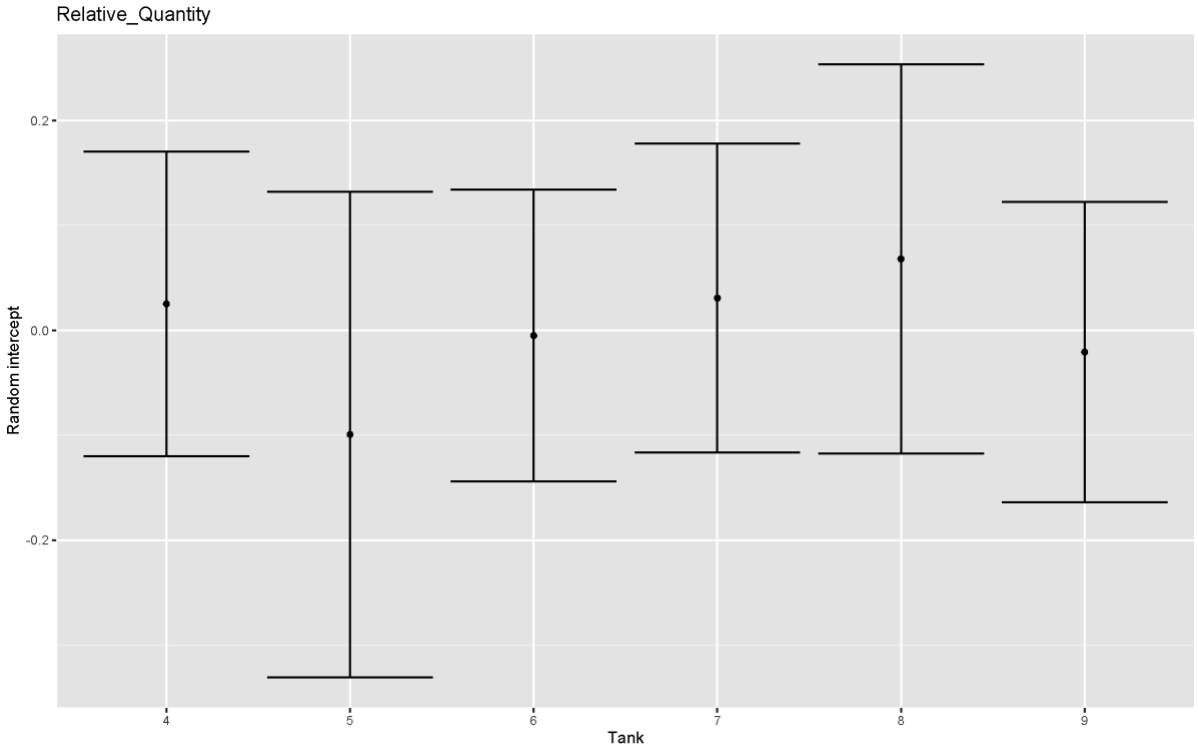

Figure A3.4: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the *nkaα1a* of Atlantic salmon under high TSS and low TSS treatment. SD= 0.08068.

nkaα1b

Table A3.13: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon *nkaα1b* data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	0.063	0.126	0.500	0.617
TreatmentLow	-0.022	0.131	-0.170	0.865
SamplingT1	-0.182	0.168	-1.080	0.280
SamplingT2	0.357	0.162	2.199	0.028
SamplingT3	0.910	0.150	6.071	<.0001
SamplingT4	0.573	0.148	3.864	<.0001
SamplingT5	0.496	0.162	3.051	0.002
SamplingT7	0.315	0.160	1.966	0.049
SamplingT8	0.486	0.167	2.904	0.004
TreatmentLow:SamplingT1	0.395	0.233	1.695	0.090
TreatmentLow:SamplingT2	0.402	0.224	1.798	0.072
TreatmentLow:SamplingT3	0.100	0.215	0.466	0.641
TreatmentLow:SamplingT4	0.252	0.211	1.199	0.231
TreatmentLow:SamplingT5	0.040	0.225	0.176	0.860
TreatmentLow:SamplingT7	0.513	0.233	2.205	0.027
TreatmentLow:SamplingT8	0.186	0.234	0.795	0.427

Table A3.14: Post-hoc Tukey HSD analysis between samplings for each treatment in the *nkaα1b* of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
T0-T1	High	1.199	0.202	1.080	0.9611
T0-T2	High	0.700	0.114	-2.199	0.3522
T0-T3	High	0.402	0.060	-6.071	<.0001
T0-T4	High	0.564	0.084	-3.864	0.0028
T0-T5	High	0.609	0.099	-3.051	0.0471
T0-T7	High	0.730	0.117	-1.966	0.5051
T0-T8	High	0.615	0.103	-2.904	0.0718
T1-T2	High	0.583	0.088	-3.566	0.0087
T1-T3	High	0.336	0.046	-7.935	<.0001
T1-T4	High	0.470	0.064	-5.558	<.0001
T1-T5	High	0.508	0.077	-4.481	0.0002
T1-T7	High	0.608	0.091	-3.339	0.0191
T1-T8	High	0.513	0.080	-4.269	0.0005
T2-T3	High	0.575	0.075	-4.241	0.0006
T2-T4	High	0.806	0.104	-1.677	0.7022
T2-T5	High	0.871	0.126	-0.957	0.9802

T2-T7	High	1.043	0.148	0.296	1
T2-T8	High	0.879	0.132	-0.857	0.9897
T3-T4	High	1.401	0.157	3.010	0.0531
T3-T5	High	1.514	0.197	3.178	0.032
T3-T7	High	1.813	0.232	4.661	0.0001
T3-T8	High	1.529	0.209	3.112	0.0393
T4-T5	High	1.080	0.139	0.599	0.9989
T4-T7	High	1.294	0.163	2.049	0.4484
T4-T8	High	1.091	0.147	0.646	0.9982
T5-T7	High	1.198	0.170	1.270	0.91
T5-T8	High	1.010	0.152	0.066	1
T7-T8	High	0.843	0.125	-1.156	0.9442
T0-T1	Low	0.808	0.130	-1.323	0.8904
T0-T2	Low	0.468	0.072	-4.937	<.0001
T0-T3	Low	0.364	0.056	-6.577	<.0001
T0-T4	Low	0.438	0.066	-5.517	<.0001
T0-T5	Low	0.585	0.091	-3.429	0.014
T0-T7	Low	0.437	0.074	-4.911	<.0001
T0-T8	Low	0.511	0.084	-4.103	0.0011
T1-T2	Low	0.579	0.072	-4.413	0.0003
T1-T3	Low	0.451	0.056	-6.453	<.0001
T1-T4	Low	0.542	0.064	-5.166	<.0001
T1-T5	Low	0.724	0.092	-2.545	0.1766
T1-T7	Low	0.541	0.077	-4.338	0.0004
T1-T8	Low	0.632	0.086	-3.375	0.0169
T2-T3	Low	0.778	0.089	-2.197	0.3531
T2-T4	Low	0.937	0.102	-0.603	0.9988
T2-T5	Low	1.251	0.147	1.909	0.5447
T2-T7	Low	0.934	0.125	-0.514	0.9996
T2-T8	Low	1.092	0.139	0.688	0.9973
T3-T4	Low	1.203	0.130	1.712	0.6798
T3-T5	Low	1.607	0.188	4.054	0.0013
T3-T7	Low	1.200	0.160	1.365	0.8733
T3-T8	Low	1.402	0.178	2.662	0.1345
T4-T5	Low	1.336	0.149	2.592	0.1587
T4-T7	Low	0.997	0.128	-0.025	1
T4-T8	Low	1.165	0.142	1.254	0.9156
T5-T7	Low	0.746	0.102	-2.149	0.3833
T5-T8	Low	0.872	0.114	-1.049	0.9669
T7-T8	Low	1.169	0.169	1.079	0.9614

Table A3.15: Post-hoc Tukey HSD analysis between treatments for each sampling point in the *nkaα1b* of Atlantic salmon data.

PARAMETERS	CONTRAST	RATIO	SE	Z-VALUE	P-VALUE
Sampling: T0	High-Low	1.089	0.198	0.469	0.6388
Sampling: T1	High-Low	0.734	0.107	-2.126	0.0335
Sampling: T2	High-Low	0.728	0.095	-2.432	0.015
Sampling: T3	High-Low	0.985	0.112	-0.128	0.8978
Sampling: T4	High-Low	0.846	0.090	-1.574	0.1154
Sampling: T5	High-Low	1.047	0.139	0.343	0.7319
Sampling: T7	High-Low	0.652	0.095	-2.947	0.0032
Sampling: T8	High-Low	0.904	0.133	-0.684	0.4942

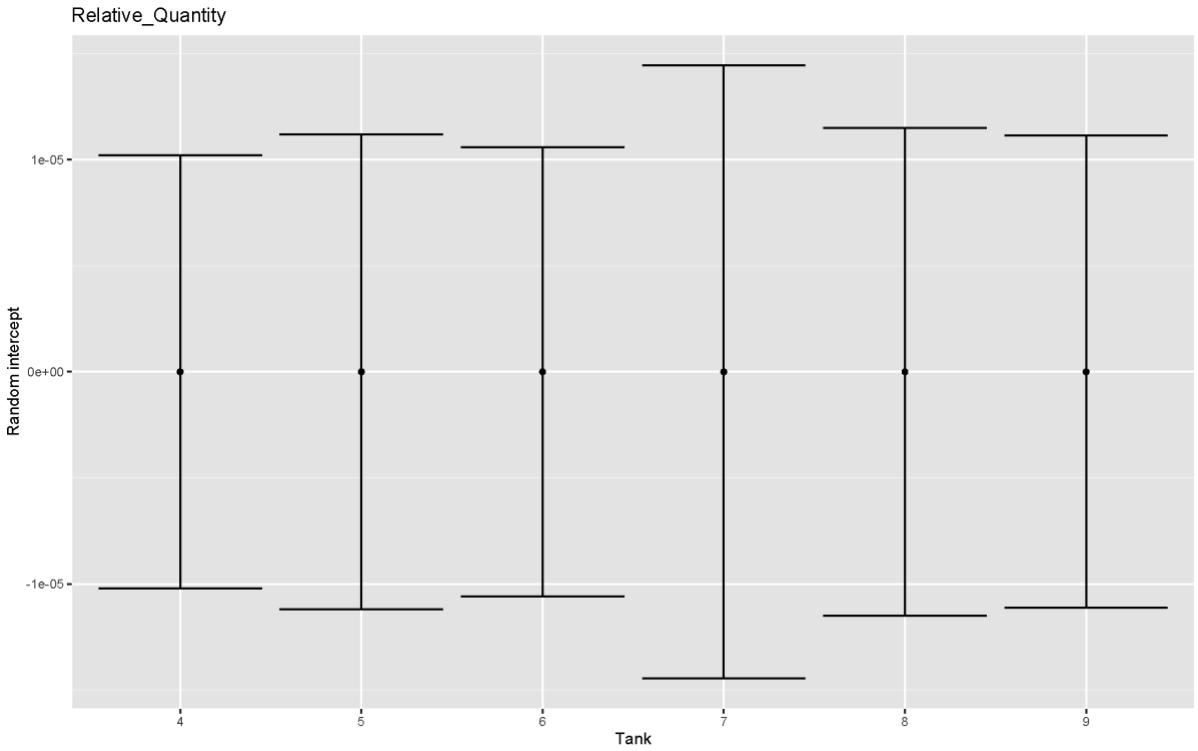

Figure A3.5: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the *nkaα1b* of Atlantic salmon under high TSS and low TSS treatment. SD= 5.15e-06.

nkcc1a

Table A3.16: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon *nkcc1a* data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	-2.327	0.121	-19.245	<.0001
TreatmentLow	-2.272	0.118	-19.268	<.0001
SamplingT1	-0.001	0.156	-0.008	0.993
SamplingT2	0.467	0.152	3.076	0.002
SamplingT3	0.768	0.145	5.293	<.0001
SamplingT4	0.434	0.144	3.021	0.003
SamplingT5	0.298	0.160	1.856	0.063
SamplingT7	-0.015	0.159	-0.097	0.923
SamplingT8	0.561	0.157	3.574	<.0001
TreatmentLow:SamplingT1	0.157	0.214	0.737	0.461
TreatmentLow:SamplingT2	0.050	0.208	0.243	0.808
TreatmentLow:SamplingT3	-0.003	0.203	-0.013	0.990
TreatmentLow:SamplingT4	0.136	0.199	0.681	0.496
TreatmentLow:SamplingT5	0.074	0.215	0.345	0.730
TreatmentLow:SamplingT7	0.241	0.233	1.033	0.301
TreatmentLow:SamplingT8	0.085	0.215	0.396	0.692

Table A3.17: Post-hoc Tukey HSD analysis between samplings for each treatment in the *nkcc1a* of Atlantic salmon data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
T0-T1	High	1.001	0.156	0.008	1
T0-T2	High	0.627	0.095	-3.076	0.0438
T0-T3	High	0.464	0.067	-5.293	<.0001
T0-T4	High	0.648	0.093	-3.021	0.0515
T0-T5	High	0.742	0.119	-1.856	0.5812
T0-T7	High	1.016	0.162	0.097	1
T0-T8	High	0.571	0.090	-3.574	0.0084
T1-T2	High	0.626	0.085	-3.471	0.0122
T1-T3	High	0.463	0.059	-6.046	<.0001
T1-T4	High	0.647	0.081	-3.464	0.0124
T1-T5	High	0.741	0.107	-2.070	0.4345
T1-T7	High	1.014	0.145	0.098	1
T1-T8	High	0.570	0.080	-3.999	0.0016
T2-T3	High	0.740	0.090	-2.462	0.2119
T2-T4	High	1.034	0.125	0.275	1
T2-T5	High	1.185	0.166	1.210	0.9292

T2-T7	High	1.621	0.225	3.479	0.0118
T2-T8	High	0.911	0.124	-0.688	0.9973
T3-T4	High	1.396	0.156	2.987	0.0567
T3-T5	High	1.600	0.212	3.547	0.0093
T3-T7	High	2.189	0.287	5.970	<.0001
T3-T8	High	1.230	0.158	1.616	0.7409
T4-T5	High	1.146	0.150	1.040	0.9683
T4-T7	High	1.568	0.203	3.466	0.0124
T4-T8	High	0.881	0.112	-1.000	0.9746
T5-T7	High	1.368	0.203	2.116	0.4042
T5-T8	High	0.769	0.112	-1.809	0.6141
T7-T8	High	0.562	0.081	-3.997	0.0016
T0-T1	Low	0.855	0.125	-1.069	0.9631
T0-T2	Low	0.596	0.085	-3.644	0.0065
T0-T3	Low	0.465	0.066	-5.407	<.0001
T0-T4	Low	0.566	0.078	-4.135	0.0009
T0-T5	Low	0.689	0.099	-2.600	0.1559
T0-T7	Low	0.798	0.136	-1.325	0.8896
T0-T8	Low	0.524	0.077	-4.395	0.0003
T1-T2	Low	0.696	0.082	-3.089	0.0421
T1-T3	Low	0.544	0.063	-5.233	<.0001
T1-T4	Low	0.661	0.074	-3.700	0.0053
T1-T5	Low	0.806	0.095	-1.825	0.6029
T1-T7	Low	0.933	0.140	-0.463	0.9998
T1-T8	Low	0.613	0.075	-3.983	0.0017
T2-T3	Low	0.781	0.087	-2.220	0.3394
T2-T4	Low	0.949	0.101	-0.487	0.9997
T2-T5	Low	1.157	0.131	1.283	0.9054
T2-T7	Low	1.340	0.196	1.999	0.4824
T2-T8	Low	0.880	0.104	-1.082	0.9606
T3-T4	Low	1.216	0.129	1.847	0.5876
T3-T5	Low	1.482	0.167	3.485	0.0116
T3-T7	Low	1.716	0.250	3.706	0.0052
T3-T8	Low	1.127	0.133	1.016	0.9722
T4-T5	Low	1.219	0.132	1.829	0.6003
T4-T7	Low	1.411	0.200	2.424	0.2295
T4-T8	Low	0.927	0.105	-0.673	0.9977
T5-T7	Low	1.158	0.171	0.996	0.9752
T5-T8	Low	0.760	0.091	-2.289	0.2993
T7-T8	Low	0.657	0.099	-2.785	0.0987

Table A3.18: Post-hoc Tukey HSD analysis between treatments for each sampling point in the *nkcc1a* of Atlantic salmon data.

PARAMETERS	CONTRAST	RATIO	SE	Z-VALUE	P-VALUE
Sampling: T0	High-Low	0.947	0.160	-0.325	0.7449
Sampling: T1	High-Low	0.809	0.106	-1.621	0.1051
Sampling: T2	High-Low	0.900	0.109	-0.867	0.3857
Sampling: T3	High-Low	0.949	0.106	-0.467	0.6408
Sampling: T4	High-Low	0.827	0.087	-1.806	0.071
Sampling: T5	High-Low	0.879	0.117	-0.971	0.3318
Sampling: T7	High-Low	0.744	0.120	-1.840	0.0658
Sampling: T8	High-Low	0.869	0.116	-1.052	0.2928

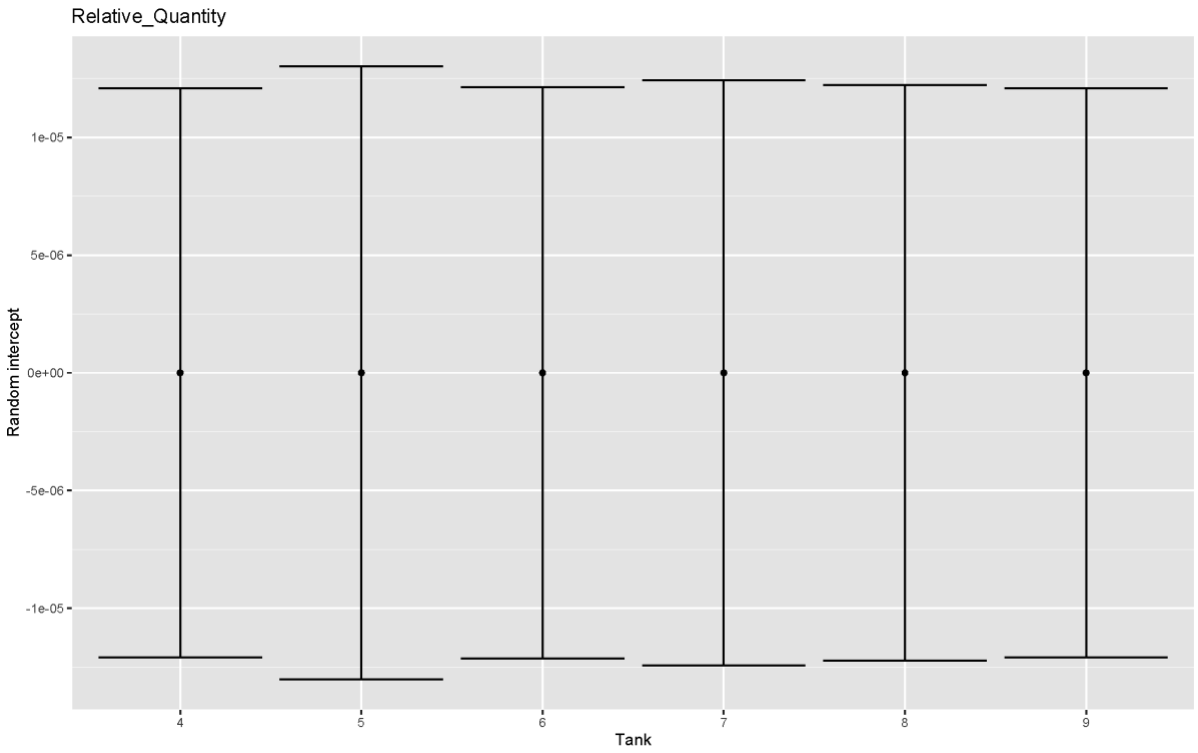

Figure A3.6: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the *nkcc1a* of Atlantic salmon under high TSS and low TSS treatment. SD= 6.159e-06.

Sodium

Table A3.19: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon sodium data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	113.544	2.249	50.480	<.0001
TreatmentLow	111.050	3.374	32.920	<.0001
SamplingT1	-3.521	2.926	-1.200	0.229
SamplingT2	-4.294	3.100	-1.390	0.166
SamplingT3	-7.895	3.279	-2.410	0.016
SamplingT4	-7.913	2.811	-2.810	0.005
SamplingT5	-12.924	3.100	-4.170	<.0001
SamplingT6	-4.186	2.975	-1.410	0.159
SamplingT7	-4.852	2.926	-1.660	0.097
SamplingT8	5.313	3.400	1.560	0.118
TreatmentLow:SamplingT1	1.618	4.842	0.330	0.738
TreatmentLow:SamplingT2	4.884	5.054	0.970	0.334
TreatmentLow:SamplingT3	10.233	5.214	1.960	0.050
TreatmentLow:SamplingT4	3.451	4.704	0.730	0.463
TreatmentLow:SamplingT5	9.910	4.924	2.010	0.044
TreatmentLow:SamplingT6	5.408	4.937	1.100	0.273
TreatmentLow:SamplingT7	7.303	4.949	1.480	0.140
TreatmentLow:SamplingT8	0.737	5.351	0.140	0.890

Table A3.20: Post-hoc Tukey HSD analysis between samplings for each treatment in the sodium of Atlantic salmon data.

PARAMETERS	TREATMENT	ESTIMATE	SE	T-RATIO	P-VALUE
T0-T1	High	1.769	4.040	0.438	1
T0-T2	High	0.600	4.260	0.141	1
T0-T3	High	4.875	4.490	1.085	0.9757
T0-T4	High	8.125	3.890	2.088	0.4851
T0-T5	High	27.600	4.260	6.474	<.0001
T0-T6	High	13.750	4.100	3.352	0.0269
T0-T7	High	11.923	4.040	2.952	0.0838
T0-T8	High	-0.571	4.650	-0.123	1
T1-T2	High	-1.169	3.780	-0.309	1
T1-T3	High	3.106	4.040	0.769	0.9975
T1-T4	High	6.356	3.360	1.894	0.6188
T1-T5	High	25.831	3.780	6.833	<.0001
T1-T6	High	11.981	3.600	3.330	0.0287
T1-T7	High	10.154	3.530	2.880	0.1008

T1-T8	High	-2.341	4.210	-0.556	0.9998
T2-T3	High	4.275	4.260	1.003	0.9852
T2-T4	High	7.525	3.620	2.077	0.4924
T2-T5	High	27.000	4.020	6.718	<.0001
T2-T6	High	13.150	3.850	3.417	0.0219
T2-T7	High	11.323	3.780	2.995	0.0748
T2-T8	High	-1.171	4.430	-0.264	1
T3-T4	High	3.250	3.890	0.835	0.9956
T3-T5	High	22.725	4.260	5.331	<.0001
T3-T6	High	8.875	4.100	2.164	0.4343
T3-T7	High	7.048	4.040	1.745	0.7176
T3-T8	High	-5.447	4.650	-1.171	0.9615
T4-T5	High	19.475	3.620	5.376	<.0001
T4-T6	High	5.625	3.430	1.639	0.7819
T4-T7	High	3.798	3.360	1.132	0.9686
T4-T8	High	-8.696	4.070	-2.135	0.453
T5-T6	High	-13.850	3.850	-3.599	0.0122
T5-T7	High	-15.677	3.780	-4.147	0.0017
T5-T8	High	-28.171	4.430	-6.361	<.0001
T6-T7	High	-1.827	3.600	-0.508	0.9999
T6-T8	High	-14.322	4.270	-3.351	0.027
T7-T8	High	-12.495	4.210	-2.966	0.081
T0-T1	Low	4.257	5.760	0.739	0.9981
T0-T2	Low	3.534	5.920	0.597	0.9996
T0-T3	Low	1.445	5.990	0.241	1
T0-T4	Low	6.271	5.650	1.109	0.9722
T0-T5	Low	16.048	5.720	2.807	0.121
T0-T6	Low	13.515	5.850	2.309	0.3427
T0-T7	Low	6.034	5.920	1.020	0.9835
T0-T8	Low	6.209	6.080	1.020	0.9835
T1-T2	Low	-0.723	3.780	-0.191	1
T1-T3	Low	-2.812	3.900	-0.722	0.9984
T1-T4	Low	2.014	3.360	0.600	0.9996
T1-T5	Low	11.791	3.460	3.406	0.0227
T1-T6	Low	9.259	3.680	2.515	0.2324
T1-T7	Low	1.777	3.780	0.470	0.9999
T1-T8	Low	1.952	4.040	0.483	0.9999
T2-T3	Low	-2.089	4.130	-0.506	0.9999
T2-T4	Low	2.737	3.620	0.756	0.9978
T2-T5	Low	12.514	3.720	3.363	0.026
T2-T6	Low	9.982	3.930	2.542	0.2197
T2-T7	Low	2.500	4.020	0.622	0.9995

T2-T8	Low	2.675	4.260	0.627	0.9994
T3-T4	Low	4.826	3.740	1.289	0.9334
T3-T5	Low	14.603	3.840	3.803	0.0061
T3-T6	Low	12.071	4.040	2.988	0.0763
T3-T7	Low	4.589	4.130	1.111	0.9719
T3-T8	Low	4.764	4.370	1.091	0.9749
T4-T5	Low	9.777	3.290	2.973	0.0795
T4-T6	Low	7.244	3.520	2.058	0.5055
T4-T7	Low	-0.237	3.620	-0.066	1
T4-T8	Low	-0.063	3.890	-0.016	1
T5-T6	Low	-2.532	3.620	-0.699	0.9987
T5-T7	Low	-10.014	3.720	-2.691	0.1588
T5-T8	Low	-9.839	3.980	-2.470	0.2541
T6-T7	Low	-7.482	3.930	-1.905	0.611
T6-T8	Low	-7.307	4.180	-1.750	0.7148
T7-T8	Low	0.175	4.260	0.041	1

Table A3.21: Post-hoc Tukey HSD analysis between treatments for each sampling point in the sodium of Atlantic salmon data.

PARAMETERS	CONTRAST	ESTIMATE	SE	T-RATIO	P-VALUE
Sampling: T0	High-Low	-0.334	6.08	-0.055	0.9564
Sampling: T1	High-Low	2.154	3.53	0.611	0.542
Sampling: T2	High-Low	2.6	4.02	0.647	0.5186
Sampling: T3	High-Low	-3.764	4.37	-0.862	0.3899
Sampling: T4	High-Low	-2.188	3.18	-0.688	0.4921
Sampling: T5	High-Low	-11.886	3.72	-3.194	0.0017
Sampling: T6	High-Low	-0.568	3.75	-0.151	0.8798
Sampling: T7	High-Low	-6.223	3.78	-1.646	0.1016
Sampling: T8	High-Low	6.446	4.65	1.386	0.1676

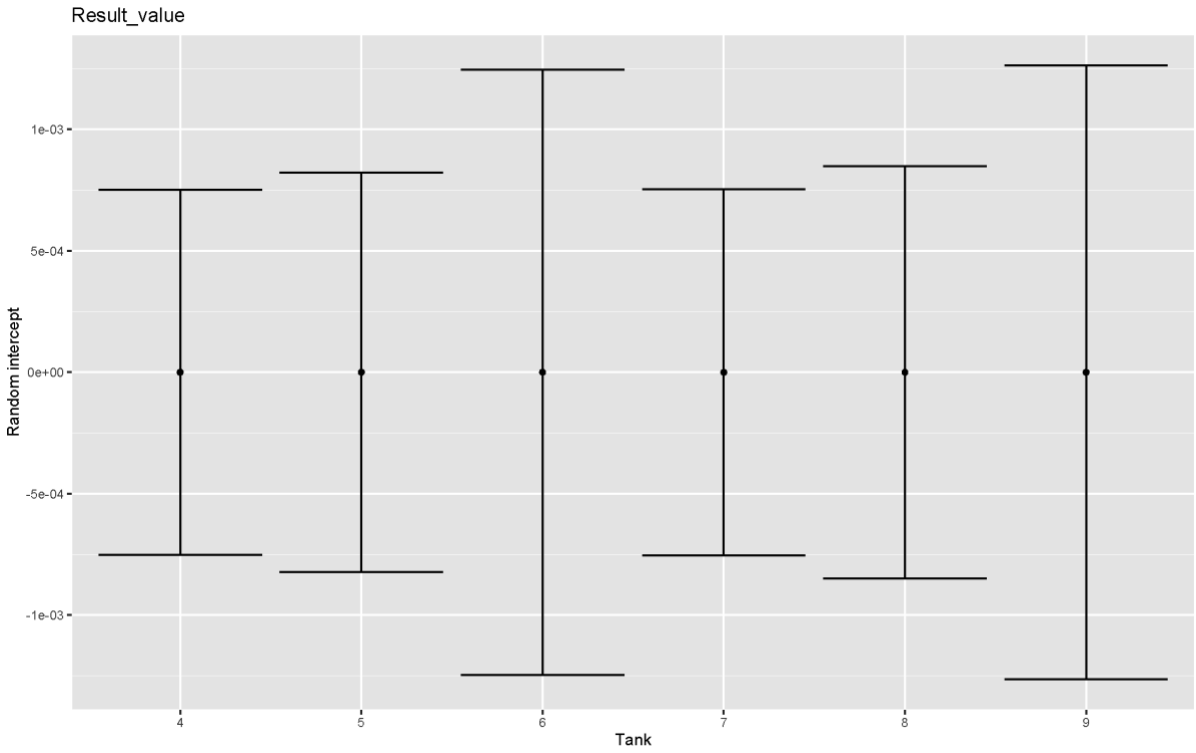

Figure A3.7: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the sodium of Atlantic salmon under high TSS and low TSS treatment. SD= 0.0003832.

Chloride

Table A3.22: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon chloride data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	158.000	3.178	49.730	<.0001
TreatmentLow	158.334	5.189	30.510	<.0001
SamplingT1	-1.769	4.039	-0.440	0.661
SamplingT2	-0.600	4.263	-0.140	0.888
SamplingT3	-4.875	4.494	-1.080	0.278
SamplingT4	-8.125	3.892	-2.090	0.037
SamplingT5	-27.600	4.263	-6.470	<.0001
SamplingT6	-13.750	4.102	-3.350	0.001
SamplingT7	-11.923	4.039	-2.950	0.003
SamplingT8	0.571	4.651	0.120	0.902
TreatmentLow:SamplingT1	-2.487	7.032	-0.350	0.724
TreatmentLow:SamplingT2	-2.934	7.292	-0.400	0.687
TreatmentLow:SamplingT3	3.430	7.489	0.460	0.647
TreatmentLow:SamplingT4	1.854	6.864	0.270	0.787

TreatmentLow:SamplingT5	11.552	7.132	1.620	0.105
TreatmentLow:SamplingT6	0.235	7.148	0.030	0.974
TreatmentLow:SamplingT7	5.890	7.163	0.820	0.411
TreatmentLow:SamplingT8	-6.780	7.659	-0.890	0.376

Table A3.23: Post-hoc Tukey HSD analysis between samplings for each treatment in the chloride of Atlantic salmon data.

PARAMETERS	TREATMENT	ESTIMATE	SE	T-RATIO	P-VALUE
T0-T1	High	3.521	2.930	1.204	0.9548
T0-T2	High	4.294	3.100	1.385	0.9021
T0-T3	High	7.895	3.280	2.408	0.2864
T0-T4	High	7.913	2.810	2.815	0.1186
T0-T5	High	12.924	3.100	4.169	0.0016
T0-T6	High	4.186	2.980	1.407	0.8939
T0-T7	High	4.852	2.930	1.658	0.7707
T0-T8	High	-5.313	3.400	-1.562	0.8236
T1-T2	High	0.773	2.840	0.272	1
T1-T3	High	4.373	3.030	1.442	0.8797
T1-T4	High	4.392	2.520	1.743	0.7189
T1-T5	High	9.403	2.840	3.313	0.0302
T1-T6	High	0.665	2.700	0.246	1
T1-T7	High	1.331	2.650	0.503	0.9999
T1-T8	High	-8.834	3.160	-2.793	0.1251
T2-T3	High	3.600	3.200	1.125	0.9697
T2-T4	High	3.619	2.720	1.331	0.9208
T2-T5	High	8.630	3.020	2.860	0.1061
T2-T6	High	-0.108	2.890	-0.038	1
T2-T7	High	0.558	2.840	0.197	1
T2-T8	High	-9.607	3.330	-2.889	0.0985
T3-T4	High	0.019	2.920	0.006	1
T3-T5	High	5.030	3.200	1.572	0.8189
T3-T6	High	-3.709	3.080	-1.204	0.9547
T3-T7	High	-3.042	3.030	-1.003	0.9852
T3-T8	High	-13.207	3.490	-3.782	0.0065
T4-T5	High	5.011	2.720	1.842	0.654
T4-T6	High	-3.727	2.580	-1.447	0.8779
T4-T7	High	-3.061	2.520	-1.215	0.9523
T4-T8	High	-13.226	3.060	-4.325	0.0008
T5-T6	High	-8.738	2.890	-3.025	0.0691
T5-T7	High	-8.072	2.840	-2.844	0.1103
T5-T8	High	-18.237	3.330	-5.485	<.0001

T6-T7	High	0.667	2.700	0.247	1
T6-T8	High	-9.499	3.210	-2.960	0.0821
T7-T8	High	-10.165	3.160	-3.214	0.0406
T0-T1	Low	1.904	3.860	0.493	0.9999
T0-T2	Low	-0.590	3.990	-0.148	1
T0-T3	Low	-2.339	4.050	-0.577	0.9997
T0-T4	Low	4.462	3.770	1.183	0.9591
T0-T5	Low	3.014	3.830	0.788	0.9971
T0-T6	Low	-1.223	3.940	-0.310	1
T0-T7	Low	-2.450	3.990	-0.614	0.9995
T0-T8	Low	-6.050	4.130	-1.464	0.8704
T1-T2	Low	-2.494	2.840	-0.879	0.9938
T1-T3	Low	-4.243	2.930	-1.450	0.8764
T1-T4	Low	2.559	2.520	1.016	0.984
T1-T5	Low	1.111	2.600	0.427	1
T1-T6	Low	-3.126	2.760	-1.131	0.9687
T1-T7	Low	-4.354	2.840	-1.534	0.8379
T1-T8	Low	-7.954	3.030	-2.623	0.1847
T2-T3	Low	-1.749	3.100	-0.564	0.9997
T2-T4	Low	5.052	2.720	1.858	0.6437
T2-T5	Low	3.604	2.790	1.290	0.9331
T2-T6	Low	-0.633	2.950	-0.215	1
T2-T7	Low	-1.860	3.020	-0.616	0.9995
T2-T8	Low	-5.460	3.200	-1.706	0.7422
T3-T4	Low	6.801	2.810	2.419	0.2804
T3-T5	Low	5.353	2.880	1.857	0.6441
T3-T6	Low	1.116	3.030	0.368	1
T3-T7	Low	-0.111	3.100	-0.036	1
T3-T8	Low	-3.711	3.280	-1.132	0.9686
T4-T5	Low	-1.448	2.470	-0.586	0.9997
T4-T6	Low	-5.685	2.640	-2.151	0.4424
T4-T7	Low	-6.913	2.720	-2.541	0.2199
T4-T8	Low	-10.513	2.920	-3.598	0.0122
T5-T6	Low	-4.237	2.720	-1.558	0.8256
T5-T7	Low	-5.465	2.790	-1.956	0.576
T5-T8	Low	-9.064	2.990	-3.031	0.0679
T6-T7	Low	-1.228	2.950	-0.416	1
T6-T8	Low	-4.827	3.140	-1.540	0.8351
T7-T8	Low	-3.600	3.200	-1.125	0.9697

Table A3.24: Post-hoc Tukey HSD analysis between treatments for each sampling point in the chloride of Atlantic salmon data.

PARAMETERS	CONTRAST	ESTIMATE	SE	T-RATIO	P-VALUE
Sampling: T0	High-Low	2.494	4.05	0.615	0.5392
Sampling: T1	High-Low	0.877	2.65	0.331	0.7408
Sampling: T2	High-Low	-2.39	3.02	-0.792	0.4295
Sampling: T3	High-Low	-7.739	3.28	-2.36	0.0194
Sampling: T4	High-Low	-0.956	2.39	-0.401	0.689
Sampling: T5	High-Low	-7.416	2.79	-2.654	0.0087
Sampling: T6	High-Low	-2.914	2.82	-1.035	0.3023
Sampling: T7	High-Low	-4.808	2.84	-1.694	0.092
Sampling: T8	High-Low	1.757	3.49	0.503	0.6155

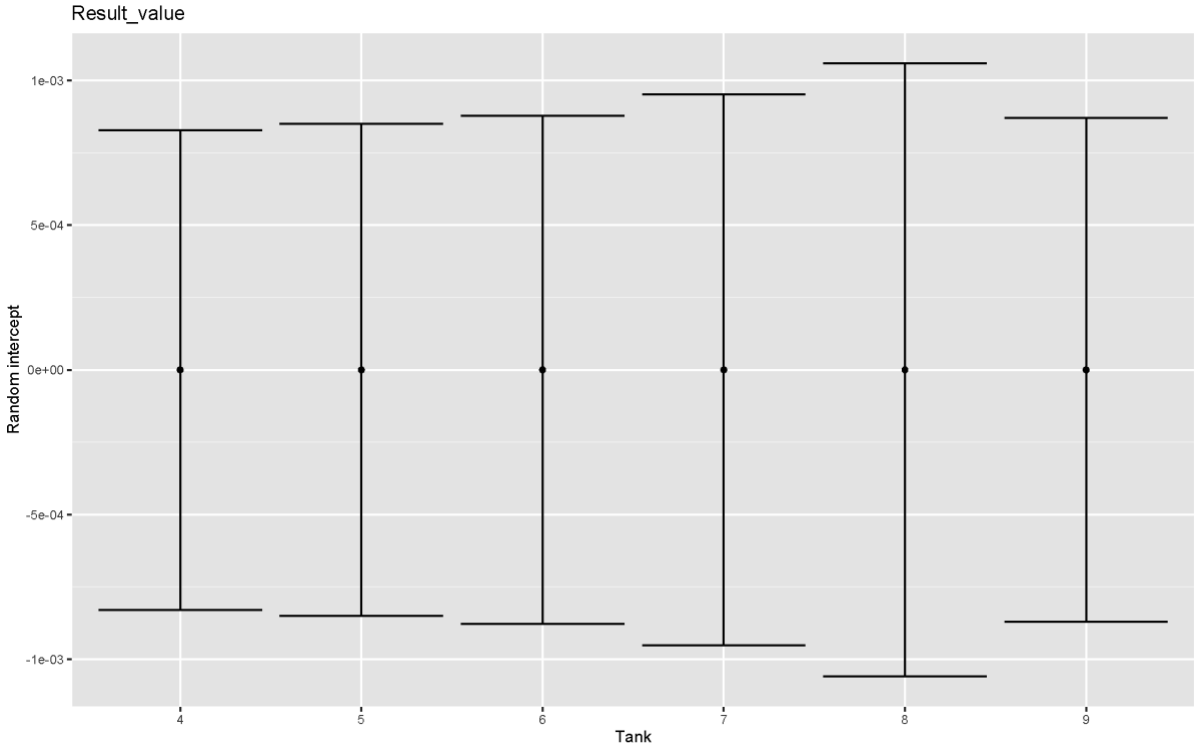

Figure A3.8: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the chloride of Atlantic salmon under high TSS and low TSS treatment. SD=0.0004228.

Simulated Transport Sodium

Table A3.25: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon sodium simulated transport data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	167.167	2.590	64.540	<.0001
TreatmentLow	168.091	2.705	62.140	<.0001
SamplingPost-Transport	13.083	3.663	3.570	<.0001
SamplingPre-Transport	-6.083	3.663	-1.660	0.097
fTreatmentLow:fSamplingPost-transport	-15.674	5.239	-2.990	0.003
fTreatmentLow:fSamplingPre-transport	-2.924	5.239	-0.560	0.577

Table A3.26: Post-hoc Tukey HSD analysis between samplings for each treatment in the sodium of Atlantic salmon simulated transport data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
3hPost-Post	High	-13.08	3.66	-3.572	0.002
3hPost-Pre	High	6.08	3.66	1.661	0.2283
Post-Pre	High	19.17	3.66	5.233	<.0001
3hPost-Post	Low	2.59	3.75	0.692	0.7691
3hPost-Pre	Low	9.01	3.75	2.405	0.0494
Post-Pre	Low	6.42	3.66	1.752	0.1943

Table A3.27: Post-hoc Tukey HSD analysis between treatments for each sampling point in the sodium of Atlantic salmon simulated transport data.

PARAMETERS	CONTRAST	ESTIMATE	SE	T-RATIO	P-VALUE
3hPost-transport	High-Low	-0.924	3.75	-0.247	0.8059
Post-transport	High-Low	14.75	3.66	4.027	0.0002
Pre-transport	High-Low	2	3.66	0.546	0.587

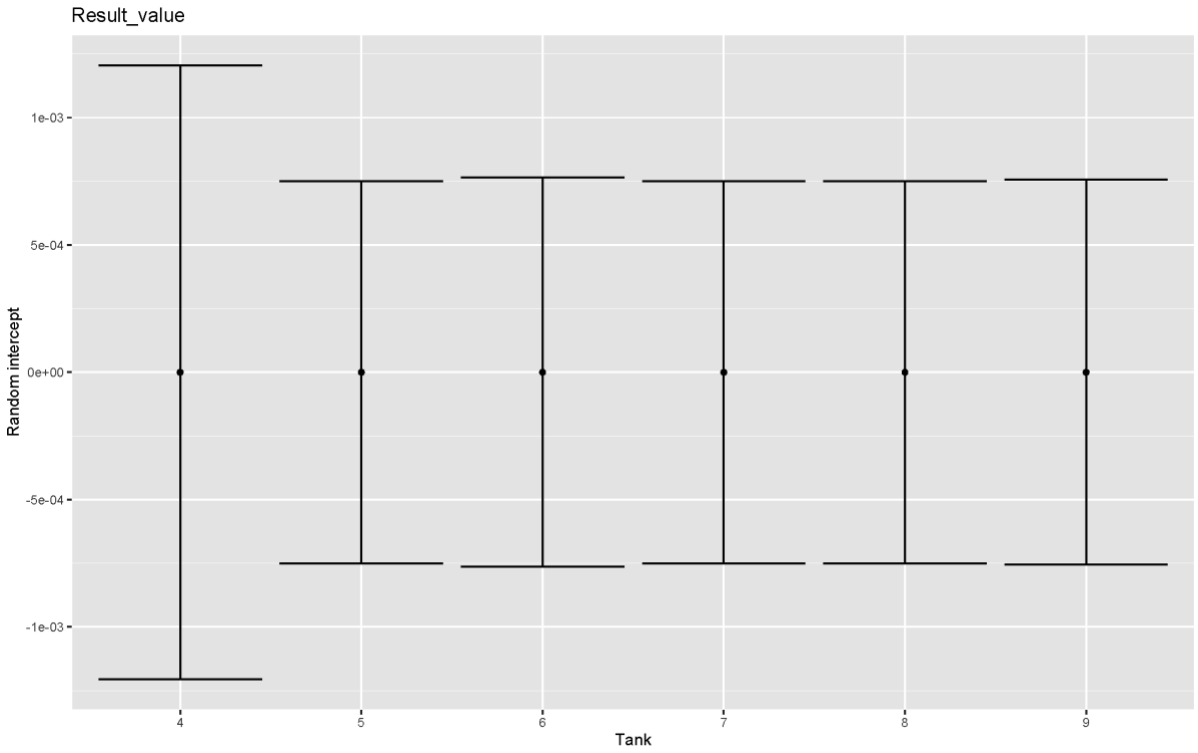

Figure A3.9: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the sodium of Atlantic salmon simulated transport under high TSS and low TSS treatment. SD= 0.0003825.

Simulated transport Chloride

Table A3.28: Parameters, estimates, and associated standard error (SE), z-value, and p-values in the model fitted to Atlantic salmon chloride simulated transport data.

PARAMETERS	ESTIMATE	SE	Z-VALUE	P-VALUE
TreatmentHigh	129.258	1.822	70.950	<.0001
TreatmentLow	127.491	1.903	67.000	<.0001
SamplingPost-Transport	11.142	2.576	4.320	<.0001
SamplingPre-Transport	-2.350	2.576	-0.910	0.362
fTreatmentLow:fSamplingPost-transport	-11.466	3.685	-3.110	0.002
fTreatmentLow:fSamplingPre-transport	-1.383	3.685	-0.380	0.707

Table A3.29: Post-hoc Tukey HSD analysis between samplings for each treatment in the chloride of Atlantic salmon simulated transport data.

PARAMETERS	TREATMENT	RATIO	SE	Z-VALUE	P-VALUE
3hPost-Post	High	-11.142	2.58	-4.325	0.0002
3hPost-Pre	High	2.35	2.58	0.912	0.6347

Post-Pre	High	13.492	2.58	5.237	<.0001
3hPost-Post	Low	0.324	2.63	0.123	0.9917
3hPost-Pre	Low	3.733	2.63	1.417	0.3384
Post-Pre	Low	3.408	2.58	1.323	0.3879

Table A3.30: Post-hoc Tukey HSD analysis between treatments for each sampling point in the chloride of Atlantic salmon simulated transport data.

PARAMETERS	CONTRAST	ESTIMATE	SE	T-RATIO	P-VALUE
3hPost-transport	High-Low	1.77	2.63	0.671	0.5047
Post-transport	High-Low	13.23	2.58	5.136	<.0001
Pre-transport	High-Low	3.15	2.58	1.223	0.226

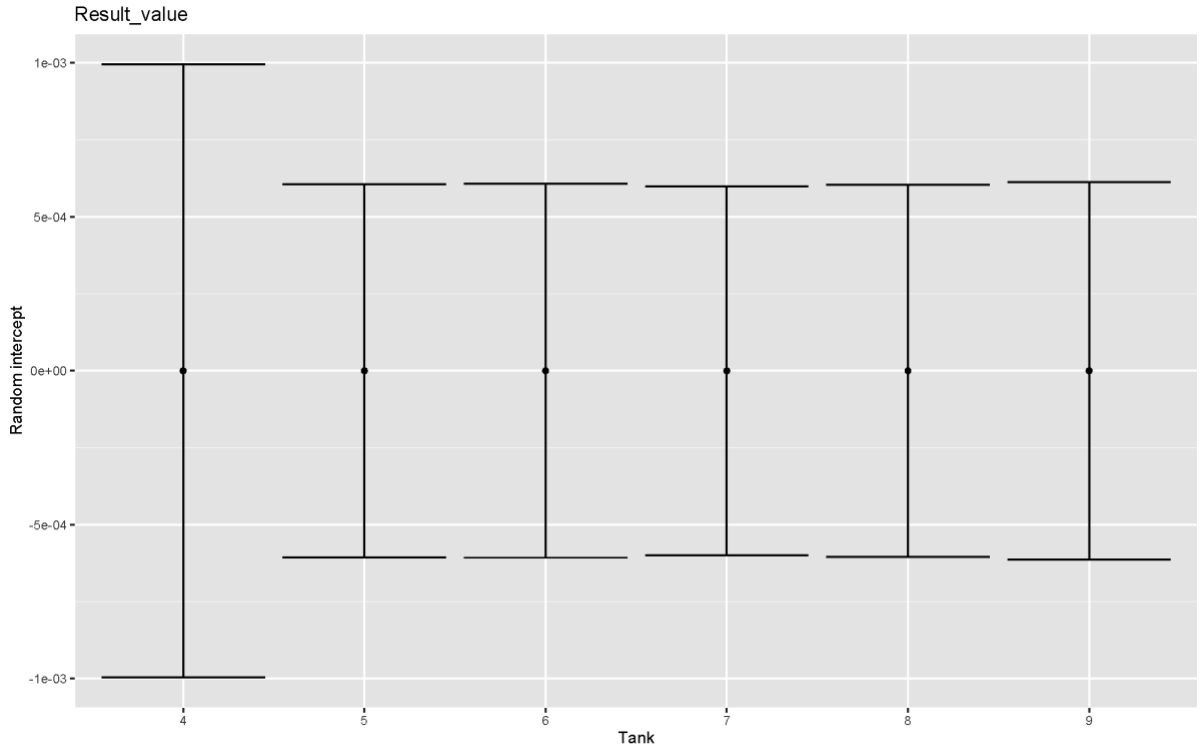

Figure A3.10: Random intercepts ± 95 % confidence interval of tank estimated in the linear mixed effects model for the chloride of Atlantic salmon simulated transport under high TSS and low TSS treatment. SD= 0.0003054.

4 Result graphs including mature male Atlantic salmon

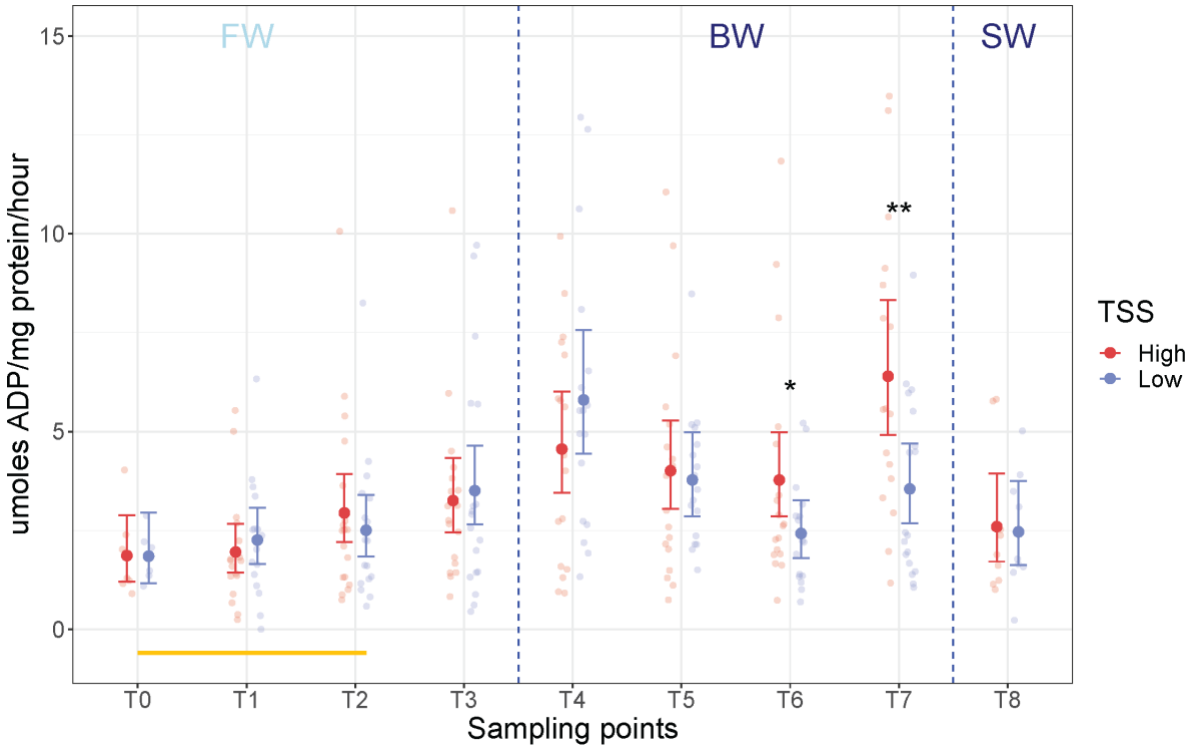

Figure A4.1: Difference in NKA protein activity levels (µmolesADP/mg Protein/hour) between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

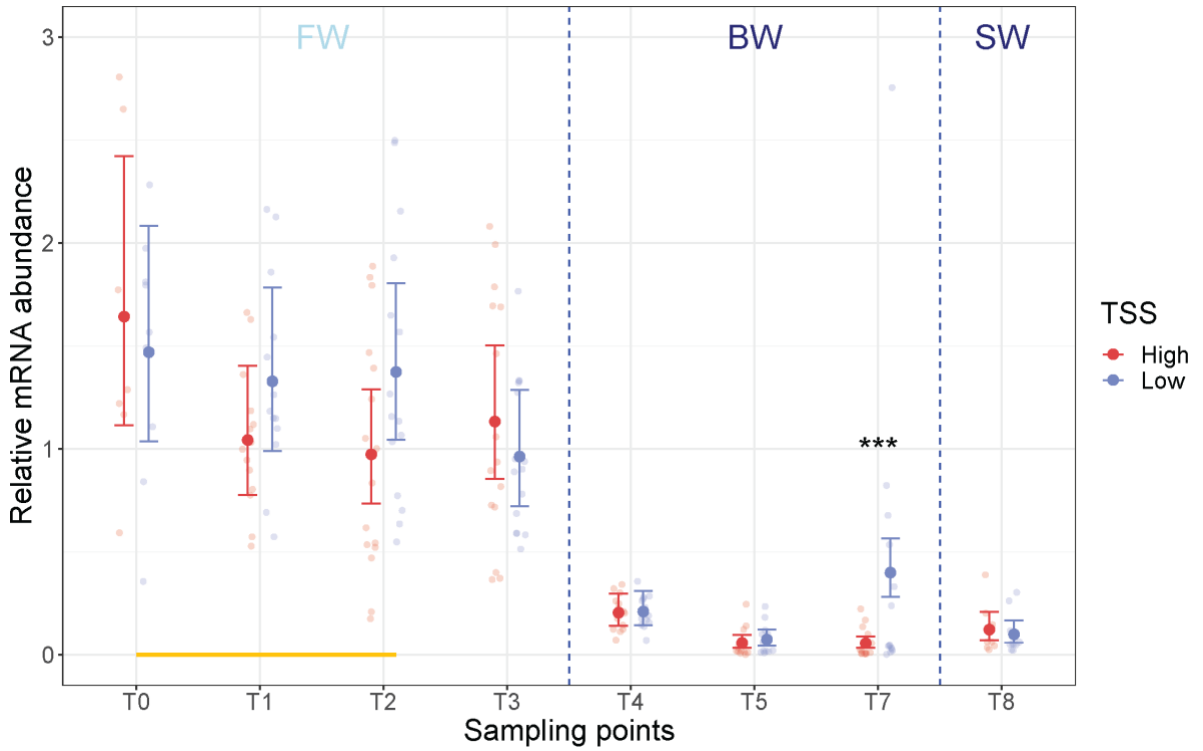

Figure A4.2: Difference in *nkaα1a* mRNA abundance between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

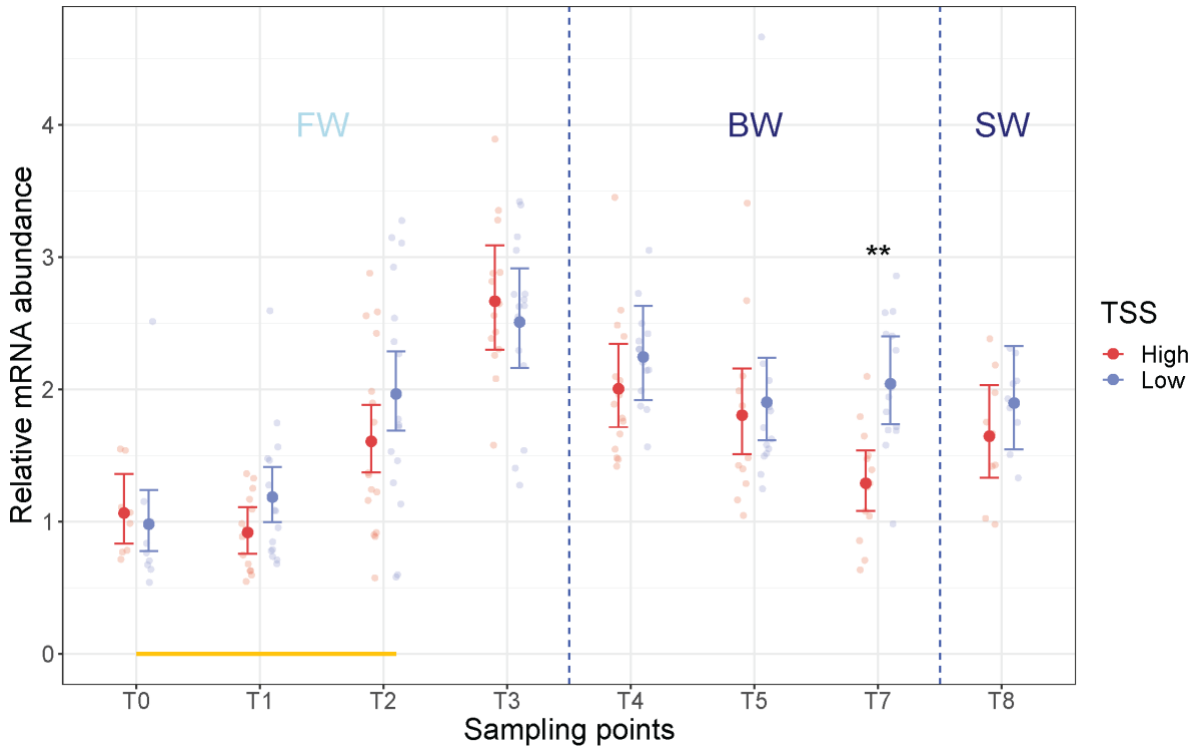

Figure A4.3: Difference in *nkaα1b* mRNA abundance between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

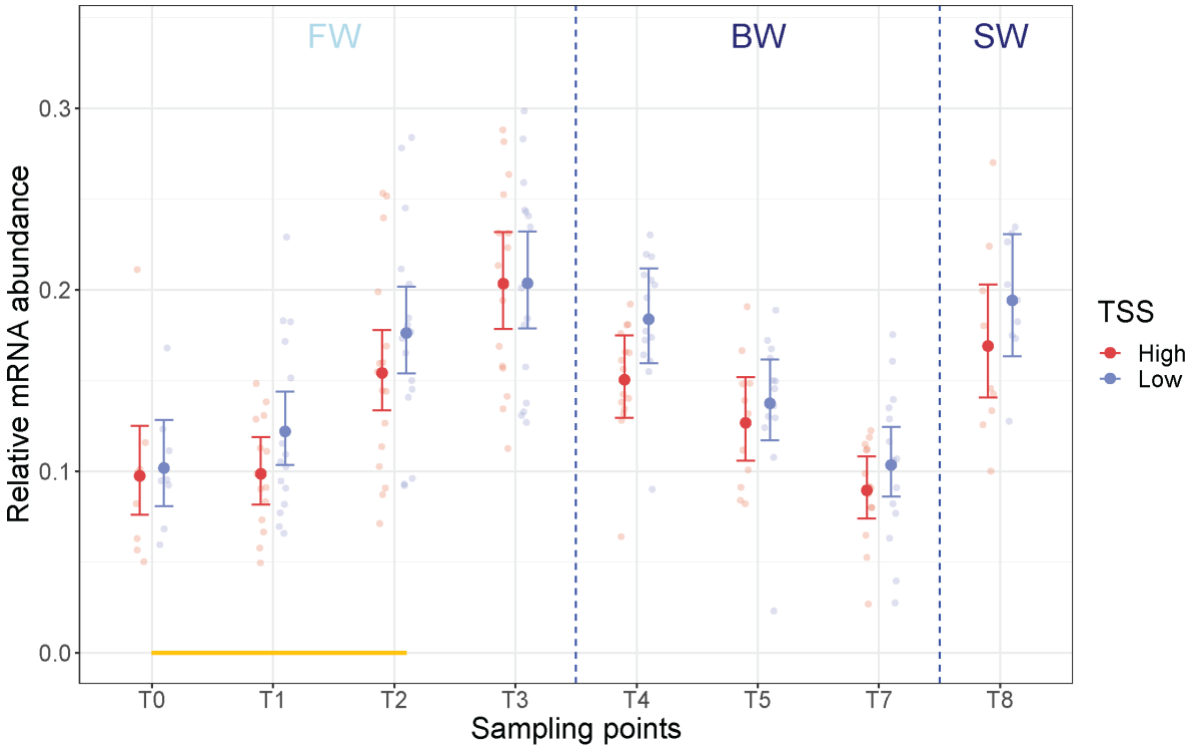

Figure A4.4: Difference in *nkcc1a* mRNA abundance between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** p<0.001, ** p<0.01, * p<0.05. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

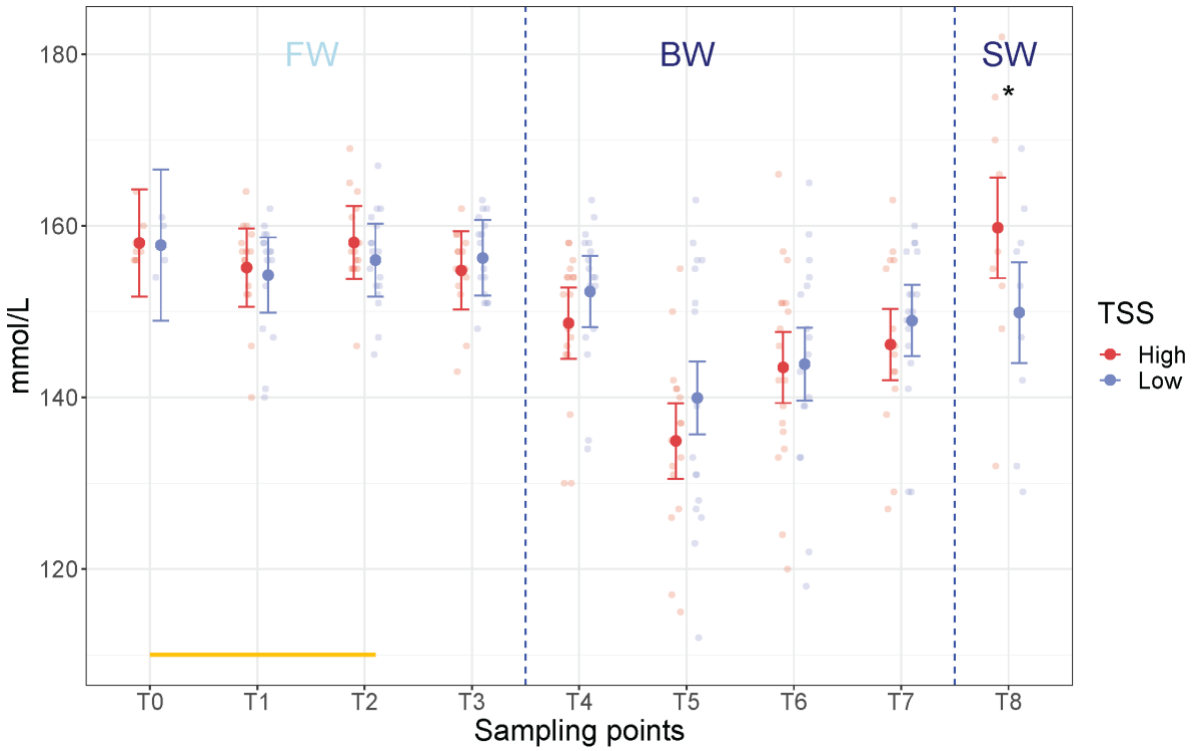

Figure A4.5: Difference in plasmatic sodium concentration (mmol/L) between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

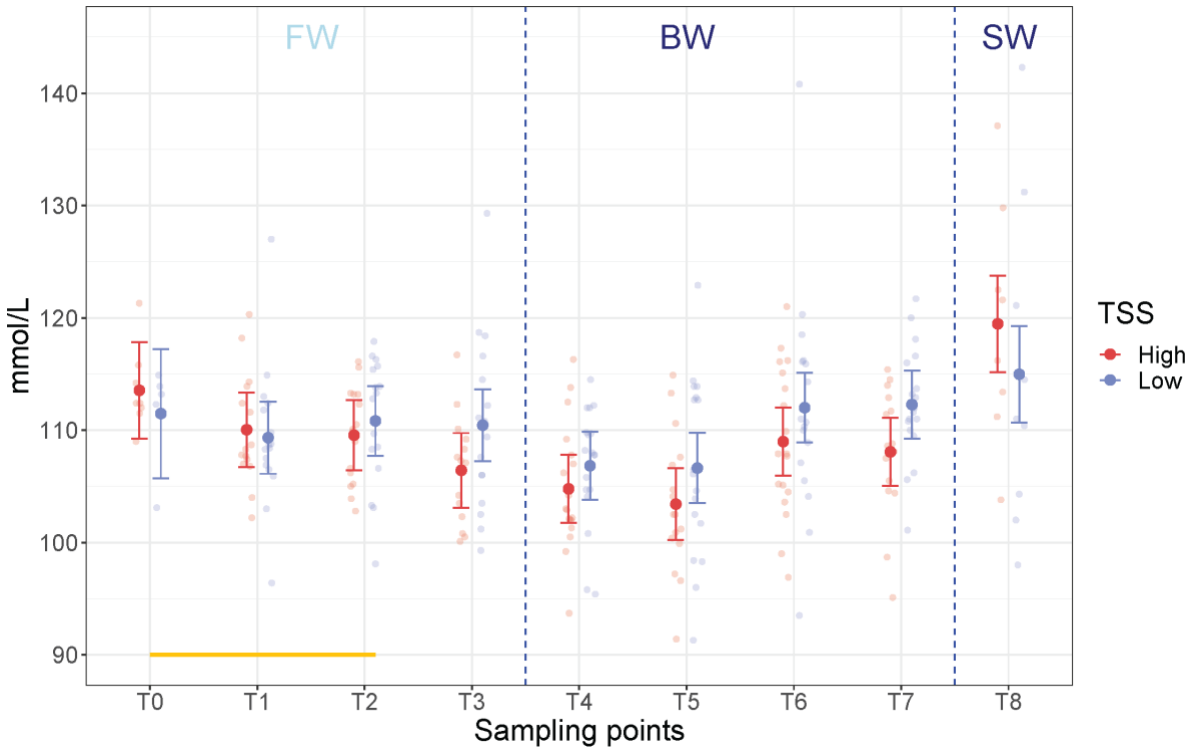

Figure A4.6: Difference in plasmatic chloride concentration (mmol/L) between treatments of juvenile Atlantic salmon in freshwater (FW), brackish water (BW) and after seawater (SW) transfer: Asterisks indicate the significant difference between high TSS/ low TSS treatment; *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Yellow line indicates winter signal photoperiod. Each sampling point is presented as mean ± 95 % confidence interval, and the raw data of each fish. Red colour indicates high TSS treatment and blue colour low TSS treatment.

5 Correlation between GSI and result

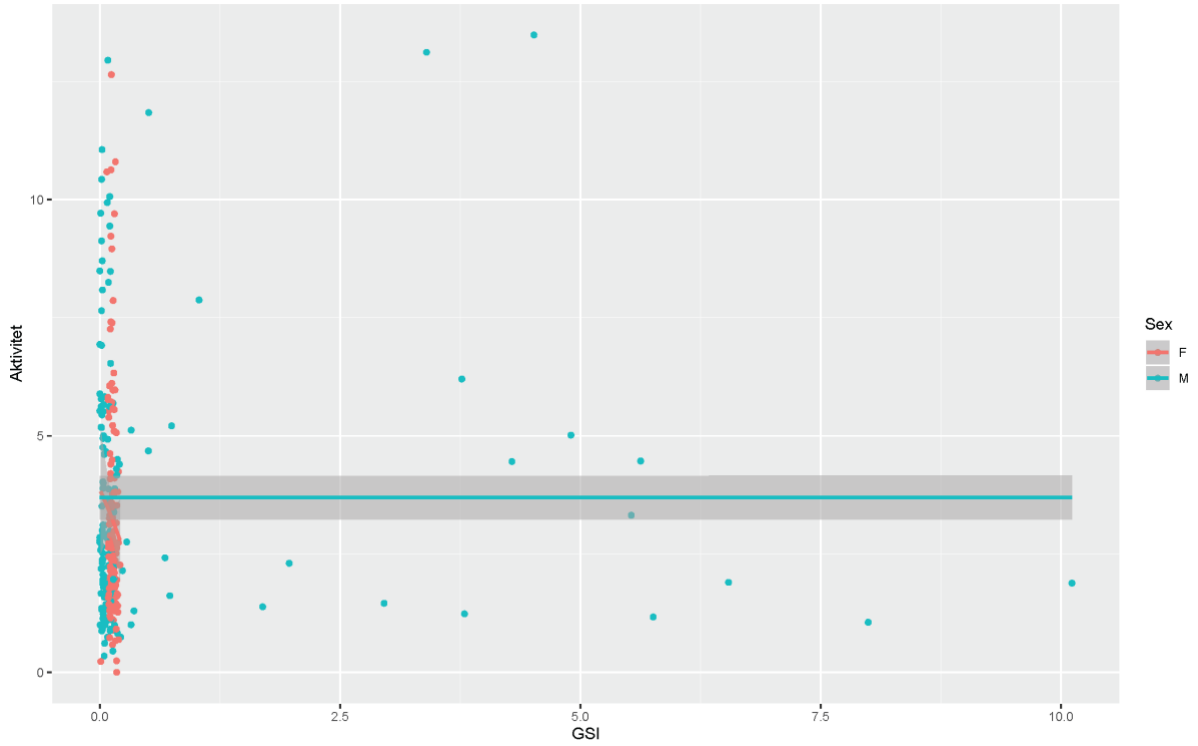

Figure A5.1: Correlation between GSI and NKA activity.

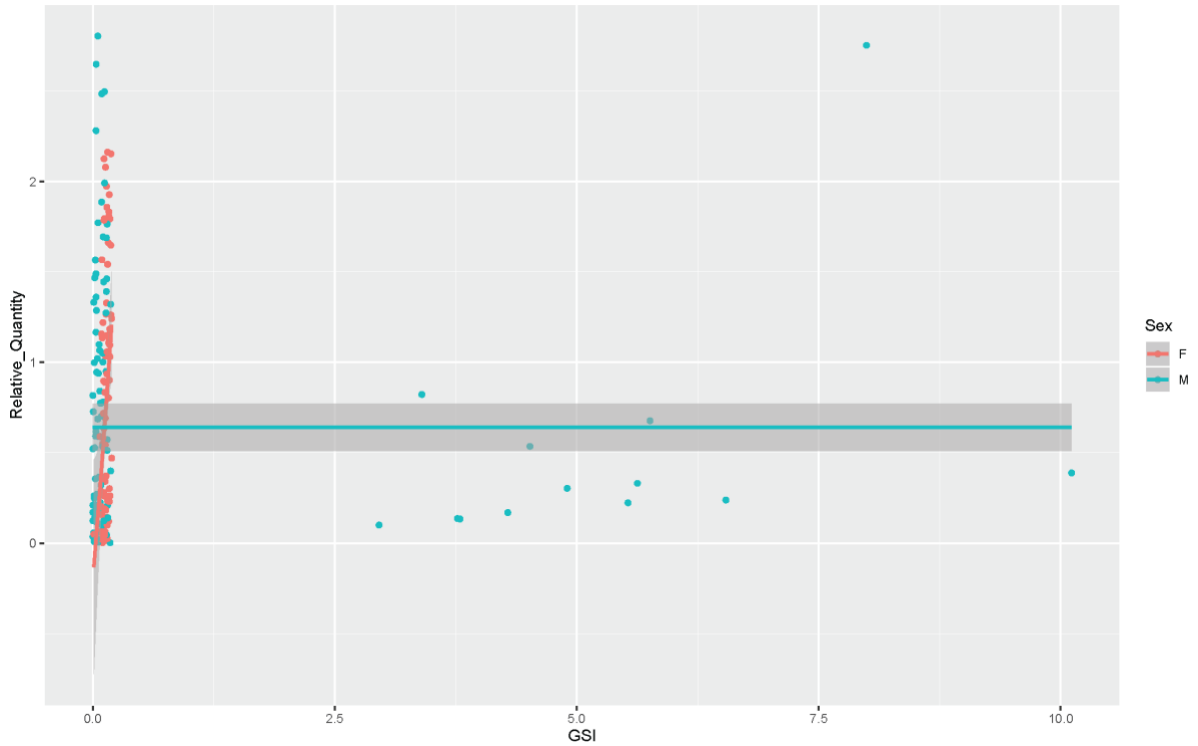

Figure A5.2: Correlation between GSI and *nkaα1a mRNA abundance*.

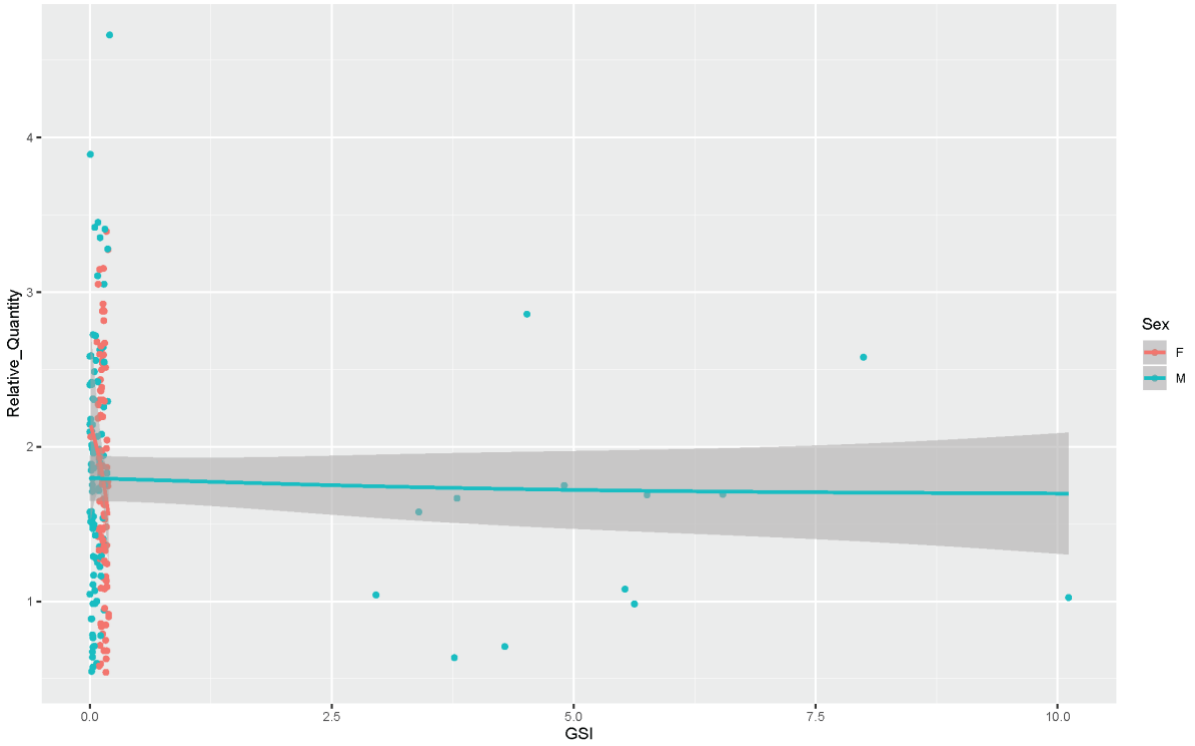

Figure A5.3: Correlation between GSI and *nkaα1b mRNA abundance*.

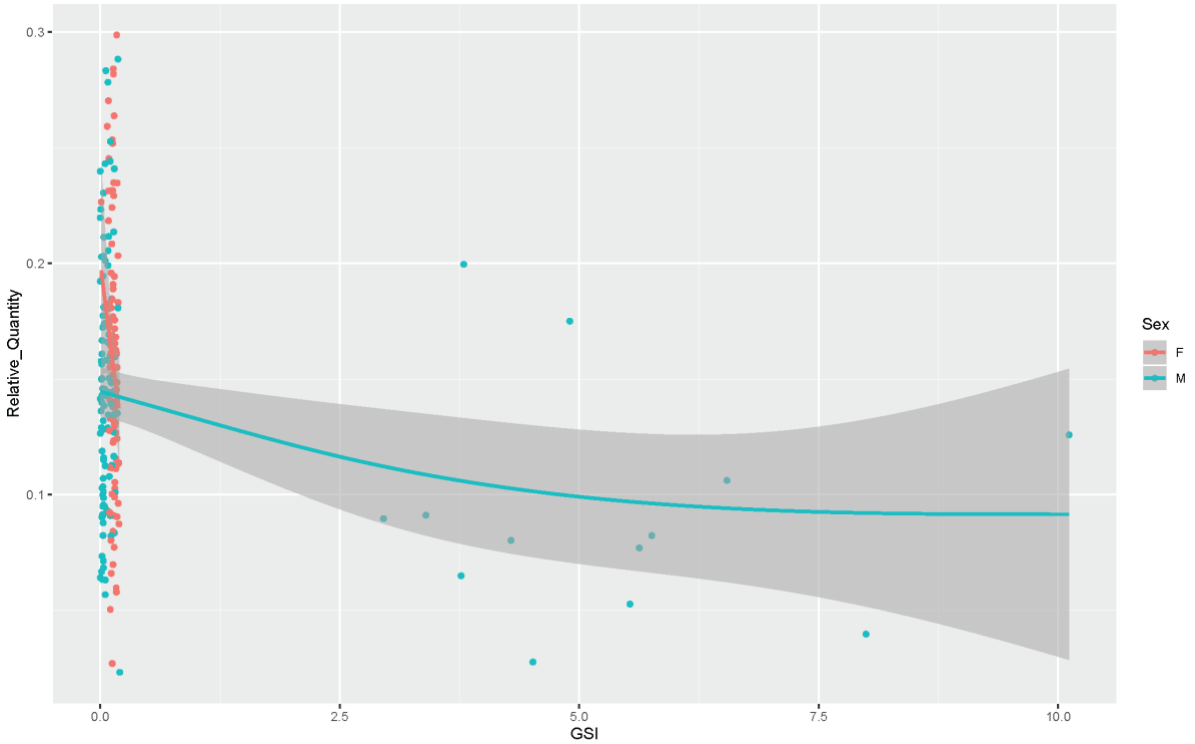

Figure A5.4: Correlation between GSI and *nkcc1a mRNA abundance*.

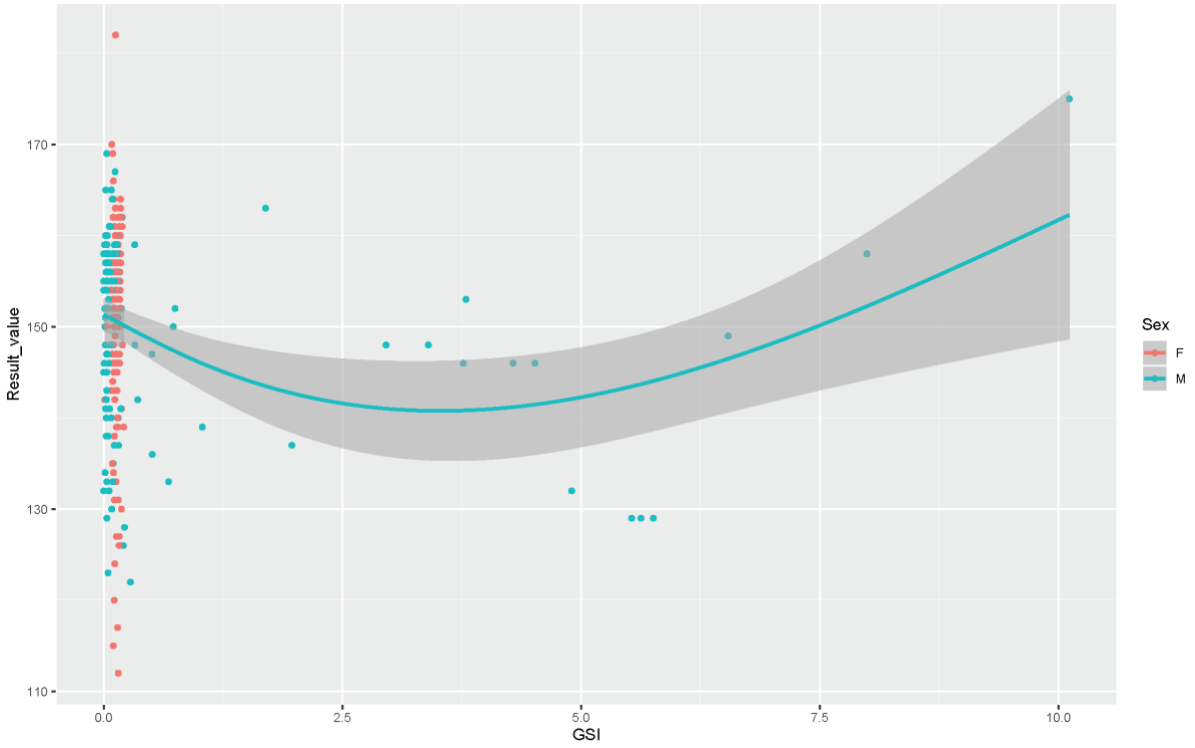

Figure A5.5: Correlation between GSI and sodium concentration.

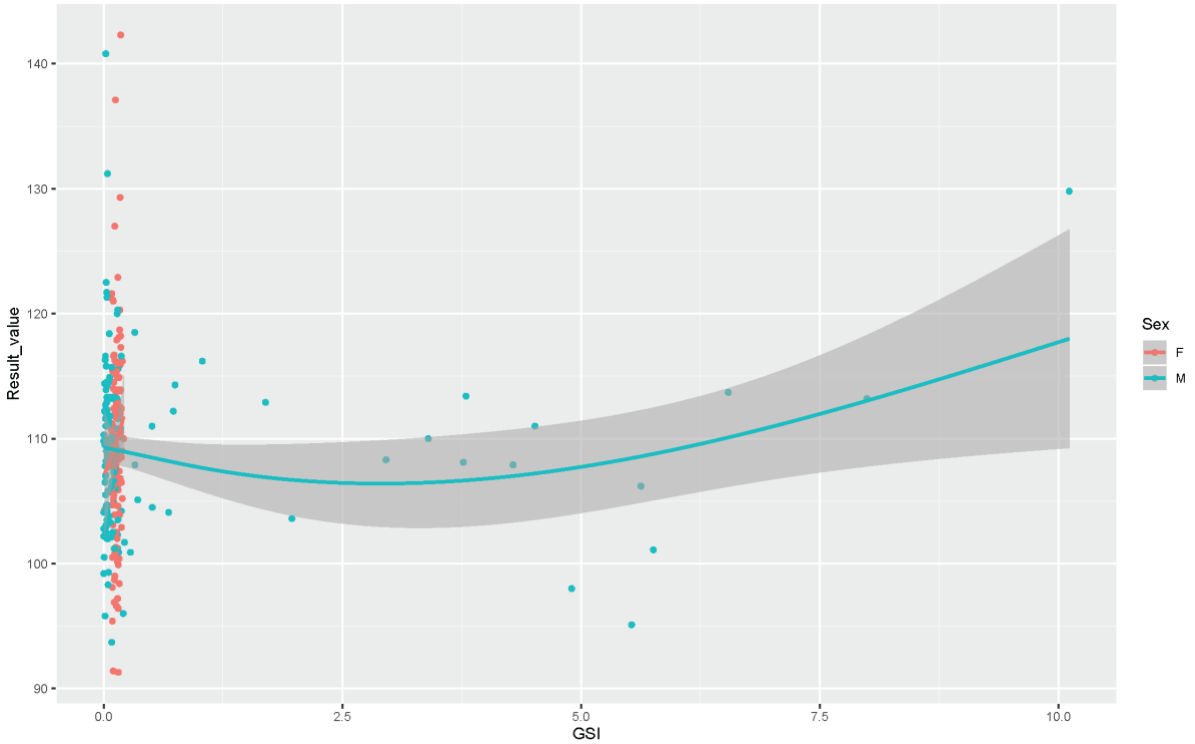

Figure A5.6: Correlation between GSI and chloride concentration.

6 Water parameters

Table A6.1: Water parameters: Mean ± SD water parameters for high TSS and low TSS.

Parameter	Units	FW phase		BW phase	
		H (n=3)	L (n=3)	H (n=3)	L (n=3)
pH	-	6.86-8.46	6.86-8.18	6.99-8.14	7.11-7.87
Temperature	°C	12.3 ± 0.1	12.4 ± 0.2	12.2 ± 0.1	12.3 ± 0.0
Sal	ppt	1.47 ± 0.07	1.42 ± 0.08	14.5 ± 1.0	15.5 ± 0.2
O_2	% sat	95.5 ± 1.5	91.1 ± 4.0	93.2 ± 1.9	92.0 ± 2.7
Alkalinity	mEq/L	0.91 ± 0.33	0.58 ± 0.03	3.23 ±0.30	1.50 ± 0.13
CO_2	mg/L	4.64 ± 0.46	3.65 ± 0.41	7.85 ± 0.12	5.76 ± 0.03
TAN	mg N/L	0.52 ± 0.10	0.30 ± 0.15	0.29 ± 0.04	0.16 ± 0.01
NO_2^--N	mg N/L	0.54 ± 0.13	0.30 ± 0.12	0.24 ± 0.05	0.10 ± 0.01
NO_3^--N	mg N/L	18.3 ± 3.9	27.6 ± 9.9	25.0 ± 6.2	47.2 ± 11.2
PO_4^{3-}-P	mg P/L	1.69 ± 0.48	1.43 ± 0.46	1.97 ± 0.81	1.53 ± 0.26
SO_4^{2-}-S	mg S/L	34.3 ± 9.6	36.2 ± 9.3	440 ± 214	352 ± 82
TSS	mg/L	6.77 ± 6.86	1.13 ± 0.82	7.31 ± 6.96	2.07 ± 1.51

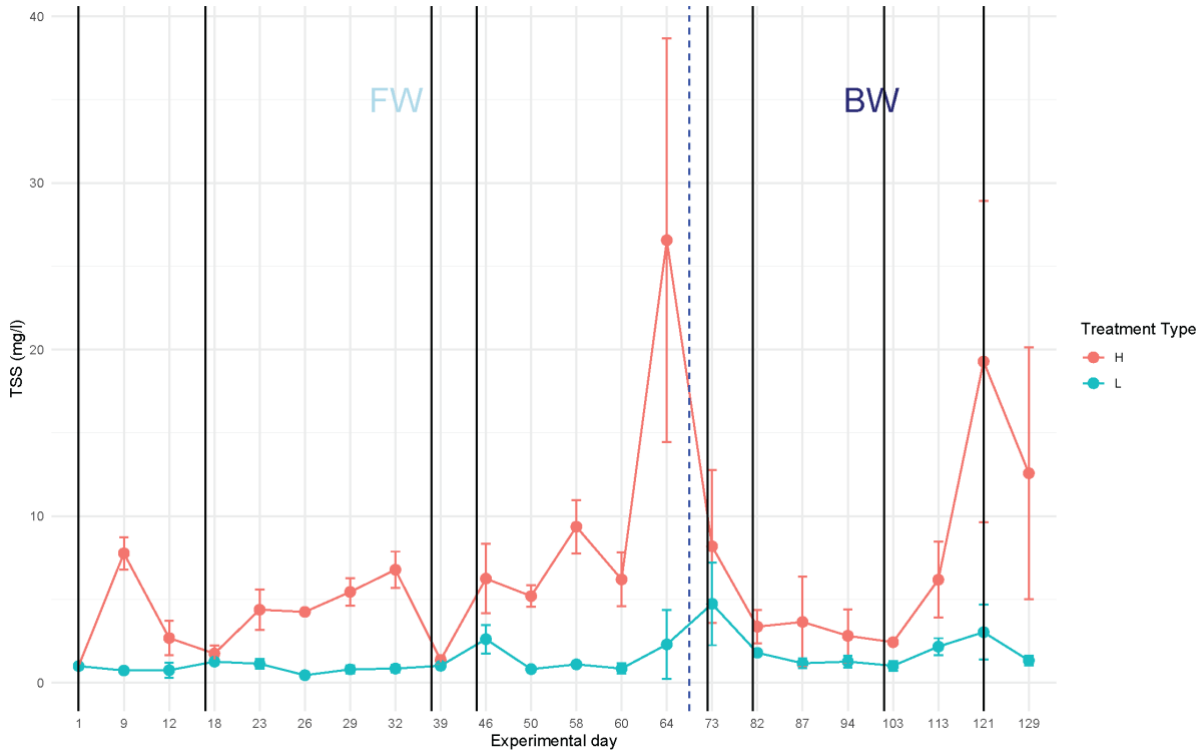

Figure A6.1: Total suspended solids (TSS) through RAS period: Mean TSS (mg/l) for high particle load (red) and low particle load (green) treatment. Black lines indicate sampling points T0-T7, and the dotted line is the switch to Brackish water.

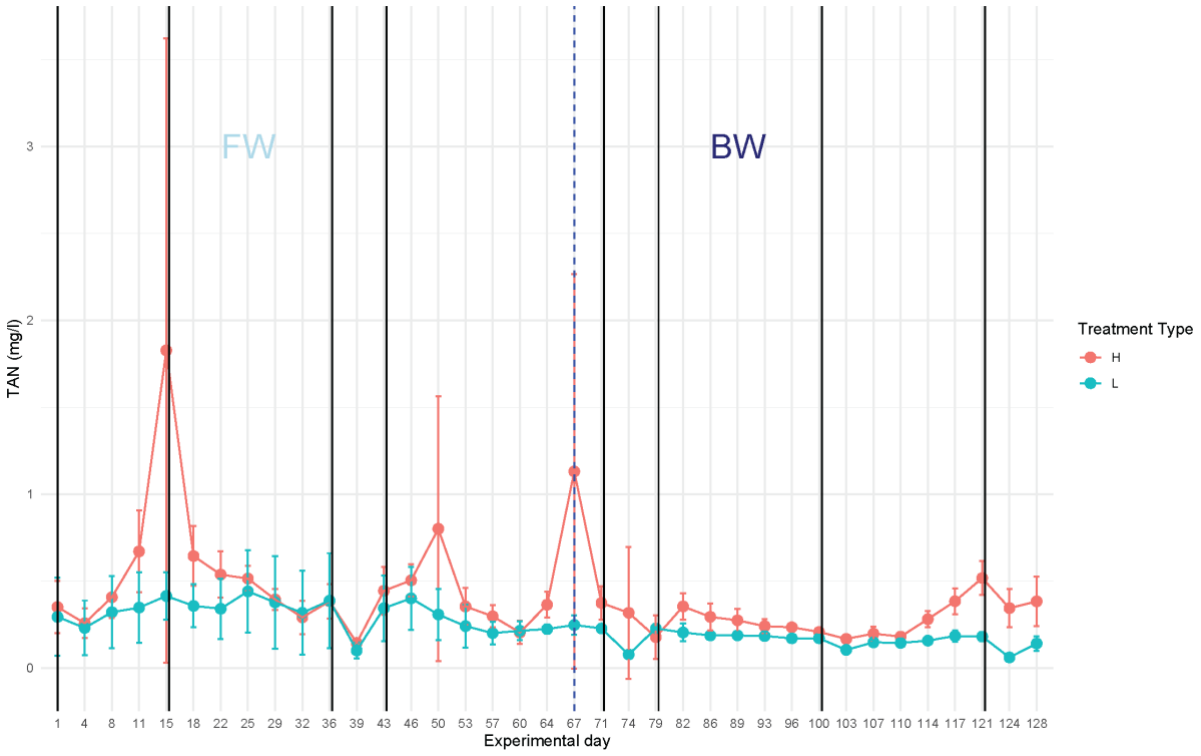

Figure A6.2: Total Ammonia Nitrogen (TAN) at different sampling points during RAS period: Mean TAN (mg/l). Black lines indicate sampling points T0-T7, and the dotted line is the switch to Brackish water.

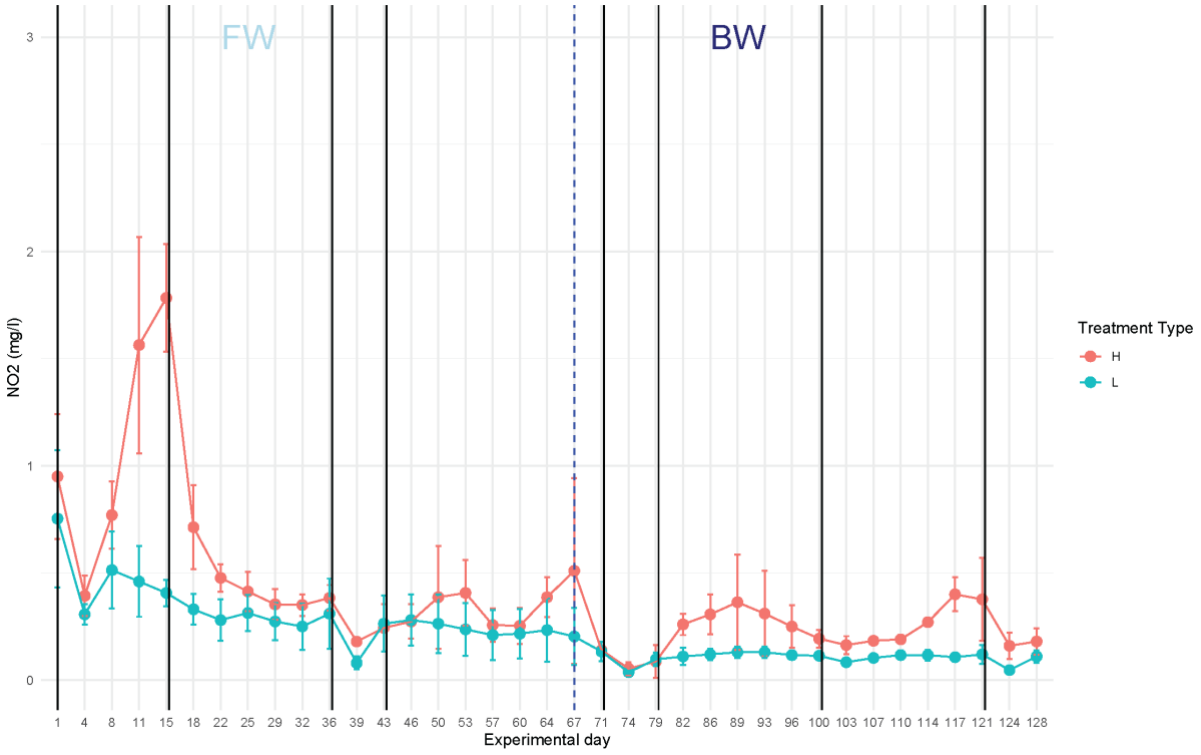

Figure A6.3: Nitrite (NO_2^-) at different sampling points during RAS period: Mean NO_2^- (mg/l). Black lines indicate sampling points T0-T7, and the dotted line is the switch to Brackish water.

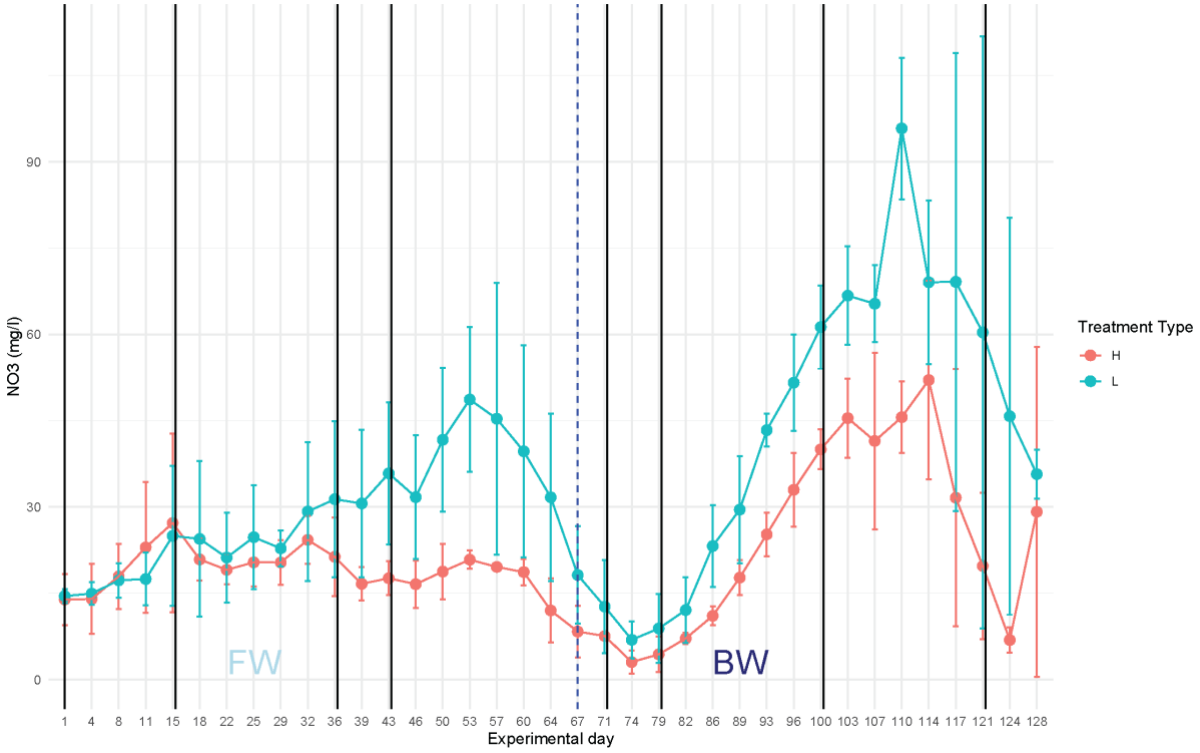

Figure A6.4: Nitrate (NO_3^-) at different sampling points during RAS period: Mean NO_3^- (mg/l). Black lines indicate sampling points T0-T7, and the dotted line is the switch to Brackish water.