Investigating Bromate Formation in Ozonated Saltwater RAS, used for rearing of Atlantic salmon (*Salmo salar*) Post-smolt

Camilla Dolmen

UNIVERSITY
OF BERGEN

Department of Biological Sciences

Master of Science in Aquaculture

June 2024

Acknowledgements

This thesis was completed at the Department of Biological Sciences (BIO) at the University of Bergen in the period of February 2023 till June 2024. This study has been carried out in collaboration with the hatchery Osan Settefisk AS in Trøndelag and the University of Bergen.

First, I would like to express my deep gratitude to my supervisors. A big thank you to my main supervisor, Professor Sigurd Stefansson at UiB, for his professional and constructive guidance as well as his quick and insightful feedback throughout the process. I am also grateful to my external supervisor, Kari Attramadal from Nofitech, whose expertise in the field has been invaluable.

Special thanks to everyone at Osan Settefisk AS, who made this master's thesis possible. I greatly appreciate their willingness to further develop the industry and their decision to invest in me and my project. I would especially like to highlight the general manager Svein Oluf Øren for his commitment to addressing the issues, as well as biologist Ida Renate Nubdal Busch and operations manager Rasmus Dolmen for their patience and availability at all times.

I must also thank Eurofins Environmental Testing Bergen for performing the water analyses, and Liv Tori Selle from Erko Settefisk AS and Maria Åkre from Hardingsmolt for their collaboration and contribution to the project through the performance of comparable water analyses.

Finally, I would like to thank my fellow students for their support and friendship over these five years. You have made the hours spent in the study hall bearable and have enriched this time with joy. I am deeply grateful for the friendships we have built, which I hope will last a lifetime. Thank you all!

Abstract

This study explores the effect of ozonation in saltwater Recirculating Aquaculture System (RAS) for Atlantic salmon post-smolt (*Salmo salar*), investing bromate formation. Norwegian aquaculture is facing a technological shift where a larger proportion of production is moving onshore. Particularly, we have seen developments in using RAS technology to produce larger smolt on land. In recent years, there have also been more post-smolt facilities allowed of producing smolt up to one kilo, shortening the time spent in the sea and thereby avoiding salmon lice and the risk of escape. Ozonation is used as a water quality improvement method in RAS systems, and if not properly managed, excessive ozonation can lead to the formation of bromate, which could potentially pose a risk to the fish.

The aim of this study was to investigate the relationship between ozonation and bromate formation in seawater in full scale systems. Water samples were collected and analysed for bromate concentration, additionally, welfare assessments of the fish were conducted.

The results indicated that ozonation in saltwater RAS leads to the formation of bromate, with concentrations increasing over the course of production. However, the bromate levels observed did not exceed 0.13 mg/L BrO_3^-, which is well below the recommended threshold for aquatic organisms. The group which experienced the highest levels of ozonation (within recommended levels), demonstrated the highest specific growth rate. Welfare assessments showed that all fish groups maintained good welfare.

In conclusion, this study confirms that ozonation in saltwater RAS results in some bromate formation and accumulation over the production period. However, these levels remain below harmful thresholds, and the use of ozone as a method to clear the water does not negatively impact fish performance. For optimal fish welfare and production efficiency, close monitoring of water quality is essential to ensure bromate levels remain safe.

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1. Introduction

1.1 The Norwegian aquaculture

The Norwegian aquaculture industry started in the early 1970s and has since experienced remarkable development, now considered a success story. In 1971, the total production of Atlantic salmon (*Salmo salar*) was 531 tons (Bjørgo *et al.*, 2011). By 2023, production had reached 1.52 million tons, contributing to a total export value of 122.5 billion NOK (Grefsrud *et al.*, 2024). Norway is a significant seafood nation globally, supplying markets world-wide with Norwegian seafood.

Since their inception in the 1970s, open sea cages have primarily been the technology used. This remains the case today. However, this has not been without challenges. Among the most significant and recognized challenges are the parasite salmon lice (*Lepeophtheirus salmonis*), escapes, disease, emissions and pollution (Afewerki *et al.*, 2023).

Continuous efforts are being made to explore new methods and technologies to address the challenges with salmon lice and optimise growth conditions has led to advancements in closed containment systems (CCS), offshore farming, and recirculating aquaculture systems (RAS). These methods effectively isolate the production process from the surrounding environment, preventing the operation from impacting natural ecosystems and vice versa.

In recent years, there has been an increasing trend towards keeping the salmon longer on land before they are transported over to the sea facilities. In 2011, the Norwegian Ministry of Fisheries and Costal Affairs authorized a trial production period for growing post-smolts up to 1 kg in land-based systems (Bakke *et al.*, 2017). The motivation behind this approach was that extending the production period on land would reduce the duration that the fish spend in marine environments, consequently decreasing their exposure to sea lice and the risk of escape events. The production of post-smolts on land is still a relatively new field of study and production may vary between different production systems. A critical issue in this context is the selection of water quality for the farming of post-smolts; for instance, salinity is a significant variable to consider. While the use of untreated seawater can compromise biosecurity in flow-through systems, concerns in RAS also relate to the effectiveness of internal water treatment processes.

1.2 Recirculating aquaculture systems

Recirculating aquaculture systems (RAS) has grown in popularity to produce smolt and offers a distinct advantage over traditional Flow Through Systems (FTS) by operating independently of the local environment. RAS allows for precise control over water quality and temperature (Dalsgaard *et al.*, 2013). This enables optimal growth and consistent production throughout the year representing an important improvement over seasonal production. Limitations in access to fresh water, have led to an increased focus on RAS for smolt production. Given that only 3% of the world's water resources are fresh water (Thaulow, 2023), RAS technology, which can recycle up to 99% of the water used (Bregnballe, 2022), addresses fresh water scarcity and enables aquaculture in areas with limited water resources.

RAS technology, however, come with their own set of challenges that can affect the overall system performance. One of the primary mechanical difficulties is the removal of suspended solids, which play a critical role in influencing the functionality of nearly every component within a RAS (Badiola *et al.*, 2012). Particularly, high levels of particulate organic matter (POM) and dissolved organic matter (DOM) strain biofilters and solid removal systems, contributing to off-flavour, colour problems, odour issues, and bacterial bloom in the system (Fjellheim *et al.*, 2016; Aguilar-Alarcón *et al.*, 2022). Decomposition of organic material leads to increased oxygen consumption and waste production (CO_2 and ammonia), affecting ammonia and nitrite conversion efficiency in the biofilter. High particle levels in the water will also reduce the effectiveness of disinfection with ozone and UV. Maintaining a constant dose of ozonation can be effective in breaking down the particles and increasing the efficiency of particle removal.

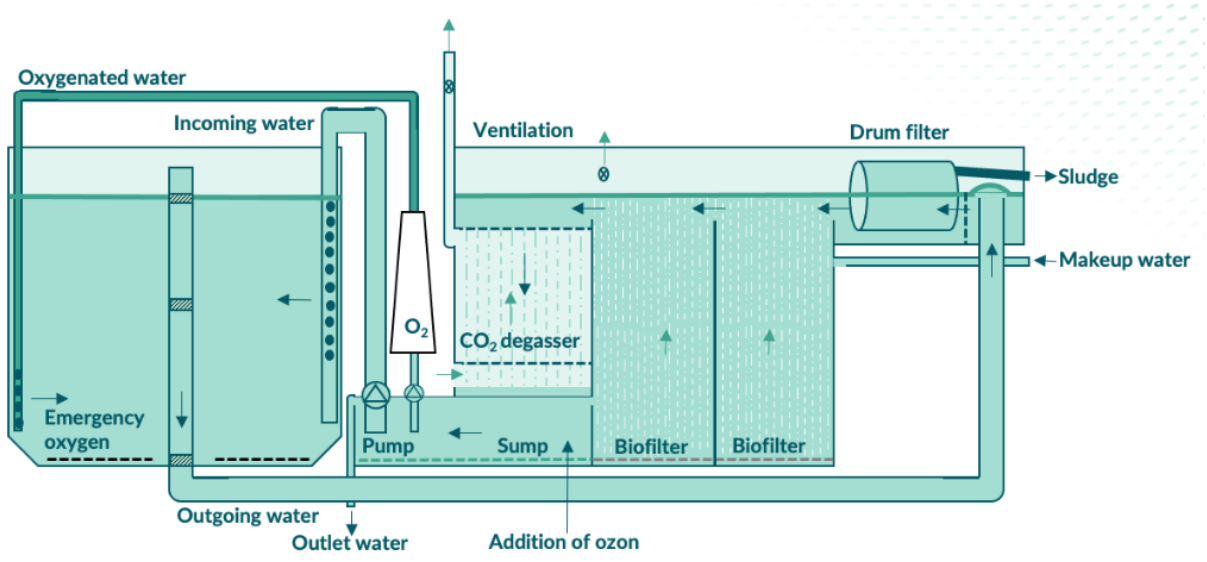


Figure 1.1: RAS setup. *The RAS setup at Osan Settefisk AS where the project was carried out. Picture: (Nofitech, 2024).*

A RAS consists of several different components, and the design and order of the units varies between the suppliers in the business. Since the water is reused, it is crucial to continuously treat the water to remove the waste produced by the fish to avoid that it accumulates over time. The first component after the fish tank is often a drum filter. The drum filter is essential for eliminating suspended solids (Dolan *et al.*, 2013). This filter incorporates a rotating microscreen that functions akin to a sieve, blocking particles that exceed the size of its pores. As the drum rotates, solids caught on the filter are transported to a backwash zone, where jets of water remove the particles, directing them into a sludge collection tray (Bregnballe, 2022).

The water then flows to the biofilter, which removes total ammonium nitrogen (TAN) through "nitrification". Ammonia is produced by the fish's metabolism of nitrogenous components, which the fish excretes through mainly the gills, as well as through urine, and feces (Liltved *et al.*, 2007). Ammonia exists in two forms: ionized ammonium (NH_4^+) and non-ionized gaseous ammonia (NH_3), collectively known as TAN, or total ammonium-nitrogen measured as nitrogen. The predominant form is largely determined by pH, at lower pH levels, ammonium predominates, whereas at higher pH levels, ammonia becomes more prevalent. Ammonia becomes toxic to fish already at low concentration, while ammonium is less harmful. This underscores the importance of having a biofilter, as it converts ammonia, via nitrite, into nitrate, facilitated by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Fjellheim *et al.*, 2016). Alkalinity, indicating the waters "buffer capacity", is crucial for maintaining stable pH levels for biofilter efficiency (Summerfelt *et al.*, 2015). Excessive alkalinity can impede the formation and excretion of ammonia, leading to potential toxicity. The biofilter has a large surface area in relation to its volume to accommodate many fixed nitrifying bacteria in a relatively small volume (Fjellheim *et al.*, 2016). AOB and NOB grow slowly and are autotrophic bacteria, meaning they use CO_2 as a carbon source for growth. They are aerobic since they require oxygen, yet they lose the competition for oxygen with the normally plentiful heterotrophic bacteria. Heterotrophic bacteria use organic material as carbon sources for growth. Therefore, the main challenge in designing an effectively functioning biological nitrification stage largely involves ensuring efficient oxygen supply for the nitrifying bacteria. This can my achieved by keeping heterotrophic growth down and ensuring effective oxygen transport to the biofilm.

A CO_2 degasser is utilized to eliminate carbon dioxide from the water. Carbon dioxide is generated through the metabolic processes of both the fish and the heterotrophic bacteria within the biofilter. Elevated levels of CO_2 can lead to a decrease in pH, adversely impacting the fish (Fjellheim *et al.*, 2016). Air is drawn in over the top and enters beneath the aerators. Exhaust fans then pull the air through the aerators and out. Water is distributed over the aerators, trickles down through them, and collects in the pump-sump. After passing through the CO_2 degasser, the water reaches the pump-sump where the disinfectant ozone is added. From there, the water is pumped into the oxygen cone, where oxygen is introduced before the water is returned to the fish tank (Bregnballe, 2022).

1.3 Ozone

Feeding makes the water in the tanks dirty, and as a result, ozonation is necessary. Ozonation removes colour and particles from the water, ensuring its clarity. Without ozonation, fish production would be challenging because the water would be dirty, making it difficult to observe the fish and, consequently, challenging to determine the appropriate feeding amount.

Ozone is formed when dry air or oxygen is subjected to electrical discharges, a process that splits oxygen molecules (Liltved and Vogelsang, 2011). The released oxygen atoms then combine with undivided oxygen molecules to create ozone. The solubility of ozone in water is determined by the partial pressure of ozone above the water surface and follows Henry's Law. This principle asserts that the relationship between the partial pressure of ozone and the mole fraction of ozone in water remains constant.

Ozone is toxic to humans when present in the air (Liltved and Vogelsang, 2011). Most people can detect the scent of ozone at concentrations as low as 0.01 ppm, although the safety threshold is set at 1 ppm in air. When seawater is treated with ozone, it rapidly oxidizes the naturally occurring bromide in seawater (65 mg/l) to form hypobromous acid (HOBr). This acid is in a pH-dependent equilibrium with the hypobromite ion (OBr^-). These secondary oxidants, along with any remaining ozone, constitute the primary active chemicals and are collectively referred to as the total residual oxidants (TRO). A portion of the hypobromite ion (OBr^-) can be further oxidized by ozone into bromate (BrO_3^-), which is formed in small quantities. Bromate is considered a potentially carcinogenic compound, however, it is not acutely toxic to fish at low concentrations, with a 96-hour LC50 value of 30 mg/l.

1.4 Bromate

Bromate (BrO_3^-) does not occur naturally in surface water, however anthropogenic activities can introduce it into the aquatic environment, primarily the reaction of ozone with bromide (Hutchinson *et al.*, 1997). Ozone chemistry in saltwater differs from freshwater due to the higher concentration of dissolved ions such as chloride and bromide (Jung *et al.*, 2017). Despite the high bromide concentration (65 mg/L) in seawater, bromate formation is not detected until an ozone dose of 5 mg/L, attributed to the low availability of residual ozone at the final step for bromate formation, because most of the ozone is consumed by the high concentration of bromide. Factors such as ozone dose, pH (with a range of 7.5 – 8.4 in saltwater), salinity and temperature influence ozone chemistry, affecting bromate formation kinetics.

Research indicates that bromate poses risks to human health, being harmful to organs such as the kidneys and brain, and has been classified as a Group 2B carcinogen by the International Agency for Research on Cancer (Dong *et al.*, 2019). Once bromate is formed, it is challenging to remove from the aqueous matrix, as it is not further oxidized.

Acute toxicity studies show that bromate is more harmful to marine organisms compared to freshwater species (Hutchinson *et al.*, 1997). For example, *Daphnia magna* (water flea) showed a 48-hour LC_{50} of 179 mg/L, whereas the planarian *Polycelis nigra* had a much higher tolerance with a 48-hour LC_{50} of 2258 mg/L. Juvenile fish and fish larvae were found to be particularly sensitive to bromate, with some species showing a 96-hour LC_{50} as low as 31 mg/L. Using the factor of 10 to extrapolate from acute to potential chronic toxicity, the existing data indicate that to safeguard aquatic organisms from prolonged adverse effects, the concentrations should ideally remain below approximately 3.0 mg BrO_3^- per liter.

In light of the potential for bromate to enter aquatic environments and its remarkable health implications, there is a critical need for a comprehensive understanding of its effects on aquatic organisms and strategies for monitoring and controlling its formation (Hutchinson *et al.*, 1997; Dong *et al.*, 2019).

1.5 Fish welfare

Ensuring good fish welfare is essential for sustainable salmon production. Aquaculture producers carry a special responsibility for fish health, as they keep fish in captivity. Sustainability during the sea phase requires ethically defensible production where animal welfare is prioritized regardless of economic value (*Lov om dyrevelferd*, 2010).
Welfare indicators are divided into two categories: group-based and individual-based. Group-based indicators include mortality rate, behaviour, appetite, growth, surface activity, water conditions, and disease (Noble *et al.*, 2018). Individual-based indicators includes factors such as sea lice, scale loss, fin damage, sexual maturity, and spinal deformities. Skin injuries and body wounds are also important measurements. Skin health is critical for homeostasis and protection against pathogens. Wound healing depends on several factors such as stress, environment, wound size and depth, and nutritional status (Jensen *et al.*, 2015). It is estimated that between 1.1 and 2.5% of Norwegian farmed salmon develop wounds, which are one of the main causes of fish mortality (Takle *et al.*, 2015). Such injuries occur during handling, internal aggression, and mechanical stresses during for example vaccination and lice treatment.

The fins and snout of salmon are susceptible to injuries, and although some injuries are considered inevitable in aquaculture, it is important to minimize their extent. Fin wear affects the organ's functionality and occurs more frequently in larger fish (Schneider and Nicholson, 1980). Fin rot leads to loss of fin tissue and can disrupt salt balance and weaken swimming function. Fin rot is not necessarily associated with high mortality, although it creates openings for other pathogens.

Deformity in salmonids is a well-known issue and a challenge that can lead to animal welfare and ethical implications. The causes are often a combination of genetic and environmental factors, including rapid growth, low phosphorus content in feed, breeding, high incubation temperature, and incorrect vaccination timing (Fjelldal and Grotmol, 2005).

Standardizing fish welfare has long been a challenge for the industry, and in this context, a specific protocol has been developed to serve as a common welfare standard (Nilsson *et al.*, 2022). This protocol builds on knowledge described in the 'Salmon Welfare Index Model' (Stien *et al.*, 2013), the Fishwell handbook, and the book 'Welfare Indicators for Farmed

Salmon: How to Assess and Document Fish Welfare' (Noble *et al.*, 2018). Common to these models is that they use the same welfare indicators that can be observed or measured to provide information about the poor, or good, welfare of the fish. Welfare indicators used in commercial daily operations are termed operational welfare indicators, while indicators that must be analysed in the laboratory are placed under laboratory welfare indicators.

1.6 Motivation and aim of study

There is a noteworthy lack of knowledge regarding the relationship between ozonation and the formation of bromate in seawater and how this might affect the salmon. Understanding this is crucial for maintaining an ideal fish production that supports good fish welfare. This study focuses on the relationship between ozonation and the formation of bromate, a thus far under-researched area, and aims to contribute new knowledge to the aquaculture industry. Water samples were sent to Eurofins Environmental Testing Bergen (which is a laboratory specialized in analysing nutrients in seawater) for bromate analysis. Additionally, ozonation was monitored, welfare assessments on the fish were conducted, and water samples for analysis of TAN, nitrite, nitrate, and alkalinity were collected.

Based on the aim of the thesis the following working hypotheses (H_A) with the corresponding null hypotheses (H_0) were developed:

(H_{A1}): Ozonation in saltwater RAS will lead to the formation of bromate in the water.
(H_{01}): Ozonation in saltwater RAS will not lead to the formation of bromate in the water.

(H_{A2}): The concentration of bromate will accumulate over the course of production.
(H_{02}): The concentration of bromate will not accumulate over the course of production.

(H_{A3}): Ozonation in saltwater RAS will reduce fish performance.
(H_{03}): Ozonation in saltwater RAS will not reduce fish performance.

2. Material and methods

2.1 Fish

Atlantic salmon post-smolts were obtained from the commercial aquaculture company Osan Settefisk AS, in central Norway. Post-smolts are defined as salmons that have completed the smoltification, signifying the transition from a freshwater-adapted fish to one with seawater tolerance. The size range in which salmon is called post-smolt is not clearly defined; however, in this master's thesis I use the term post-smolts for sizes up to 1 kg. In this project, I have followed three different groups of post-smolts at Osan Settefisk. For the third group (G3), fertilized roe with origin at Veestseøra (site number: 24096) was acquired from AquaGen and reared at the Osan Settefisk RAS facility following standard commercial protocols. For the second group (G2), we received fertilized roe with origin at Leikvangbukta (site number: 10246) from MOWI, and for the first group (G1), it was a mix of roe from both AquaGen (Veesteøra) and MOWI (Leikvangbukta).

2.2 Experimental facility

The stages of this experiment were conducted at Osan Settefisk AS, in Nærøysund, in central Norway. The company use RAS technology (see further below).

Roe hatched at approximately 500 degree-days Celsius, and alevins were maintained in hatching cabinets until the absorption of the yolk sac, which occurred at around 370 degree-days Celsius post-hatching. The resulting fry were moved to the start feeding department, housed in tanks with a diameter of 6 meters (47 m^3). In the start feeding department the light control was LD24:0, meaning that it was light for 24 hours, and dark for 0 hours. When the fish reached approximately 7 grams, they were sorted by size and placed in tanks with 8-meter diameter (133 m^3) in the fry department. The light control in the fry section was LD12:12, meaning that it was light for 12 hours, and dark for12 hours. When the fish reached around 45 grams, they underwent vaccination and further sorting by size before being transferred to the smolt department. In the smolt department, they were placed in tanks with a diameter of 12 meters (430 m^3). Here, the light regime LD12:12 continued, until there was about one month left before the fish were to be transferred to the post-smolt department. Then it switched back to LD24:0. This resulted in a total duration of LD12:12 of about 3 months,

which, in combination with LD24:0, led the fish to smoltify. Once the fish were smoltified, they were moved to the post-smolt department, where the light was constant LD24:0. At this point, the fish had surpassed 100 grams. In the post-smolt department, the tanks have a diameter of 20 meters (2250 m^3). It was from this department the fish were extracted for sampling.

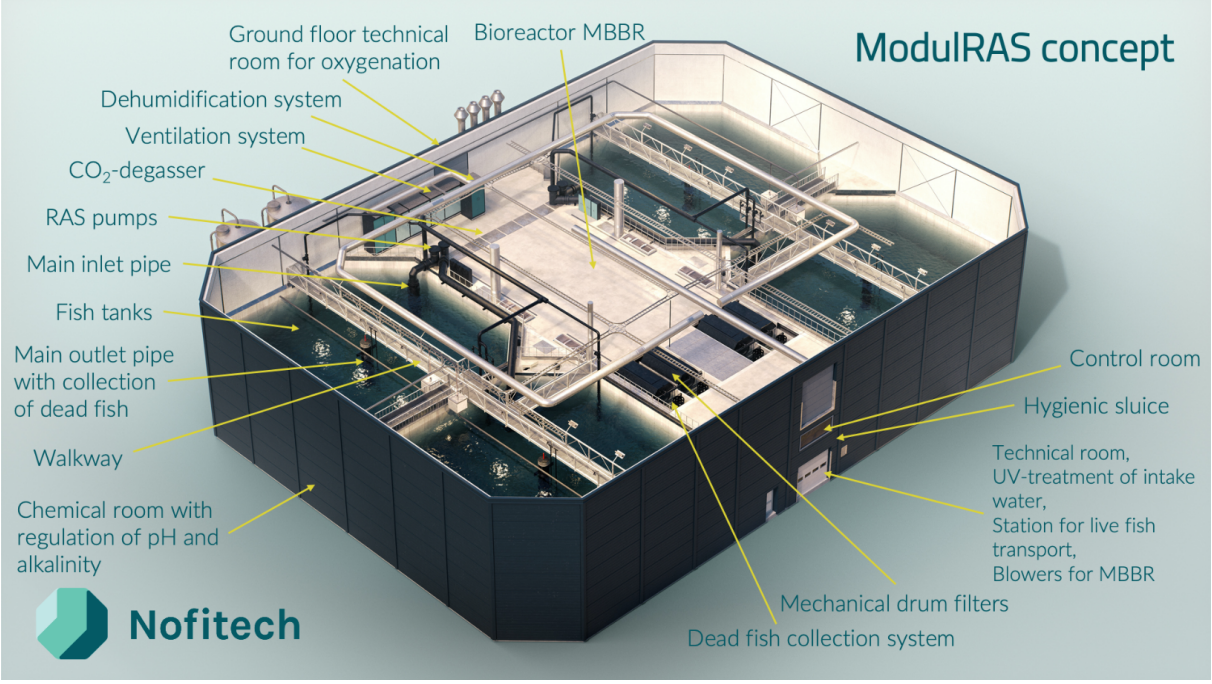


Figure 2.1: Illustration of the post-smolt facility: *Illustration showing the four tanks and the two separate RAS modules between the tanks. The various components are marked with a yellow arrow. Moving bed biofilm reactor is abbreviated as MBBR in the figure. Picture: (Nofitech, 2024).*

The post-smolt facility (Figure 2.1) is constructed by Nofitech. The facility consists of 2 independent RAS systems. Each system consists of 2 large fish tanks, each with a capacity of 2250 m^3. After the tanks, the water passes through 50 µm drum filters (CM Aqua, DTU Science Park, Agern Allé 5A, 2970 Hørsholm, Denmark). The biofilter in each RAS is divided into two chambers in series, each approximately 400 m^3, with biomedia from Biowater featuring a specific surface area of 828 m^2/m^3 and approximately 45% fill rate. At the bottom of the biofilters, there is air from blowers from Kaeser (KAESER KOMPRESSORER AS, Verpetveien 38, 1543 Vestby, Norway). The aerator is designed in several parallel chambers as a counter-current trickling filter degasser with random packed media (Hiflow ring 90-7 from RVT Process Equipment GmbH, Im Greies 15, D-96364

Marktrodach, Germany). RAS pumps pump the water from the pump-sump back to the tanks, and a partial stream goes through oxygen cones, supplying the tanks with a highly oxygenated water stream, regulated by two O_2 sensors in the tank and set points set by the breeder. Ozone treatment provided by Redox (Redox, Industriveien 24, 6530 Røsand, Norway) to both the skimmer and the pump-sump.

2.3 Experimental design

The samplings conducted in connection with this master's project was carried out at Osan Settefisk AS. As mentioned earlier, three different groups of post-smolts were studied to ensure a broad dataset. First, the general factors for experimental design that apply to all three groups are described, then the differences in temperature and water quality for each group are described.

Following smoltification, the fish were transferred to the post-smolt section, consisting of four large tanks of 2250 m^3 each (n = 200.000–320.000/tank, biomass up to 60 kg/m^3). The water temperature in the tanks varied according to the sea temperature during the period when the fish were to be moved to the sea, besides this it was generally around 12 degrees Celsius (see "2.3.1 Farming conditions" for temperature figures). The temperature is regulated using seawater exchange or heat pumps. Full feeding was maintained during this period, with a dimensioned water exchange of 300 liters per kilogram of feed and LD24:0 (light for 24 hours, darkness for 0 hours).

The feeding was adjusted daily based on the fish's appetite. This was done by checking drainpipes for any feed spillage and physically inspecting the tanks to ensure the fish were consuming the provided feed. Changes in feeding, was recorded using the program "Aquateknikk" on a dedicated feeding PC, which then adjusted the feeding automatically. Each tank was equipped with two feeding machines that controlled the feed supply. Alternatively, feeding was also calculated based on estimates of specific growth rate (SGR) = 1.7% to ensure adequate feeding.

The water was ozonated, and the amount of ozone added was determined based on the feeding. Ozonation was adjusted to around 10g per kilogram of feed.

2.3.1 Farming conditions

The pH was between 7.3 – 7.5 and the salinity of seawater was 25 ‰. The oxygen level was automatically adjusted with a setpoint of 90%. Feeding was at 100% during this period, meaning the fish have consumed as much as they desired. Throughout the experiment, salmon were fed 4 mm formulated commercial feed from EWOS®. These conditions applied for all three groups.

G1 was monitored from February 15, 2023, to May 7, 2023. During this period the temperature was mostly 12°C (Figure 2.2). However, to match the actual sea temperature, it was gradually adjusted down to 8,5°C before the fish was transferred to the sea on May 7. This was achieved by decreasing the temperature by 0,3°C per day leading up to the transfer.

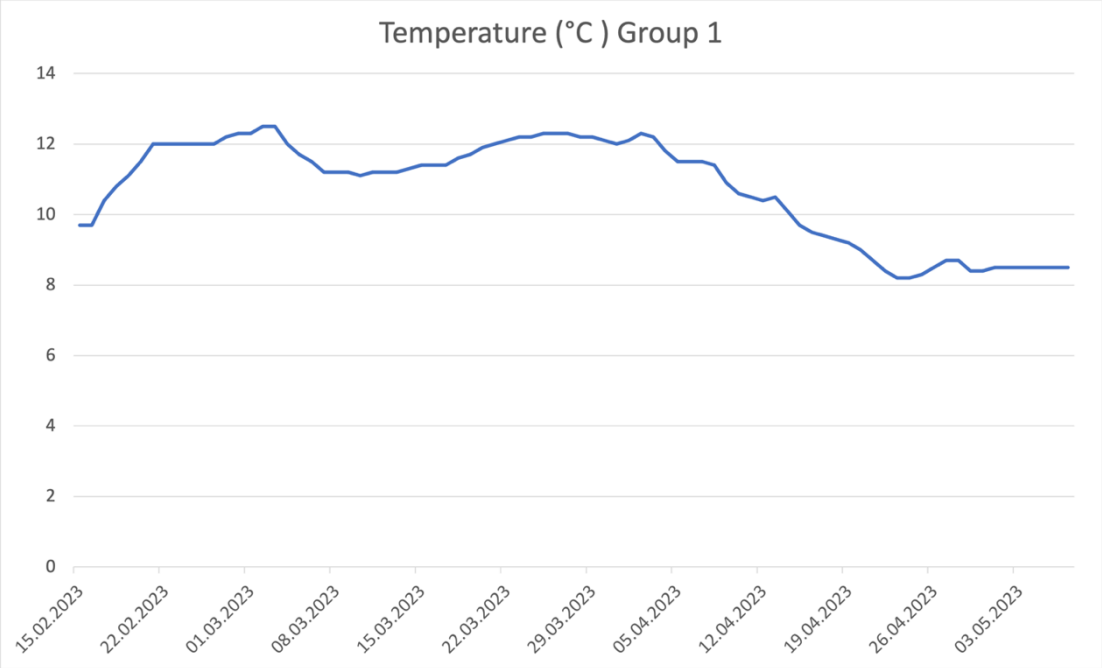


Figure 2.2: Temperature. *Temperature (°C) in the tanks for G1 (15.02.2023 – 07.05.2023).*

G2 was monitored from Mai 31, 2023, to July 24, 2023. During this period the temperature maintained approximately 12°C in the tanks throughout the entire period (Figure 2.3), as the sea temperature was also 12°C at the time.

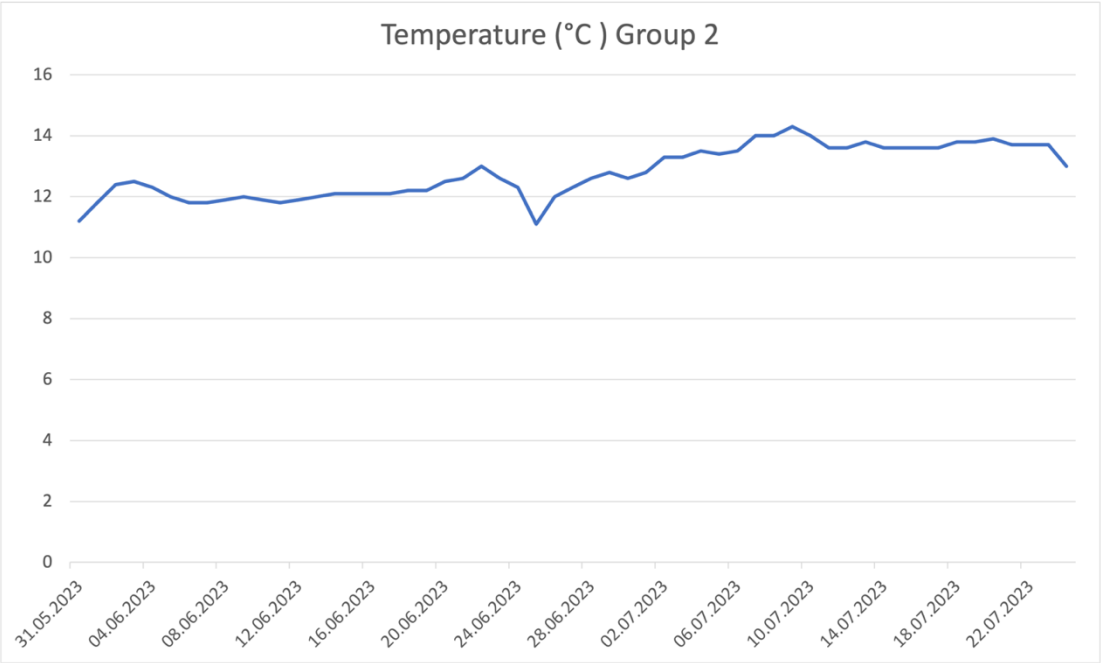


Figure 2.3: Temperature. *Temperature (°C) in the tanks for G2 (31.05.2023 – 24.07.2023).*

G3 was monitored during the period from September 25, 2023, to December 6, 2023. The temperature was mostly 12 °C (Figure 2.4). However, to match the actual sea temperature, it was gradually adjusted down to 7 °C before the fish was transferred to the sea on December 6. This was achieved by decreasing the temperature by 1 degree Celsius per week leading up to the transfer.

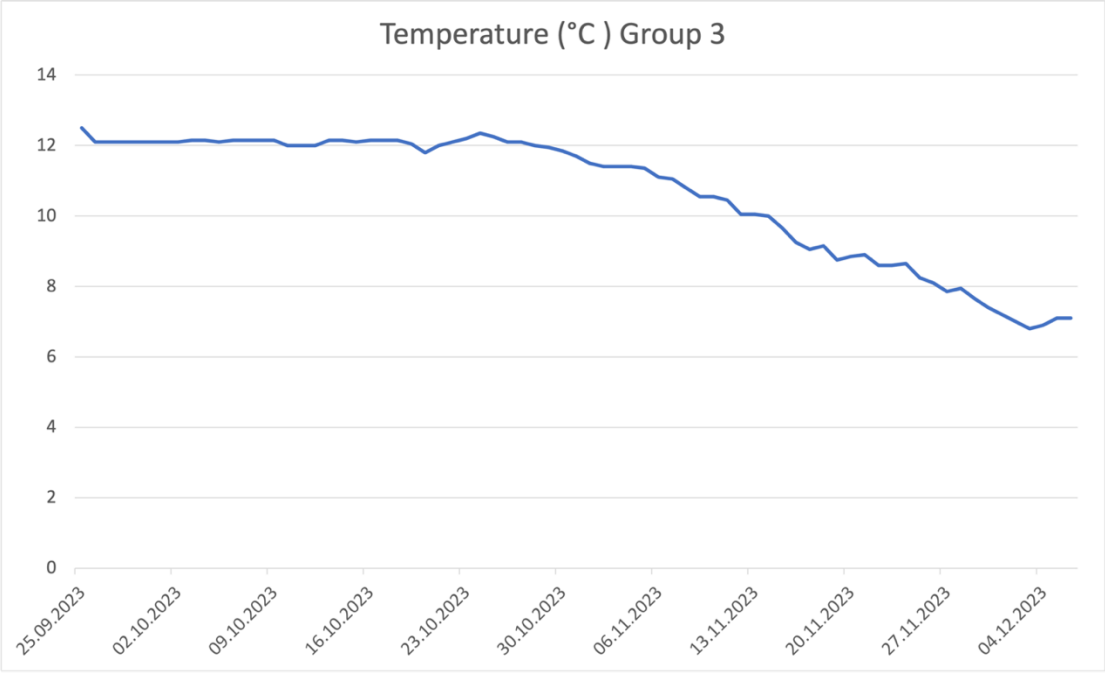


Figure 2.4: Temperature. *Temperature (°C) in the tanks for G3 (25.09.2023 – 06.12.2023).*

For water quality, our measurements included TAN, nitrite, nitrate, and alkalinity. These measurements were conducted separately for each RAS system, several times a week.

Figure 2.5 – 2.7 shows the measurements made for TAN, nitrite, nitrate, and alkalinity. The individual dots represent observed data points for different parameters over time. The smooth lines through the data points are loess curves, which are used to show a trend in the data without assuming a specific form for the relationship between the variables.

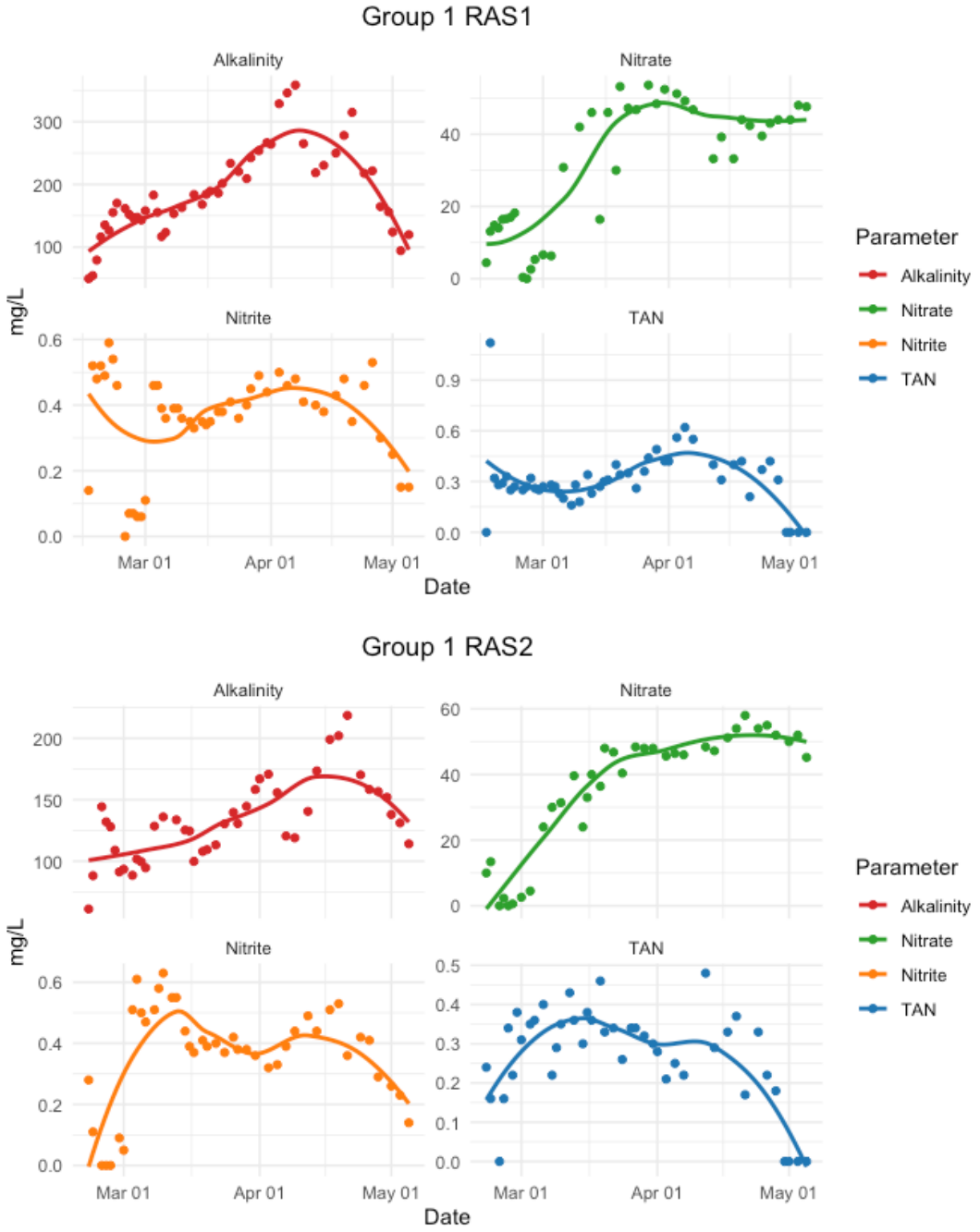


Figure 2.5: Water quality. *The figure shows the measurements made for TAN, nitrite, nitrate, and alkalinity for RAS1 and RAS2, G1 (15.02.2023 – 07.05.2023). The measurements are*

presented as a scatter plot with a fitted curve that represents a smoothed line through the data points.

For G2, only RAS1 was included in the experiment, as the fish in the tanks belonging to RAS2 during this period were in the facility for a short period of time.

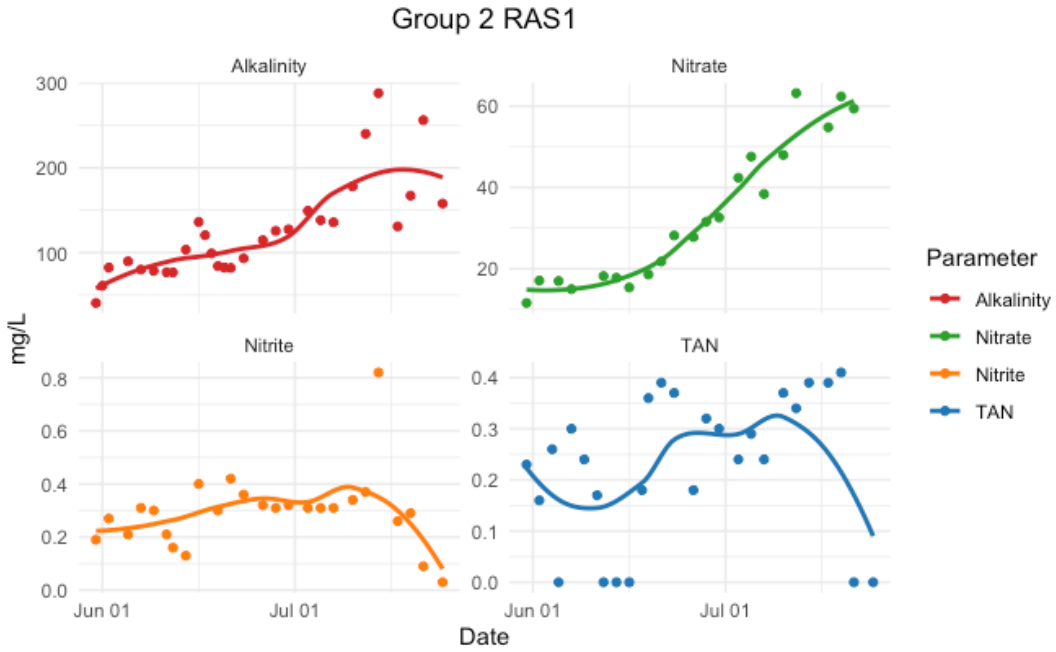


Figure 2.6: Water quality. *The figure shows the measurements made for TAN, nitrite, nitrate, and alkalinity for RAS1, G2 (31.05.2023 – 24.07.2023). The measurements are presented as a scatter plot with a fitted curve that represents a smoothed line through the data points.*

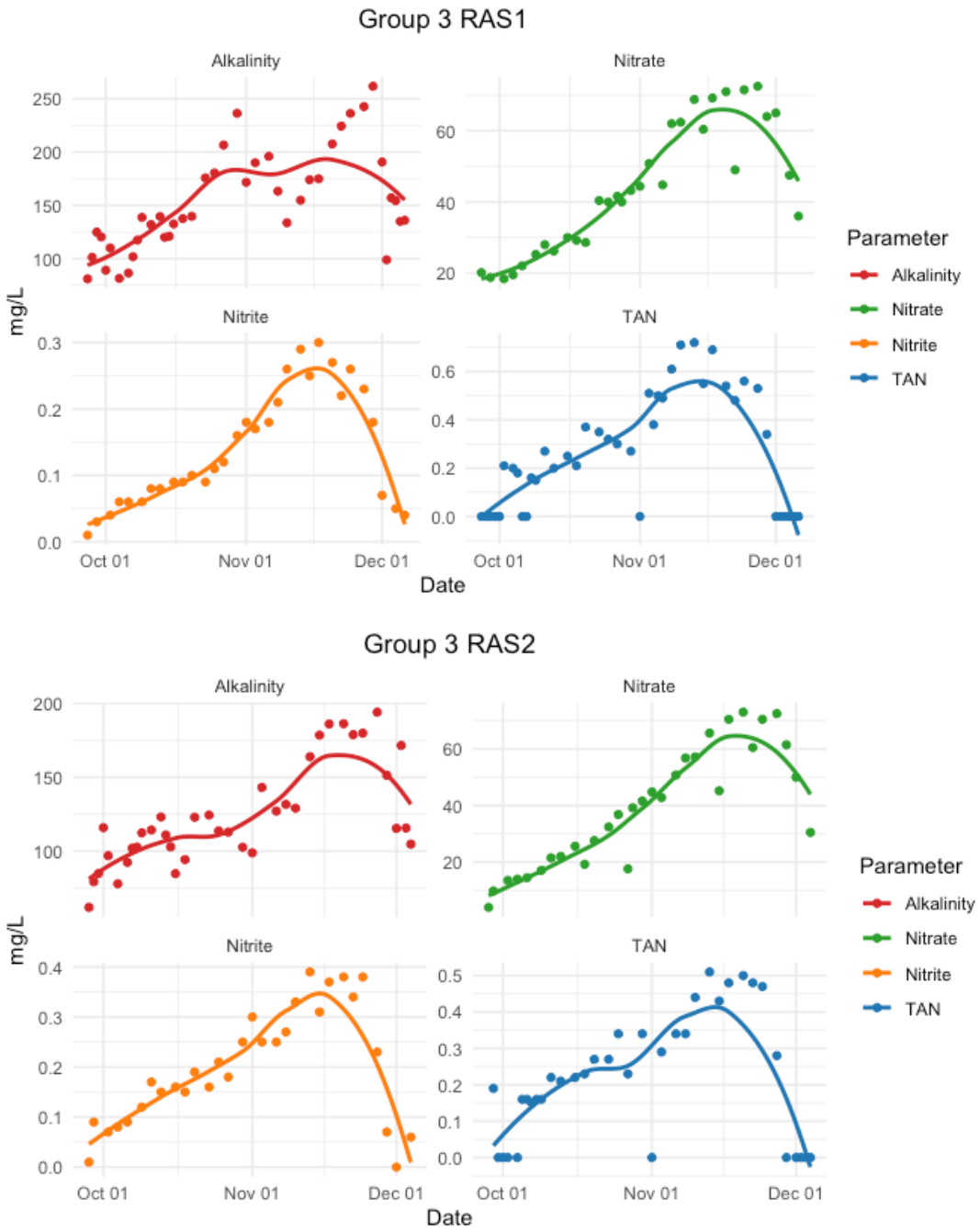


Figure 2.7: Water quality. *The figure shows the measurements made for TAN, nitrite, nitrate, and alkalinity for RAS1 and RAS2, G3 (25.09.2023 – 06.12.2023). The measurements are presented as a scatter plot with a fitted curve that represents a smoothed line through the data points.*

2.3.2 Welfare tests

Throughout the experiment, three individual samplings were conducted for each group (except for G2, see further below). These three measurements were distributed as follows: one at the beginning of post-smolt production, one midway through, and one at the final stage, just before the fish were transferred to the sea. For each sampling, a total 50 fish (with some exceptions mentioned below) per tank were randomly selected for measurements of length (measured in millimetres), weight (measured in grams), and welfare indicators. All fish were anesthetized before sampling and quickly revived in a recovery tank after the measurements were completed. The anaesthetic Tricaine (Tricaine Pharmaq vet powder for bath solution 1000 mg/g) was used.

The initial welfare assessment for G1 was carried out prior to the relocation of fish from the smolt department to the post-smolt department. Specifically, fish from smolt tank 8 (SM8) were transferred to post-smolt tank 1 (PS1), those from SM6 to PS2, SM7 to PS3, and SM5 to PS4. Only 20 fish were measured from each tank for G1, this was adjusted up to 50 fish for G2.

The fish in G2 were monitored for 2 months before being transferred to the sea facility, hence only 2 welfare assessments were conducted for these fish. There was a human error where the first welfare measurement was conducted on fish in the wrong tank relative to the experiment. Fortunately, the fish in the tanks measured in June belonged to the same fish group, and the fish in the respective tanks have followed the same development. Therefore, the welfare measurements are included in the results, as they can serve as an estimate of how this fish group performed.

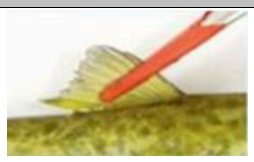
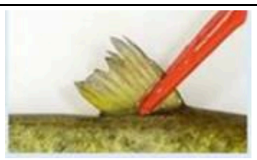
Throughout the study, welfare measurements were sometimes coordinated with the facility's routine sampling, which occasionally led to numbers exceeding 50. This is reflected in the results, arguing that a higher number of fish provides a more robust assessment of welfare. Here are the exceptions from the standardized practice of 50 fish per tank:

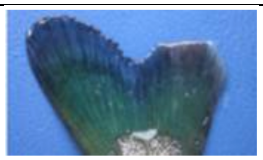
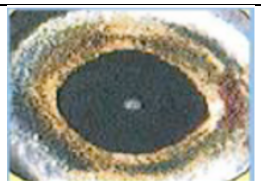
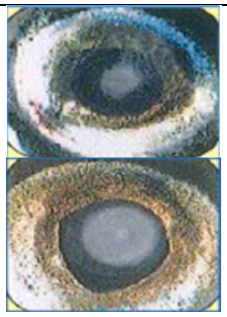
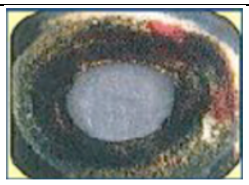
Exceptions from 50 fish:

- G1 = 20 fish
- PS4G2 = 60 fish
- PS1G2 = 70 fish
- PS3G2 = 60 fish
- PS2G2 = 61 fish
- All four tanks for the last measurement for G3 (November/December) contained 60 fish each.

The fish's welfare was documented in an Excel spreadsheet. These measurements encompass the following parameters: weight in grams, length in millimetres, healed damage on dorsal fin, active damages on the dorsal fin, gill cover, pectoral fins, pelvic fins, cataract, wounds, caudal fin, skeletal deformity, slime layer, and loss of scales. The damages are graded on a scale from 0 to 3, where 0 are no damages at all and 3 are serious damage. Table 2.1 show how the different parameters were graded.

Table 2.1: Welfare indicators for Atlantic salmon. *The images in the table illustrate what creates score 1, 2 and 3. Picture: (SalMar, 2023).*

	1	2	3
Healed damage on dorsal fin			
Active damage on dorsal fin			
Pectoral fin			
Pelvic fin	Picture missing. Use pectoral fin as reference.	Picture missing. Use pectoral fin as reference.	Picture missing. Use pectoral fin as reference.

Caudal fin			
Gill cover Right and left are added together for a total score (max. 6)			
wounds			
Cataract 0: all black lens			
Skeletal deformity 0: no deformity 1: hunchback 2: short tail 3: scoliosis (zigzag back)			
Loss of scales 0: no loss 1: loss of individual shells 2: shell loss in small areas (up to crown piece size). under 10% of the fish 3: over 10% of the fish has loss of scales			
Slime layer graded 0-2	Picture missing. 1: partly absent. a little dry	Picture missing. 2: completely absent. dry	

2.4 Sampling protocol

The seawater samples used in this study were collected from the production water in the post-smolt facility at Osan Settefisk AS. The pH was between 7.3 – 7.5 and the salinity of the sampled seawater were 25 ‰. Eurofins Environment Testing Bergen conducted the analyses for measuring bromate and turbidity, while I carried out the analyses for measuring TAN, nitrite, nitrate, and alkalinity.

Throughout the experiment, a total of 18 shipments of water samples were sent to Eurofins Environment Testing Bergen for the analysis of bromate and turbidity. "*Eurofins Environment in Bergen specializes in analysing nutrients in seawater. The laboratory holds accreditation in accordance with ISO 17025 standards*" (Eurofins, 2024). Each shipment of water samples consisted of a total of 6 sample bottles of water. The sampling points were: the inlet to the tank, the outlet from the tank, and the main pumps. These three measurement points applied to both RAS-system 1 and RAS-system 2. The exception here is G2, as only RAS1 was monitored and therefore 3 sample bottles of water were sent for each shipment.

In addition, the aquaculture companies Hardingsmolt AS and Erko Settefisk AS submitted water samples to Eurofins, allowing the results from Osan to be compared with other facilities. These water samples were also analyzed for bromate and turbidity formation. Hardingsmolt submitted water samples at two different times: one midway through production and another at the end of production. They selected approximately the same sampling points as Osan: after the drum filter, outlet, and main pump for both RAS-system 1 and RAS-system 2. Erko Settefisk AS submitted one water sample at the end of the production cycle. The water was drawn from the pump-sump (Representing the inlet to the fish tank).

2.5 Analytical procedures

2.5.1 Bromate

Reference method: SS-EN ISO 17294-2:2016

I have translated the method from Swedish to English. The method was carried out in accordance with the standard procedure at Eurofins.

Samples are injected into a chromatographic system (HPLC/IC), where the mobile phase and separation of analytes occur through a chromatographic column. The sample is then transferred to the ICP-MS system, where: a continuous plasma is generated by argon gas passing through a radiofrequency field, thereby ionizing it. The sample (in aqueous solution) is injected into the plasma as a fine mist. The plasma evaporates the water, breaks down compounds, and ionizes atoms. Ions are separated from the plasma and directed into a vacuum chamber through a series of columns with progressively smaller openings.

The ion stream passes through a series of ion lenses that focus the beam and remove photons as well as uncharged particles/atoms. The ions pass through a quadrupole that selects ions with a specific mass/charge. The selected ions collide with a detector, generating a measurable electrical voltage. By comparing the measured voltage with the voltage generated at a known concentration of the analyte (with the same mass), the concentration of the analyte in the sample can be calculated. Instrument calibration consists of 6 points. The calibration curve is evaluated for linearity and is also tested by running several control samples. The samples are evaluated for correct integration, retention time, and sufficient resolution between peaks.

2.5.2 Turbidity

Reference method: NS-EN ISO 7027-1:2016

I have translated the method from Norwegian to English. The method was carried out in accordance with the standard procedure at Eurofins.

The method applies to the measurement of turbidity for all types of water that are free from particles that settle rapidly. Turbidity is a measure of the water's ability to scatter or absorb light and is often taken as an indication of how cloudy the water is. Turbidity is a function of dissolved material and suspended particles. Settleable material is not normally included as part of turbidity measurement, as it is given time to settle at the bottom of the measuring cuvette before the sample is measured. Turbidity is determined by comparing light scattering (nephelometric determination) in the sample under given conditions against a calibration-solution. Suspended particles scatter incident light, which is measured at an angle of 90° to the incident light. Light scattering is determined by the number, size, color, and refractive index of the particles.

2.5.3 TAN, nitrite, nitrate, and alkalinity

Water samples were done three times a week (Monday, Wednesday, and Friday) by personnel in the RAS, with potential further follow-up if any values surpass the specified limits, including TAN, nitrate, nitrite, and alkalinity with various test kits from MERCK KGaA (64271 Darmstadt, Germany).

Table 2.2: Recommended Limits for Water Quality Parameters. *This table outlines the recommended concentration limits for the water quality parameters measured in this study.*

Parameters	Recommended limits (mg/L)
Alkalinity	100 - 400
Nitrite	< 0.5
Nitrate	< 100
TAN	< 2

2.5.3.1 TAN – Total ammonium-nitrogen

Procedure for water sample

The test kit "Ammonium Cell Test" (1.14558.0001) from MERCK KGaA (64271 Darmstadt, Germany) was used.

The process began with the pipetting of 1.0 ml of the water sample, which was then transferred to a test tube with the corresponding QR code. Next, 1 dose of NH_4-1K was added to the test tube. The cap was screwed on, and the sample was thoroughly mixed. The sample was then allowed to stand for 15 minutes to accommodate the reaction time.

Subsequently, the test tube was placed in the spectrophotometer (Merck Spectroquant Prove 100), ensuring the QR code faced the specified location for QR code reading. The result of the sample was displayed on the spectrophotometer screen. Upon completion of the analysis, the test tube, along with its contents and sealed cap, was placed in the designated container for proper handling of chemical waste.

2.5.3.2 Nitrite

Procedure for water sample

The test kit “Nitrite Test” (1.14776.0001) from MERCK KGaA (64271 Darmstadt, Germany) was used.

The analysis commenced with the pipetting of 5.0 ml of the water sample into a clean test tube. One measuring spoon of Nitrite 1 (NO_2-1) was then added to the test tube. After capping the test tube, the sample was mixed thoroughly until the powder completely dissolved. A pH strip was used to verify that the sample registered a pH of 2.0-2.5. The sample was left to stand for 10 minutes to accommodate the reaction time.

Subsequently, the lid of the spectrophotometer was opened, and the Nitrite (NO_2^-) method was selected by adjusting the AutoSelector. The sample was then carefully transferred from the test tube to the cuvette (10-mm), making sure the cuvette's frosted side faced the operator so the light beam would pass through the clear side.

Upon completion of the analysis, the chemicals were disposed of responsibly by pouring them into the labelled container. The test tube and cuvette were then thoroughly washed with deionized water, with the wash water being collected in the same container to ensure proper disposal.

2.5.3.3 Nitrate

Procedure for water sample

The test kit “Nitrate test in seawater” (1.14942.0001) from MERCK KGaA (64271 Darmstadt, Germany) was used.

The analysis was initiated by pipetting 5.0 ml of NO_3-1 into a clean test tube, followed by the addition of 1.0 ml of the water sample to the same test tube. The cap was then placed on the test tube, and the contents were mixed well. Subsequently, 1.5 ml of NO_3-2 was pipetted and added to the mixture, the cap was placed back on, and the mixture was mixed thoroughly.

After ensuring a thorough mix, the sample was allowed to stand for 15 minutes to facilitate the reaction time. Two microspoons of NO_3-3 were then added to the test tube, the cap was replaced, and the contents were mixed well again. The sample was left to stand for an additional 60 minutes to allow for the reaction time.

Progressing to the spectrophotometric analysis, the lid of the spectrophotometer was opened, and the Nitrate (NO_3^-) method was selected by adjusting the AutoSelector. The sample was carefully transferred from the test tube to the cuvette (10-mm), ensuring that the clear sides of the cuvette were kept clean and dry, using lens paper when necessary.

After the analysis was completed, the chemicals were poured into the labelled container for proper disposal. The test tube and cuvette were then thoroughly washed with deionized water, with the rinse water also being collected in the same container to ensure proper chemical waste management.

2.5.3.4 Alkalinity

Procedure for water sample – Alkalinity

Alkalinity was determined using a titration method. Alkalinity in a water sample was analysed using hydrochloric acid (HCl). Once the amount of hydrochloric acid used is known, the alkalinity can be calculated. The calculation typically involves using the formula:

$$Alkalinity\ (mg/L\ as\ CaCO_3) = \frac{Volume\ of\ HCl\ used\ (L) \times Concentration\ of\ HCl\ (mol/L) \times Equivalent\ weight\ of\ CaCO_3}{Volume\ of\ water\ sample\ (L)}$$

The 'Titrisoft 3.5.2' program on the computer automatically performs these calculations and displays the results on the screen.

Firstly, the pH sensor was removed from its storage case and cleaned with battery water. Then it was positioned in the stand and 100 ml of the water sample was poured into a glass beaker. After placing the stirring magnet in the beaker, the beaker was positioned correctly, and the titration machine (TitroLine 7000) was started. It was important to verify that the titrator was properly connected to the computer before proceeding with the 'Titrisoft 3.5.2' program. The titration process was conducted following the program's instructions. Upon completion, the alkalinity value was displayed on the screen, indicating the end of the titration. Then the glass beaker, magnet, and pH sensor were cleaned and returned to their designated storage locations.

Figure 2.8: The titration machine. *The glass beaker is filled with the water sample and the magnetic stir bar is placed in the beaker, along with the pH meter and the pipette containing hydrochloric acid. Picture: (Camilla Dolmen, 2023).*

2.6 Biometric calculations

Fulton's equation for condition factor (CF) was used to calculate the condition factor for each fish.

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

The weight-specific growth rate (SGR) was calculated by using this formula:

$$SGR = \frac{\ln\left(Weight_F(g)\right) - \ln\left(Weight_I(g)\right)}{T_2 - T_1} * 100$$

Where $Weight_F$ is the average final weight, and $Weight_I$ is the average initial weight between two sampling times T.

2.7 Statistical analysis

The data was structured using Microsoft Excel (version 16.66.1). All figures and models were fitted using R Studio (version 2022.07.1) and GraphPad Prism (version 10.2.0). The statistical analyses were also preformed using both GraphPad Prism and R Studio. Using GraphPad Prism a Simple linear regression was performed to demonstrate the correlation between the dosage of ozone applied per hour and the resultant concentration of bromate. To investigate the differences in the condition factor and weight across the tanks, a one-way ANOVA was performed in R Studio. Measurements from the sampling points were compared. Following the ANOVA, a Tukey's HSD post-hoc test was utilized to examine the pairwise differences between the tanks and sampling points. Statistical significance was assessed using p-values, with the threshold for significance established at $p < 0.05$.

3. Results

The results could not be recorded as planned due to malfunctions in the ozonation information display for both G1 and G2. Consequently, data on the ozone added to these two groups are missing. Therefore, the main focus of the results regarding ozonation will be on G3, while G1 and G2 will be used to compare the measurements made on bromate and turbidity. For the results regarding fish welfare all three groups are represented.

3.1 Ozon added and concentration of bromate

Over a period of 70 days (from September 28 to December 6), the addition of ozone and the formation of bromate in G3 were measured and recorded. Initially, the bromate concentration was below the measurable limit (< 0.002 mg/L), which was recorded as the detection limit of 0.002 mg/L in the data analysis. The formation of bromate increased from < 0.002 mg/L at the baseline on September 7, 2023, to 0.110 mg/L at the final measurement on November 27, 2023, for RAS1, and from < 0.002 mg/L at the baseline on September 19, 2023, to 0.099 mg/L at the final measurement for RAS2. Figure 3.1 shows that the three measurement points for bromate (inlet, outlet, and main pump) closely follow each other. The trend appears to be a slightly higher concentration of mg/L bromate in the outlet than in the inlet and main pump for RAS1. This also applies for RAS2 in the second measurement, after that they followed each other, except for the fourth measurement where the bromate at the inlet is slightly higher than the rest.

Ozone addition began at a low level and gradually increased to 0.16 mg/L for RAS1 and 0.14 mg/L for RAS2. About 10 days before the fish were transferred to the sea facility, the ozone addition decreased before being completely turned off for the last six days. The reason for the reduction in ozone addition towards the end is that feeding is stopped to fast the fish a few days before they are transferred, and therefore, ozonation also ends as there is no longer a need to ozonate the water when the fish is no longer being fed.

For a day-by-day table of ozonation, see Appendix 7.1 Table 1.

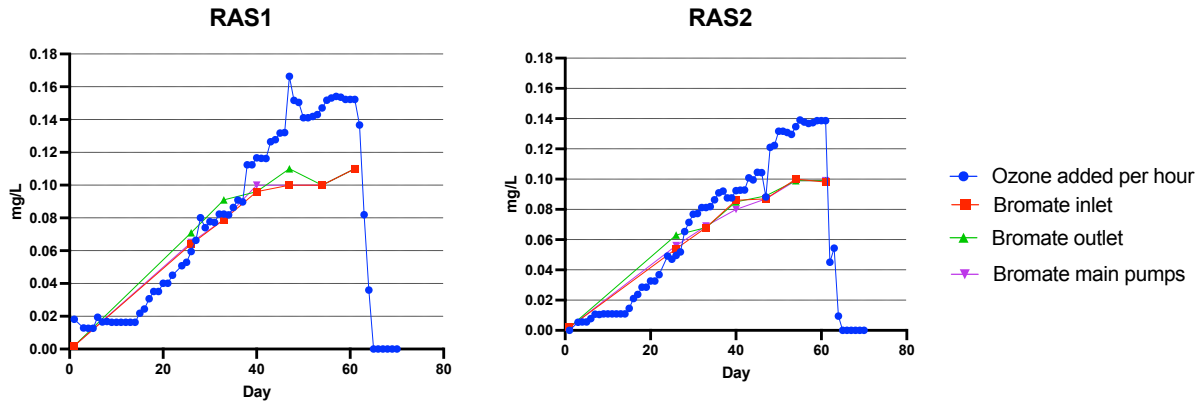


Figure 3.1: Added Ozone and Bromate Concentration at Different System Points. *The graph illustrates the relationship between the quantity of ozone added per hour (mg/L) and the concentration of bromate (mg/L) detected at various points in the system on specific days. The blue line represents the ozone dosage, while the concentration of bromate is traced at the inlet water (red line), the outlet water (green line), and the main pumps (purple line). The values below the detection limit are entered as the detection limit of 0.002mg/L. The temporal distribution of bromate concentrations reflects the variations in ozone treatment across RAS1 and RAS2, G3.*

Figure 3.2 shows relationship between the dosage of ozone applied per hour and the resultant concentrations of bromate in RAS1 and RAS2 for G3. Both systems were subjected to a similar range of ozone dosages over the study period, allowing for a controlled comparison of the resulting bromate formation. Preliminary analyses indicated a dose-dependent increase in bromate concentrations for each system. Notably, the increment in bromate levels per unit of ozone added appeared distinct between the two systems, suggesting system-specific factors that may influence bromate formation.

Linear regression provided a statistically significant model for each RAS, with RAS1 ($p = 0.0024$) demonstrating a correlation where bromate concentration increased by an average of 0.6216 mg/L per mg/L of ozone added, while RAS2 ($p = 0.0015$) exhibited a slightly higher increase of 0.6640 mg/L per mg /L of ozone added. For clarity in the reporting and clarification of data below the detection limit, a conservative estimate of 0.002 mg/L was employed. The subsequent figure (Figure 3.2) provides a graphical representation of these findings, illustrating the differential responses of the two RAS systems to ozonation, highlighting the relationship between ozone dosage and bromate concentration through their respective regression lines and statistical indicators.

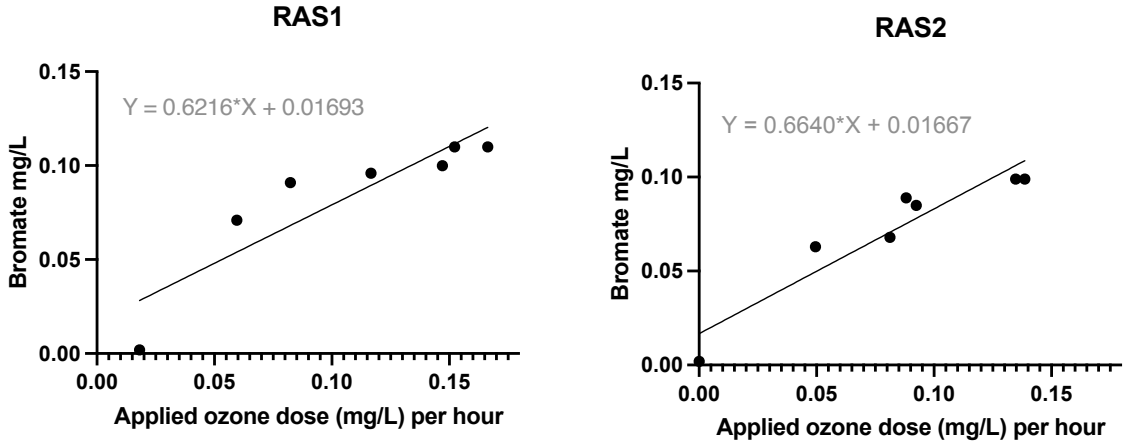


Figure 3.2: Linear Relationship Between Ozone Dosage and Bromate Concentration. *This composite graph delineates the relationship between the applied ozone dose per hour (mg/L) and the measured concentration of bromate (mg/L) at the outlet water for both RAS1 and RAS2, G3. The linear regression lines, represented by solid lines, are described by the equations Y = 0.6216X + 0.01693 for RAS1 and Y = 0.6640X + 0.01667 for RAS2, respectively. Both regression models exhibit statistically significant positive correlations, with p-values of 0.0024 for RAS1 and 0.0015 for RAS2, indicating that the concentration of bromate increases by 0.6216 mg/L and 0.6640 mg/L for each additional mg/L of ozone dosed in RAS1 and RAS2, respectively. Values below the detection limit are treated as the detection limit of 0.002 mg/L for the purposes of this analysis.*

3.1.1 Water exchange

To maintain nitrate levels below 75 mg/L, make-up water was continuously added to the system to dilute the concentration. This addition of new water also results in the dilution of bromate. The average estimated daily water exchange rate is 13% ± 0.057% for RAS1 and 13% ± 0.072% for RAS2 (For estimated daily water exchange rates, see Appendix 7.2 Table 2 and 3). Tables 3.1 – 3.2 display the measured bromate values at the inlet, outlet, and main pump, as well as the estimated actual production of bromate, accounting for dilution. This adjustment is made by taking the measured value and multiplying it by the dilution factor. For example, the measured bromate at the inlet on October 23, 2023, was 0.064 mg/L, with the estimated actual production calculated as 0.064 mg/L × 1.13 = 0.072 mg/L.

Table 3.1: Measured Bromate and Estimated Bromate Production at Various System Points for RAS1. *The table shows the measured value of bromate (mg/L) at the inlet, outlet, and main pumps, as well as the estimated actual production of bromate, accounting for dilution, for RAS1, G3.*

Date	Bromate (mg/L) - inlet	Estimated actual production of bromate (mg/L) - inlet	Bromate (mg/L) - outlet	Estimated actual production of bromate (mg/L) - outlet	Bromate (mg/L) - main pump	Estimated actual production of bromate (mg/L) - main pump
07.09.23	<0,0020	0,002 *(0,002*1,13)*	<0,0020	0,002 *(0,002*1,13)*	<0,0020	0,002 *(0,002*1,13)*
23.10.23	0,064	0,072 *(0,064*1,13)*	0,071	0,080 *(0,071*1,13)*	0,065	0,073 *(0,065*1,13)*
30.10.23	0,079	0,089 *(0,079*1,13)*	0,091	0,103 *(0,091*1,13)*	0,079	0,089 *(0,079*1,13)*
06.11.23	0,096	0,110 *(0,096*1,13)*	0,096	0,108 *(0,096*1,13)*	0,100	0,113 *(0,100*1,13)*
13.11.23	0,100	0,113 *(0,100*1,13)*	0,110	0,124 *(0,110*1,13)*	0,100	0,113 *(0,100*1,13)*
20.11.23	0,100	0,113 *(0,100*1,13)*	0,100	0,113 *(0,100*1,13)*	0,100	0,113 *(0,100*1,13)*
27.11.23	0,110	0,124 *(0,110*1,13)*	0,110	0,124 *(0,110*1,13)*	0,110	0,124 *(0,110*1,13)*

Table 3.2: Measured Bromate and Estimated Bromate Production at Various System Points for RAS2. *The table shows the measured value of bromate (mg/L) at the inlet, outlet, and main pumps, as well as the estimated actual production of bromate, accounting for dilution, for RAS2, G3.*

Date	Bromate (mg/L) - inlet	Estimated actual production of bromate (mg/L) - inlet	Bromate (mg/L) - outlet	Estimated actual production of bromate (mg/L) - outlet	Bromate (mg/L) - main pump	Estimated actual production of bromate (mg/L) - main pump
19.09.23	<0,0020	0,002 *(0,002*1,13)*	<0,0020	0,002 *(0,002*1,13)*	<0,0020	0,002 *(0,002*1,13)*
23.10.23	0,054	0,061 *(0,054*1,13)*	0,063	0,071 *(0,063*1,13)*	0,056	0,063 *(0,056*1,13)*
30.10.23	0,068	0,077 *(0,068*1,13)*	0,068	0,077 *(0,068*1,13)*	0,069	0,078 *(0,069*1,13)*
06.11.23	0,086	0,097 *(0,086*1,13)*	0,085	0,096 *(0,085*1,13)*	0,080	0,090 *(0,080*1,13)*
13.11.23	0,087	0,098 *(0,087*1,13)*	0,089	0,101 *(0,089*1,13)*	0,087	0,098 *(0,087*1,13)*
20.11.23	0,100	0,113 *(0,100*1,13)*	0,099	0,112 *(0,099*1,13)*	0,099	0,112 *(0,099*1,13)*
27.11.23	0,098	0,111 *(0,098*1,13)*	0,099	0,112 *(0,099*1,13)*	0,099	0,112 *(0,099*1,13)*

3.1.2 Bromate Levels at Comparative Facilities

Hardigsmolt AS submitted water samples for analysis to Eurofins to facilitate a comparison with the results obtained at the Osan facility, thus benchmarking against another operational setting. The fish were housed in the facility for a duration of seven weeks (from September 8, 2023, to October 28, 2023). The initial sample was dispatched for testing after 17 days of residence, with the final sample sent five days preceding the fish transfer. During this interval, the fish in the tank belonging to RAS1 exhibited growth from 138 grams to 256 grams, while the fish in the tank belonging to RAS2 exhibited growth from 141 grams to 263 grams. During the sampling period, the rate of make-up water addition was approximately 25 m^3/h for each RAS, with the total volume of water per system estimated at approximately 2500 m^3. Salinity levels exhibited variability throughout the study duration, with a noticeable increase from 15‰ to 23.6‰ in the two weeks preceding the final sample collection. Ozonation was manually adjusted; however, it is estimated to have operated at approximately 550 grams per hour. This rate corresponds to an ozone dosage of 21 grams per kilogram of feed. Table 3.3 displays the outcomes of the bromate assays. Initial measurements indicated a marginally higher bromate level in RAS2 when compared with RAS1; however, this trend reversed by the final collection, with RAS1 exhibiting the highest aggregate bromate concentration. Relative to the findings from the Osan site, the data bore a striking resemblance. Both facilities reported a peak bromate concentration of 0.110 mg/L in the week leading up to the fish dispatch and a minimum value of 0.098 mg/L in the same timeframe. This suggests a highly parallel evolution in bromate profiles.

Table 3.3: Bromate Concentration at Various System Points at Hardingsmolt. *The table shows the measured value of bromate (mg/L) after drumfilter, outlet, and main pumps, for both RAS1 and RAS2 at Hardingsmolt.*

Date	RAS1			RAS2		
	Bromate (mg/L) - After drumfilter	Bromate (mg/L) - Outlet	Bromate (mg/L) - Main pump	Bromate (mg/L) - After drumfilter	Bromate (mg/L) - Outlet	Bromate (mg/L) - Main pump
25.09.2023	0.057	0.051	0.045	0.059	0.060	0.054
23.10.2023	0.100	0.110	0.098	0.100	0.099	0.098

Erko Settefisk AS represents the second comparative aquaculture facility in this study to submit water samples for analysis to Eurofins. Employing an alternative ozonation method, they utilize liquid ozone (LOZ), a fluid manifestation of free radical oxygen operating

analogously to gaseous ozone (O3). This agent is introduced at the water's surface. The fish were maintained at the facility for a total of thirteen weeks (from September 26, 2023, to December 28, 2023). During this interval, the fish exhibited growth from 140 grams to 580 grams. During the sampling period, the rate of make-up water addition was approximately 300-400L/min, with the total volume of water per system estimated at approximately 3000 m^3. Notably, the salinity level was recorded at 15‰, in contrast to the 25‰ observed at the Osan facility. On December 20th, a sample of water from the pump-sump was dispatched for analytical evaluation, revealing a bromate concentration of 0.015 mg/L. This value is over sevenfold lower than those recorded at both Osan and Hardigsmolt, suggesting a reduced propensity for bromate formation in facilities employing LOZ. However, it is imperative to acknowledge that this conclusion is predicated on a singular sample, thereby limiting its comparative validity.

3.2 Turbidity

In water quality management, the recommended target turbidity range is typically set between 2-3 Formazin Nephelometric Units (FNU). Values below 2 FNU may suggest that ozonation could be scaled back, whereas measurements exceeding 3 FNU typically warrant an increase in ozonation. Table 3.4 presents the measured turbidity values for G3 at the Osan facility. The initial sampling was conducted on different dates for RAS1 and RAS2 due to staggered operational start times. For the final three measurements, it was not feasible to collect water from the main pumps. The turbidity was consistently below 2 FNU, except for the inlet and outlet water for RAS2 on November 13, 2023. This generally indicates that the water is very clear, suggesting that the current ozonation level may be slightly higher than necessary.

Table 3.4: Measured Turbidity at Various System Points. *This table enumerates the turbidity values, expressed in Formazin Nephelometric Units (FNU), recorded at various points of RAS1 and RAS2, G3. The data captures the turbidity at the inlet, outlet, and main pump locations, reflecting the effectiveness of particulate matter removal through ozonation. Notably, these readings aid in evaluating the clarity of the water and the potential need for adjustments in ozonation levels. The 'NA' indicates that data were not applicable at those sampling times.*

Date	RAS1			RAS2		
	Turbidity (FNU) inlet	Turbidity (FNU) outlet	Turbidity (FNU) main pump	Turbidity (FNU) inlet	Turbidity (FNU) outlet	Turbidity (FNU) main pump
07.09.23	0.57	0.64	0.57	NA	NA	NA
19.09.23	NA	NA	NA	0.34	0.38	0.36
23.10.23	0.88	0.96	1.1	0.80	0.94	1.1
30.10.23	0.66	1.1	0.55	0.50	0.75	0.48
06.11.23	0.93	1.1	0.99	1.6	1.6	1.5
13.11.23	1.4	1.7	NA	2.3	2.0	NA
20.11.23	0.88	1.1	NA	0.75	1.2	NA
27.11.23	1.2	1.3	NA	1.5	1.5	NA

3.2.1 Turbidity at Comparative Facilities

Hardigsmolt AS submitted water samples for turbidity analysis to Eurofins to facilitate a comparison with the results obtained at the Osan facility. Table 3.5 displays the outcomes of the turbidity assays. The turbidity consistently exceeded 3 FNU, and on two occasions, it was also above 4 FNU (after the drum filter and at the outlet on October 23, 2023). These measurements indicate that the water was relatively turbid, suggesting that there may have been a need for increased ozonation.

Table 3.5: Measured Turbidity at Various System Points at Hardingsmolt. *This table enumerates the turbidity values, expressed in Formazin Nephelometric Units (FNU), recorded at various points of RAS1 and RAS2 at Hardingsmolt. The data captures the turbidity in the water after drumfilter, at the outlet, and main pump locations, reflecting the effectiveness of particulate matter removal through ozonation. Notably, these readings aid in evaluating the clarity of the water and the potential need for adjustments in ozonation levels.*

Date	RAS1			RAS2		
	Turbidity (FNU) after drumfilter	Turbidity (FNU) outlet	Turbidity (FNU) main pump	Turbidity (FNU) after drumfilter	Turbidity (FNU) outlet	Turbidity (FNU) main pump
25.09.2023	3.1	3.2	3.0	3.5	3.1	2.8
23.10.2023	3.8	3.7	3.5	4.4	4.1	3.8

Erko Settefisk AS represents the second comparative aquaculture facility in this study to submit water samples for analysis to Eurofins. Employing an alternative ozonation method, they utilize liquid ozone (LOZ), a fluid manifestation of free radical oxygen operating analogously to gaseous ozone (O_3). This agent is introduced at the water's surface. On

December 20th, a sample of water from the pump-sump was dispatched for analytical evaluation, revealing a turbidity concentration of 2.2 FNU. This indicates a turbidity that lies between the values observed at Osan and at Hardingsmolt. The turbidity is within the threshold limits, suggesting optimal ozonation.

3.2.2 Bromate and Turbidity Levels for Group 1 and Group 2

As previously mentioned, data collection did not proceed as planned due to malfunctions with the ozonation display for both G1 and G2, resulting in the absence of recorded ozone addition data for these groups. Nevertheless, water samples were submitted to Eurofins for the analysis of bromate and turbidity. These results, presented in Tables 3.6 – 3.7, may serve as a comparative basis for G3.

Due to differing initiation dates, a water sample from RAS2 was only submitted on February 20, 2023. Bromate concentrations remained below the detectable threshold (< 0.0020 mg/L) until April 11, 2023. The trend in bromate formation was relatively consistent across both RAS systems. The highest concentration recorded for G1 was 0.096 mg/L in the inlet water of RAS2 at the final measurement; however, RAS1 demonstrated higher values overall.

Measurements of turbidity commenced on April 11, 2023, following a project request to include turbidity in the monitoring parameters. In G1, turbidity predominantly remained within the target range of 2-3 FNU, except for four instances where it was recorded below 2 FNU. The turbidity was highest in the outlet water for both RAS1 and RAS2, which is consistent with expectations because the outlet water often contains fish waste, resulting in increased cloudiness compared to the inlet and main pump.

Table 3.6: Comparative Measurements of Bromate and Turbidity in RAS1 and RAS2, G1. *This table summarizes the concentrations of bromate (mg/L) and the corresponding turbidity levels (FNU) at the inlet, outlet, and main pump stages of RAS1 and RAS2, G1. The 'NA' indicates that data were not applicable at those sampling times. Note that turbidity measurements began on April 11, 2023, upon the request to expand monitoring parameters within the project scope. Initial bromate levels were below the detection threshold of 0.002 mg/L, with a gradual increase observed over time. The highest recorded turbidity levels were*

observed at the outlet points, which aligns with the expected increase in particle concentration post-treatment.

Date	RAS1						RAS2					
	Inlet		Outlet		Main pump		Inlet		Outlet		Main pump	
	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)
20.02.2023	NA	NA	NA	NA	NA	NA	< 0.002	NA	< 0.002	NA	< 0.002	NA
06.03.2023	< 0.002	NA	< 0.002	NA	< 0.002	NA	< 0.002	NA	< 0.002	NA	< 0.002	NA
11.04.2023	0.084	1.5	0.072	1.5	0.078	1.5	0.064	2.1	0.071	2.1	0.071	2.1
17.04.2023	0.074	1.9	0.073	3.3	0.080	2.2	0.076	2.2	0.072	2.5	0.074	2.4
25.04.203	0.088	2.0	0.088	2.3	0.080	2.1	0.096	1.6	0.080	1.7	0.089	1.7

In G2, only RAS1 was monitored since the fish in the RAS2 tanks were present in the facility for to brief a period to warrant tracking. It should be noted that the bromate value recorded on June 28, 2023, as seen in Table 3.7 (indicated by an asterisk), is lower than that of the previous date. This is likely due to the analysis of an outdated sample. These water samples were stored in the Osan laboratory refrigerator and were sent for analysis along with other samples approximately one month after collection. Given that the storage duration for water samples intended for bromate analysis is one month, it is probable that these samples slightly exceeded this time frame before analysis, resulting in a decreased measured value. Therefore, the actual bromate level on June 28, 2023, may have been higher than what is recorded in the table. Discounting this discrepancy, we observe a gradual increase in bromate levels, starting from below the detectable limit and reaching 0.13 mg/L in the main pump during the final measurement — the highest recorded level for bromate across all groups (G1, G2, and G3). Turbidity levels for G2 were generally lower compared to G1 and G3, never exceeding 1.4 FNU, which suggests a higher degree of ozonation for this group. This may also explain the observed highest bromate concentration within this group.

Table 3.7: Comparative Measurements of Bromate and Turbidity in RAS1, G2. *This table details the measurements of bromate concentration (mg/L) and turbidity (FNU) at the inlet, outlet, and main pump stages within RAS1, G2. An asterisk (*) denotes a likely deprecated sample value for bromate on June 28, 2023, potentially affected by extended storage time prior to analysis. The progression of bromate levels and the consistently low turbidity readings throughout the sampling period are indicative of the water treatment efficacy and ozonation intensity.*

Date	RAS1					
	Inlet		Outlet		Main pump	
	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)	Bromate (mg/L)	Turbidity (FNU)
30.05.2023	< 0.002	1.3	< 0.002	1.6	< 0.002	1.3
19.06.2023	0.044	0.57	0.054	0.73	0.048	0.71
28.06.2023*	0.0055	1.1	< 0.002	1.4	< 0.002	1.4
06.07.2023	0.084	1.0	0.086	1.1	0.097	1.1
17.07.2023	0.12	1.1	0.12	1.3	0.13	1.1

3.3 Parameters for Fish Production

There was variation in both the number of fish and in mortality rates among the tanks. Mortality remained below 0.7% for all tanks (table 3.8), indicating good aquaculture conditions and effective management of fish welfare throughout the study period. The highest mortality rate is observed in PS2G2 (0.63%), followed by PS4G1 (0.36%) and PS3G1 (0.33%). The lowest mortality rate is observed in PS4G3 (0.07%), followed by PS1G3 (0.09%) and PS2G3 (0.09%). G3 generally has lower mortality rates compared to G1 and G2.

Table 3.8: Fish Mortality and Tank Density Across Different Groups During the Experimental Period. *The table provides an overview of the number of fish per tank for all groups, as well as the number of fish that died during the experimental period, and the density in the tanks at both the start and the end.*

Tanks and group	Number of fish	Mortality	Mortality rate (%)	Density start kg/m^3	Density end kg/m^3
PS1G1	276 698	635	0.23	19.6	57.3
PS2G1	277 226	666	0.24	20.7	58.7
PS3G1	275 631	923	0.33	18.8	40.7
PS4G1	287 708	1 048	0.36	20.1	49.3
PS1G2	316 228	403	0.13	17.7	40.9
PS2G2	316 305	1 993	0.63	16.7	37.2
PS1G3	250 751	221	0.09	10.7	38.5
PS2G3	250 228	231	0.09	12.4	43.0
PS3G3	249 692	268	0.11	11.2	36.3
PS4G3	250 263	179	0.07	11.3	35.0

3.4 Morphological values

Morphological values within the study were based on the calculation and recording of condition factor (CF), weight and specific growth rate (SGR). In G2, the fish were observed for two months preceding their release into the sea, during which only two welfare assessments could be executed. A human error occurred during the initial welfare assessment, resulting in measurements being taken from fish in a tank that was not part of the experimental design. Consequently, it is not feasible to evaluate the progression of CF or SGR for this group, as the development between the two measurements cannot be accurately tracked due to the discrepancy in fish populations. Therefore, the morphological values results will concentrate on Groups 1 and 3, where consistent and reliable data were obtained.

3.4.1 Condition factor

To explain the physiological state and growth conditions, an analytical assessment of the CF was performed across G1 and G3. Each group consisted of fish distributed across four tanks (PS1, PS2, PS3, and PS4), and the condition factors were calculated at specific time intervals to determine variations within and between these groups.

For G3, an Analysis of Variance (ANOVA) revealed no significant differences in the condition factors across the tanks ($p = 0.302$). This implies that the tanks' environmental conditions or other non-measured factors did not affect the growth conditions of G3.

The ANOVA results for G1 highlighted significance in the CF ($p = 0.0165$), suggesting that the tank conditions may indeed exert an influence on the physiological state of the fish. To elucidate the pairwise differences between tanks, a post-hoc Tukey HSD test was conducted. The Tukey test demonstrated a significant discrepancy in CF between PS4 and PS3 , indicating that PS4 presented a lower CF relative to PS3. This distinct difference points to potential variations in environmental or biological factors that may be affecting growth efficiency or physiological wellbeing differentially within these tanks.

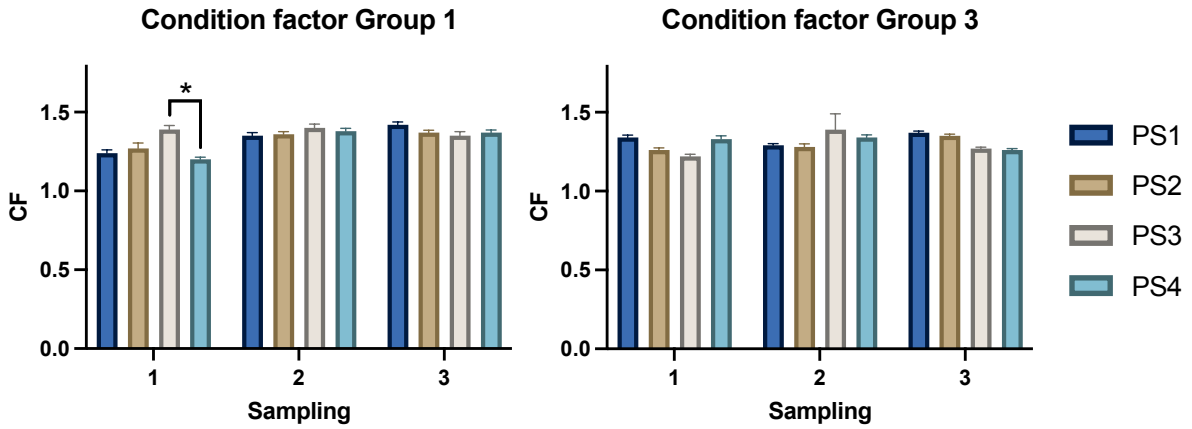


Figure 3.3: Comparison of Condition Factors. *The bar chart delineates the CF for G1 on the left and G3 on the right. Each set of bars represents a tank within the respective groups (PS1, PS2, PS3, and PS4). The error bars represent the standard error of the mean, providing a visual representation of the variability within each tank. Results for G1 highlighted significant disparities in the CF ($p = 0.0162$), an asterisk (*) above the bars between PS3 and PS4 in G1 indicates a significant difference at $p < 0.05$, highlighting different growth conditions between these tanks, while G3 revealed no significant differences in the CF across the tanks ($p = 0.302$). $n = 20$ for each tank in G1, and $n = 50$ for each tank in G3 (with an exception for the third sampling where $n = 60$ for each tank).*

3.4.2 Weight

The differences in growth were measured by changes in weight across all four tanks, for both G1 and G3 (Figure 3.4). Each group was sampled across three different times to assess growth patterns. The analysis of variance (ANOVA) was conducted separately for each group to assess the differences in mean weight across the four tanks.

The ANOVA results for G1 indicated significant differences in the weight among the tanks ($p = 0.0155$). Post-hoc comparisons using Tukey's HSD suggested significant weight reductions in PS3 compared to PS1 ($p = 0.021$), and PS3 compared to PS2 ($p = 0.047$), with PS3 showing consistently lower weights. No other comparisons were statistically significant, suggesting similar growth conditions or genetic influences between the other groups (PS1 vs. PS2, PS4 vs. PS1, PS4 vs. PS2, and PS4 vs. PS3). Similarly, the ANOVA for G3 showed significant differences among tank groups ($p = 0.0132$). However, post-hoc testing revealed a different pattern, with the only significant difference being between PS4 and PS2 ($p = 0.014$),

where PS4 was significantly lighter than PS2. All other group comparisons did not yield statistically significant differences.

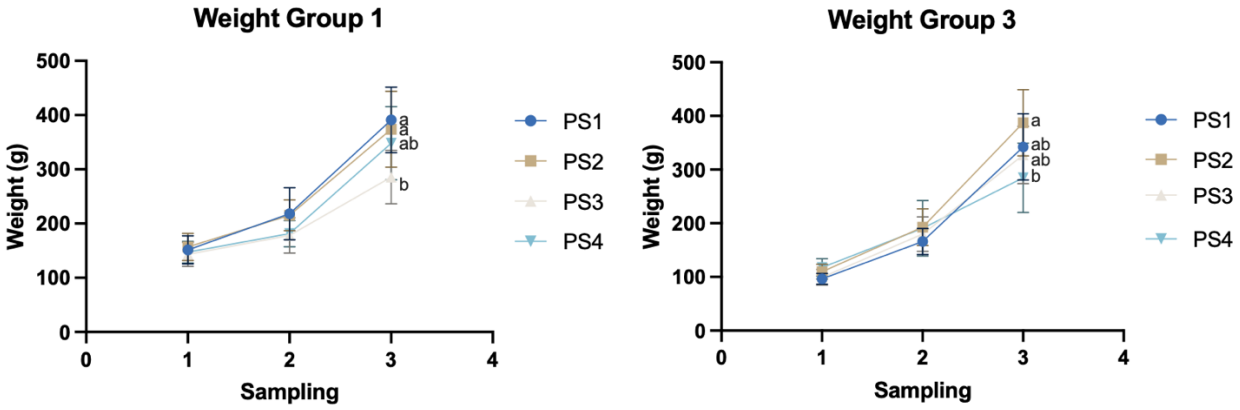


Figure 3.4: Comparative Growth Patterns. *G1 and G3 showing the mean weights (measured in grams) in tanks PS1, PS2, PS3, and PS4. Data points are connected by lines to illustrate the trend over time, highlighting the differences in growth patterns among the tanks within each group. The error bars represent the standard error of the mean, providing a visual representation of the variability within each tank. Different letters indicate significant differences ($p < 0.05$). G1 depicts significant differences in weight, particularly lower weights observed in PS3 compared to PS1 and PS2. G3 Shows a less varied growth pattern, with the only significant difference noted between PS4 and PS2, suggesting different influences or conditions affecting the tank PS4. $n = 20$ for each tank in G1, and $n = 50$ for each tank in G3 (with an exception for the third sampling where $n = 60$ for each tank).*

3.4.3 Specific Growth Rate

SGR was calculated for salmon across four tanks (PS1, PS2, PS3, PS4) within two distinct groups, G1, and G3, during different sampling times. For G1 all tanks had an increase in SGR from T1_T2 to T2_T3. The visual representation of these rates can be seen in Figure 3.5.

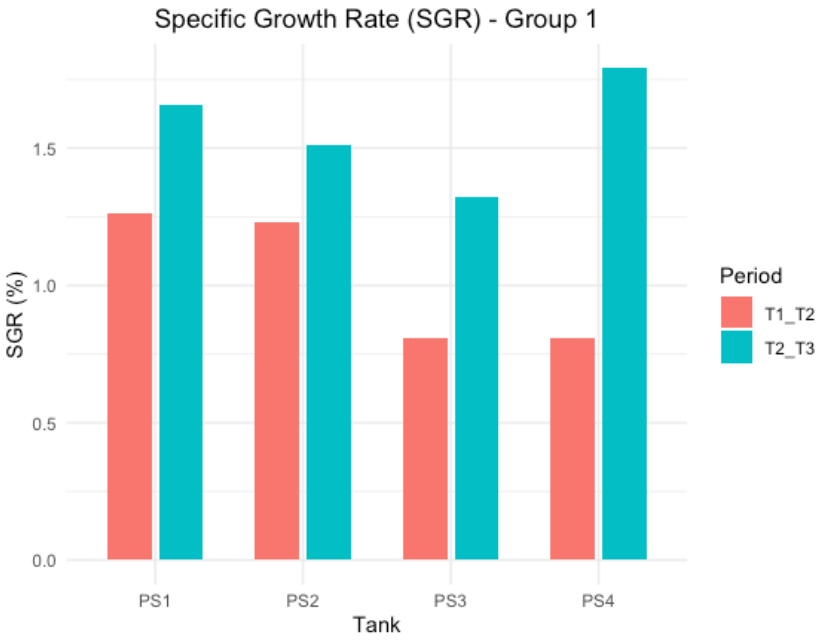


Figure 3.5: Specific Growth Rate for Group 1. *The graph illustrates the SGR between sampling 1 and 2 (T1_T2), and sampling 2 and 3 (T2_T3) for each tank (PS1, PS2, PS3, PS4) in G1.*

SGR for G3 showed an increase for PS1 and PS2, while PS3 and PS4 showed a decrease between T1_T2 and T2_T3 (figure 3.6). The SGR for G3 were generally higher than the SGR for G1.

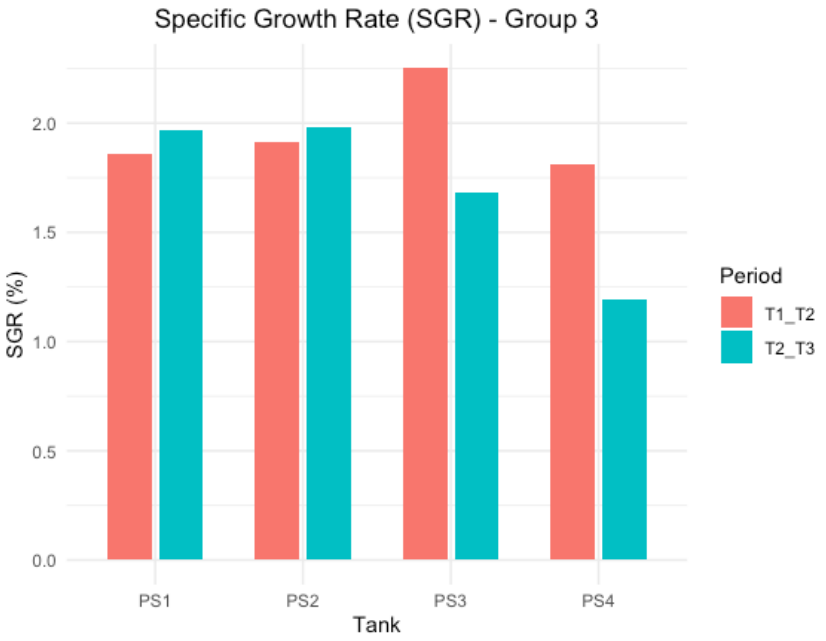


Figure 3.6: Specific Growth Rate for Group 3. *The graph illustrates the SGR between sampling 1 and 2 (T1_T2), and sampling 2 and 3 3 (T2_T3) for each tank (PS1, PS2, PS3, PS4) in G3.*

3.5 Fish welfare

Figures 3.7 to 3.11 present fish welfare data in donut charts, with each donut representing measurements from the fish in the respective tank. Fish welfare is presented as the average percentage of fish in each tank that received a welfare score from 0 to 3 (see chapter '2.3.2 Welfare tests' for details on the number of fish measured per tank). The operculum scores range from 0 to 6 because each operculum was measured separately and the scores were summed together. The figures are categorized into Group 1, Group 2, and Group 3, with each row indicating the month during which the scoring was conducted.

3.5.1 Active damage on dorsal fin

The distribution of active damage on the dorsal fin shows that the fish in the tanks mostly had scores of 0 or 1. Overall, 43.4% scored 0, indicating that there was no damage, while 44.5% had a score of 1. In G1, no fish received a score of 3, nevertheless there were some instances of this in both G2 and G3. In G3, it is observed that the proportion of fish with no damage on the dorsal fin gradually decreased throughout the year, and more fish acquired active damage on the dorsal fin.

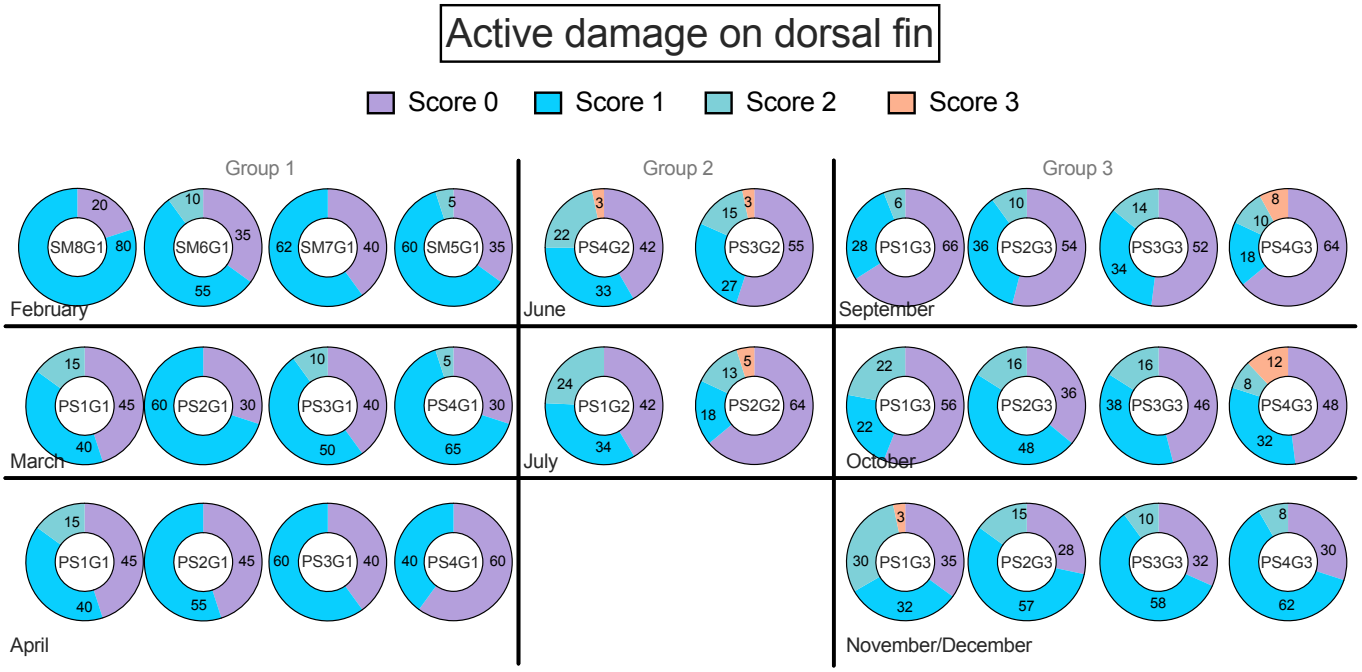


Figure 3.7: Percentage distribution of active damage on dorsal fin*. Each chart corresponds to a specific tank, with scores ranging from 0, indicating no damage, to 3, signaling severe*

damage. Each row corresponds to a monthly scoring. The fish's movement from smolt (SM) to post-smolt (PS) tanks reflected vertically, connecting origins and destinations (e.g., SM8 to PS1). G1 shows no instances of score 3, in contrast to G2 and G3. The trend in G3 highlights a decrease in fish with undamaged dorsal fins over time and an increase in active dorsal fin damage.

3.5.2. Healed damage on dorsal fin

In total, 72% of all the fish were awarded a score of 0, indicating no damage. The average percentage distribution was also quite consistent across the different groups; approximately 76% in G1 scored 0, around 63% in G2, and about 71% in G3. G1 generally exhibited healthier dorsal fins compared to G2 and G3. Only 1.7% of G1 received a score of 2, and none were scored at 3. In contrast, a higher percentage of fish in G2 and G3 were given scores of 2 and 3. This pattern was mirrored in the observations of active dorsal fin damage as well.

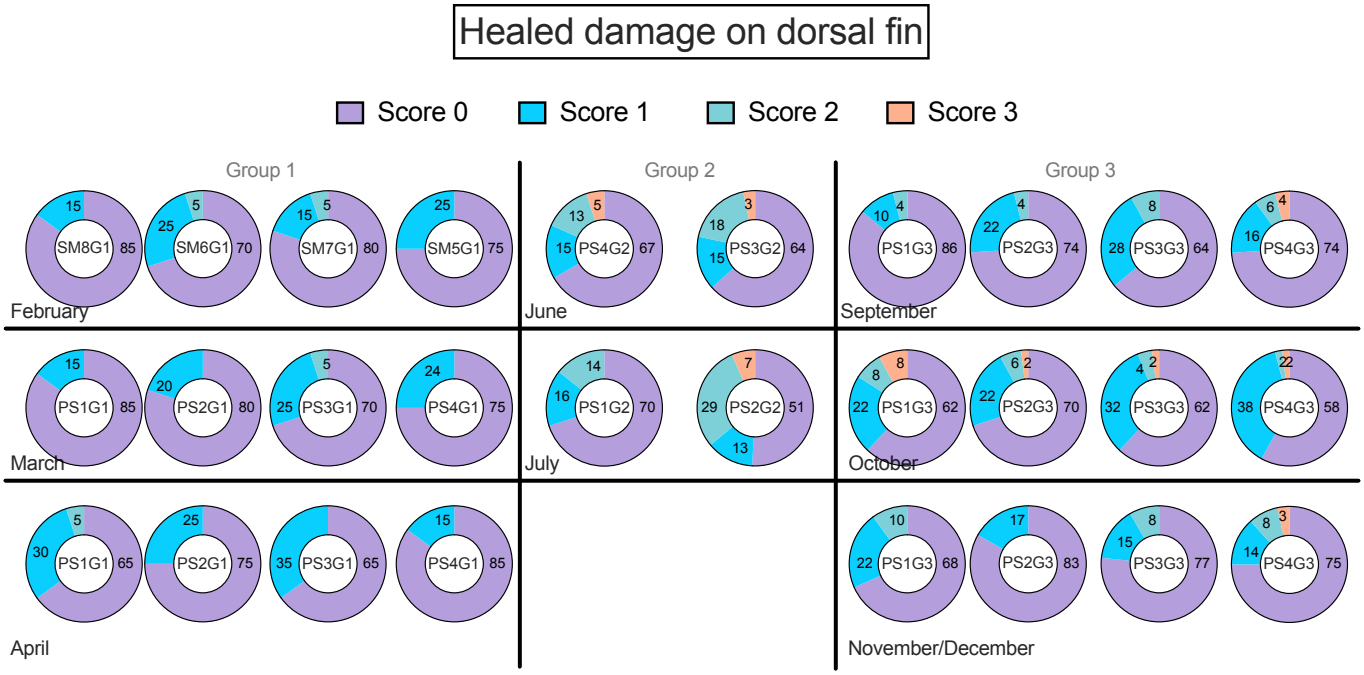


***Figure 3.8: Percentage distribution of healed damage on dorsal fin**. Each chart corresponds to a specific tank, with scores ranging from 0, indicating no damage, to 3, signaling severe damage. Each row corresponds to a monthly scoring. The fish's movement from smolt (SM) to post-smolt (PS) tanks reflected vertically, connecting origins and destinations (e.g., SM8 to PS1). G1 displayed the healthiest dorsal fins with a mere 1.7% scoring a 2 and no instances of score 3. Conversely, G2 and G3 exhibited more instances of scores 2 and 3, indicating more noteworthy damage.*

3.5.3 Pectoral fin

For G1, the average percentage distribution for pectoral fin condition shows the highest occurrence of score 0 in all tanks (> 65%), whereas less than 40% of the fish in G3 achieved a score of 0. The initial measurement for PS4G3 in September is notable, with 40% of the fish receiving a score of 3, indicative of severe pectoral fin damage. However, such results were not observed in the same tank in the subsequent monthly measurements.

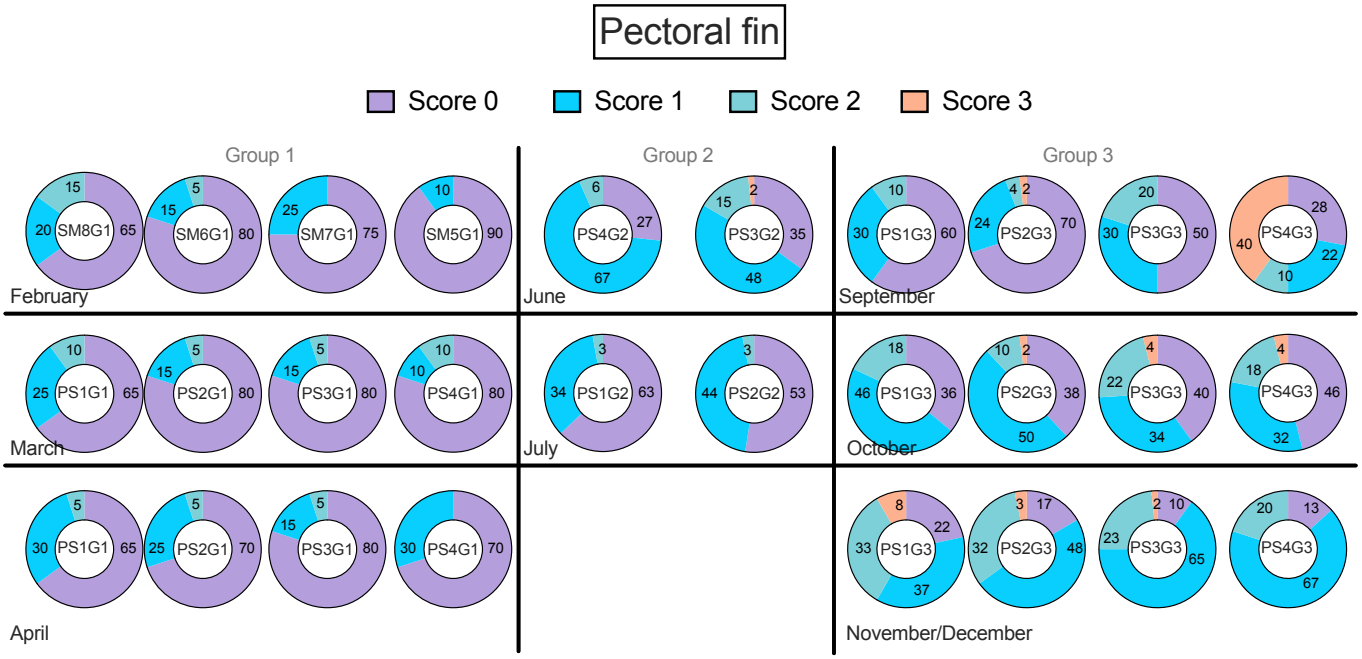


Figure 3.9: Percentage distribution of pectoral fin*. Each chart corresponds to a specific tank, with scores ranging from 0, indicating no damage, to 3, signaling severe damage. Each row corresponds to a monthly scoring. The fish's movement from smolt (SM) to post-smolt (PS) tanks reflected vertically, connecting origins and destinations (e.g., SM8 to PS1). G1 consistently exhibits a predominance of score 0, with over 60% of fish in each tank showing no signs of harm. In stark contrast, fewer than 40% of fish in G3 achieve a score of 0.*

3.5.4 Operculum

Opercula were scored from 0 to 6, accounting for each side scored separately and then summed. Among a total of fish, only one (from PS1G2) received a score of 6. Scores of 5 were also rare, with only 2 instances, both in G3. The average percentage distribution of operculum scores reveals that 'score 0' was predominant across all fish groups, with over 50% achieving this score except for PS1G3 and PS2G3 in September and PS3G3 in October and

November/December. G1 stood out, having a maximum observed score of '2'. Generally, the fish in G3 displayed somewhat poorer operculum conditions compared to those in G1 and G2.

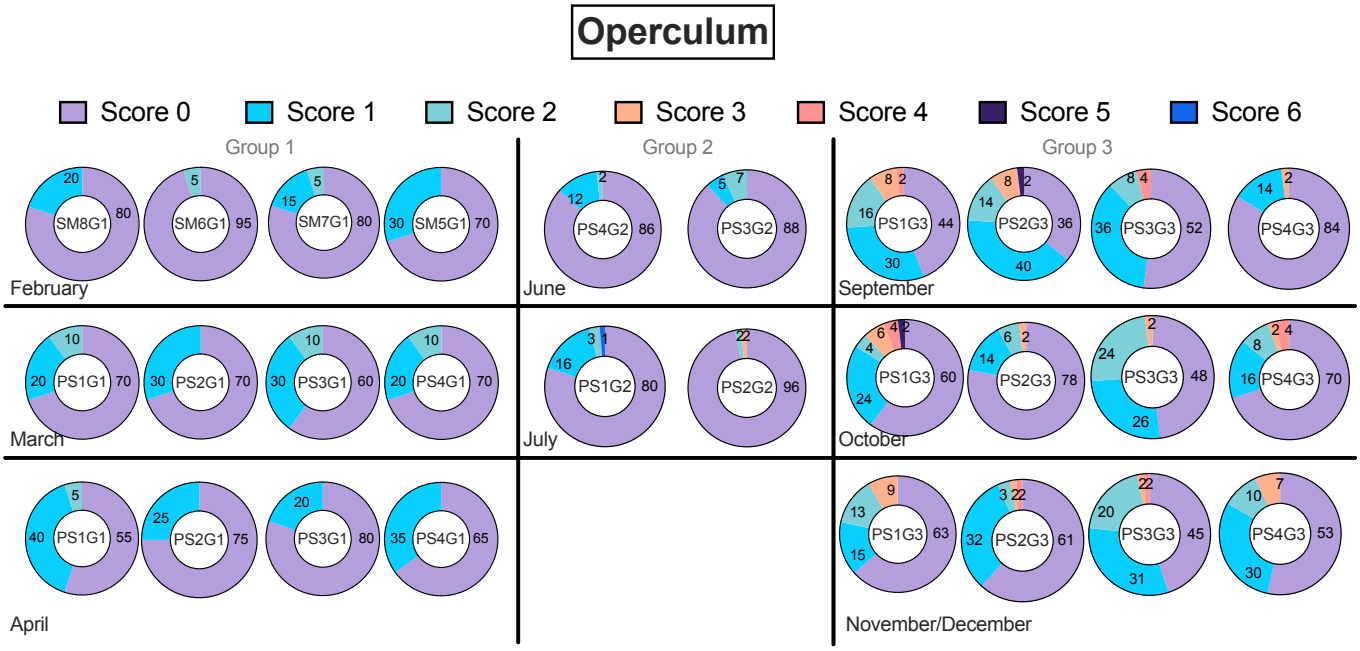


Figure 3.10: Percentage distribution of operculum. *Each chart corresponds to a specific tank, with scores ranging from 0, indicating no damage, to 6, signaling severe damage. Each row corresponds to a monthly scoring. The fish's movement from smolt (SM) to post-smolt (PS) tanks reflected vertically, connecting origins and destinations (e.g., SM8 to PS1). Most of the fish tanks consistently exhibited a prevalence of score 0, representing over 50% of fish in most cases. G1 distinguishes itself with none of its members exceeding a score of 2, suggesting healthier opercula relative to G2 and G3.*

3.5.5 Loss of scale

The average percentage distribution of scale loss indicates varying degrees among the fish. The greatest variation was observed in G2, where the values was distributed as follows: score 0 at 10.4%, score 1 at 34.3%, score 2 at 39.8%, and score 3 at 15.5%. It is evident that scale loss in G3 increased over the period.

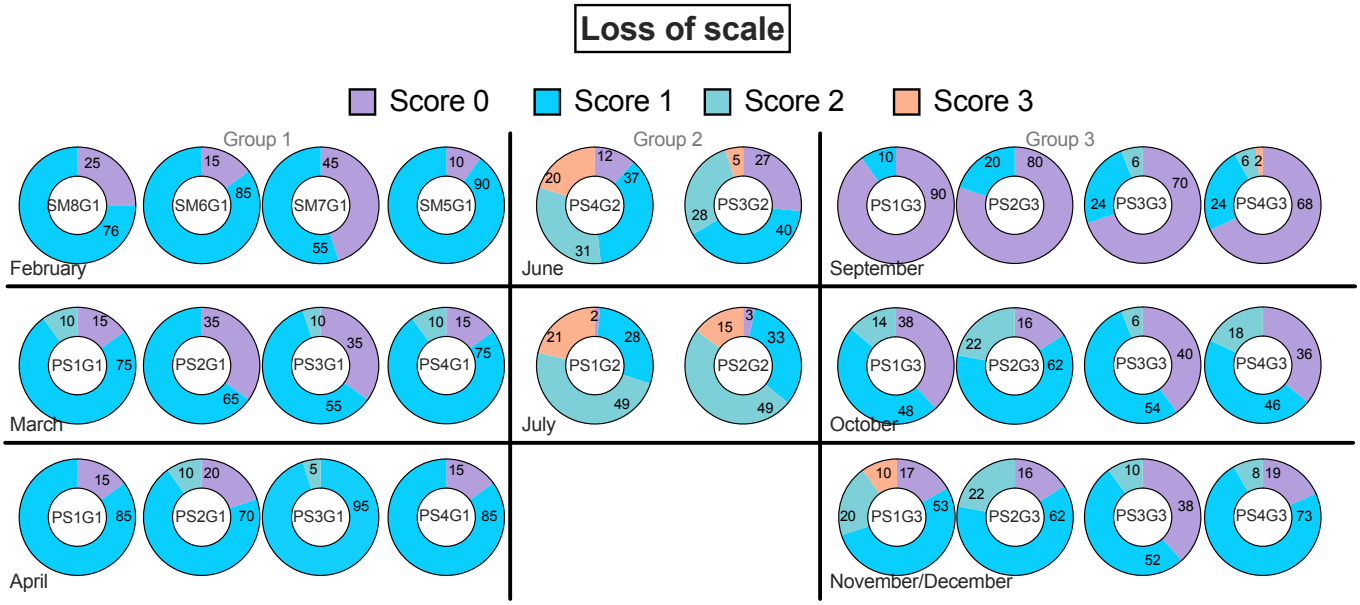


Figure 3.11: Percentage distribution of loss of scale. *Each chart corresponds to a specific tank, with scores ranging from 0, indicating no damage, to 3, signaling severe damage. Each row corresponds to a monthly scoring. The fish's movement from smolt (SM) to post-smolt (PS) tanks reflected vertically, connecting origins and destinations (e.g., SM8 to PS1). G2 exhibits the widest range of scale loss. G3 demonstrates a clear increase in scale loss as the period progresses.*

3.5.6 Wounds, Caudal fin, Skeletal deformities, Slime layer, Pelvis fin, Cataract

Generally, there were negligible findings (score 0) concerning factors such as wounds, caudal fin condition, skeletal deformities, slime layer, pelvic fin, and cataract. Consequently, these results are consolidated into a table format (table 3.9). In the table, the data from all tanks have been combined for each assessment period. The results are presented in percent (%).

Table 3.9: Welfare Assessment Scores for Various Health Indicators Across Groups and Months. *Summary of welfare assessment scores for wounds, caudal fin condition, skeletal deformity, slime layer, pelvic fin, and cataract across Groups 1, 2, and 3 over various months. The scores range from 0, indicating no damage, to 3, signaling severe damage. Most observations for each factor resulted in a score of 0, signifying the absence of issues.*

		Wounds	Caudal fin	Skeletal deformity	Slime layer	Pelvic fin	Cataract
G1	*February*	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 100	Score 0 ≈ 100	Score 0 ≈ 100	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 83 Score 1 ≈ 17
	March	Score 0 ≈ 91 Score 1 ≈ 9	Score 0 ≈ 97 Score 1 ≈ 3	Score 0 ≈ 100	Score 0 ≈ 100	Score 0 ≈ 93 Score 1 ≈ 6 Score 2 ≈ 1	Score 0 ≈ 89 Score 1 ≈ 8 Score 2 ≈ 1 Score 3 ≈ 2
	April	Score 0 ≈ 83 Score 1 ≈ 17	Score 0 ≈ 96 Score 1 ≈ 4	Score 0 ≈ 100	Score 0 ≈ 100	Score 0 ≈ 73 Score 1 ≈ 21 Score 2 ≈ 6	Score 0 ≈ 80 Score 1 ≈ 16 Score 2 ≈ 0 Score 3 ≈ 4
G2	*June*	Score 0 ≈ 94 Score 1 ≈ 5 Score 2 ≈ 1	Score 0 ≈ 85 Score 1 ≈ 15	Score 0 ≈ 100	Score 0 ≈ 100	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 80 Score 1 ≈ 20
	July	Score 0 ≈ 77 Score 1 ≈ 22 Score 2 ≈ 1	Score 0 ≈ 87 Score 1 ≈ 13	Score 0 ≈ 100	Score 0 ≈ 99 Score 1 ≈ 0 Score 2 ≈ 1	Score 0 ≈ 100	Score 0 ≈ 38 Score 1 ≈ 34 Score 2 ≈ 26 Score 3 ≈ 2
G3	*September*	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 86 Score 1 ≈ 10 Score 2 ≈ 3 Score 3 ≈ 1	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 100	Score 0 ≈ 97 Score 1 ≈ 2 Score 2 ≈ 0 Score 3 ≈ 1	Score 0 ≈ 93 Score 1 ≈ 6 Score 2 ≈ 1
	October	Score 0 ≈ 93 Score 1 ≈ 6 Score 2 ≈ 1	Score 0 ≈ 98 Score 1 ≈ 2	Score 0 ≈ 99 Score 1 ≈ 0 Score 2 ≈ 0 Score 3 ≈ 1	Score 0 ≈ 91 Score 1 ≈ 9	Score 0 ≈ 95 Score 1 ≈ 2 Score 2 ≈ 1 Score 3 ≈ 2	Score 0 ≈ 68 Score 1 ≈ 23 Score 2 ≈ 9
	November/December	Score 0 ≈ 91 Score 1 ≈ 7 Score 2 ≈ 1 Score 3 ≈ 1	Score 0 ≈ 93 Score 1 ≈ 7	Score 0 ≈ 100	Score 0 ≈ 99 Score 1 ≈ 1	Score 0 ≈ 93 Score 1 ≈ 3 Score 2 ≈ 3 Score 3 ≈ 1	Score 0 ≈ 86 Score 1 ≈ 13 Score 2 ≈ 1

4. Discussion

4.1 Discussion of Methods

This study was conducted in a fullscale RAS post-smolt facility. Osan Settefisk AS operates conventional production of smolt, emphasizing high quality and optimal conditions for the fish. This study aimed to observe the effects of ozonation and its potential to lead to bromate formation in the water. Ideally, the experimental setup would have included a control group without ozone treatment for a direct comparison of water quality. However, due to the critical role of ozonation in maintaining water quality and fish health, it was not possible for Osan to permit a setup where half of the production did not use ozone as a water treatment. Consequently, this study followed three different groups of fish (G1, G2, and G3), each consisting of several tanks. Groups G1 and G3 additionally used two separate RAS systems (RAS1 and RAS2), with conditions being approximately the same for both systems. This methodological limitation means that the study could not directly compare with an untreated control group.

Water quality measurements and fish welfare assessments began in February 2023. At this time, not all components were in place at the post-smolt facility yet. It is reasonable to state that the experiments started before the facility was fully prepared, and before all experimental conditions were established. This resulted in the ozonation information screen not being operative for G1. Despite extraordinary pressure on the equipment suppliers, they were unable to operationalize the system for G2, even though this was promised. Fortunately, the issue was resolved before G3, the final group, was monitored. This lack of information and the complexity of the technical solutions significantly impacted the study's execution.

Additionally, the ozonation injection point was changed before the final group. This change was necessary due to difficulty achieving uniform ozonation, and occasions of ozone presence in the air, which required temporary pause of ozonation for health and safety reasons for the employees at the facility. After changing the injection point from before the biofilter to the pump-sump, and shortening the pipe where the injection occurred, a stable and safe ozonation process was achieved without any instances of ozone in the air. This adjustment was made to minimize the residence time in the pipe, as my supervisor and I hypothesized that the lengthy

pipe section allowed the ozone more time to react with the bromide in the seawater, potentially leading to increased bromate formation.

The parameters TAN, nitrite, nitrate, alkalinity, temperature, pH, and CO2 were continuously measured in this study. However, it is important to acknowledge that these measurements were part of a conventional production process, where continuous adjustments were made to optimize conditions. For instance, if nitrate levels approached predetermined threshold values, the amount of water added was adjusted to dilute the nitrate concentration, resulting in temporarily reduced measurement values. Similarly, the temperature can be gradually adjusted towards the end of the production period to prepare the fish for the transition to seawater temperatures. These adjustments implied that the measured values of environmental parameters were the results of targeted modifications to maintain optimal growth conditions. This practice could potentially complicate the interpretation of the data, as it becomes challenging to distinguish the effects of "natural" environmental variations from those induced by human interventions.

In this study, the number of fish and the size of the individuals varied between groups and within the different tanks, resulting in different densities. Although there are no legal limits for density in post-smolt facilities, Ytrestøyl *et al.* (2023) recommend an upper limit of 75 kg/m^3. In our study, the highest observed densities in groups G1, G2, and G3 were respectively 58.7 kg/m^3, 40.9 kg/m^3, and 40.0 kg/m^3. These values are well below the recommended maximum, indicating that the fish had relatively plenty space. However, standardizing the quantity and size of fish within industrial frameworks can be challenging, potentially introducing variation in experimental conditions, and affecting fish welfare.

During the study, the number of fish that were weighed, measured, and assessed for various welfare indicators varied between groups. The main reason for this variation was that I personally led the planning and implementation of the project with support from the employees at Osan Settefisk and my supervisors. Initially, I based the number of fish on previous experiences where 20 fish per tank were considered appropriate. After completing the first group and presenting initial results to my advisor, it was recommended to increase the number to 50 fish per tank to better represent the welfare status within the tanks.

PS1 and PS2 were part of RAS system 1 (RAS1), while PS3 and PS4 belonged to RAS system 2 (RAS2). In the statistical analyses of CF and weight, significant differences between the tanks (PS1-PS4) within each of the groups (G1 and G3) were controlled. It would have been of interest to also perform comparisons between the groups, however, this would have limited value, since the fish material and rearing conditions differed between the groups. The fish had been graded as fry and were distributed according to size when being transferred to the smolt department; hence, the four PS tanks could not be considered as replicates preventing comparison between G1 and G3. It was also observed that there were size differences between tanks, which led to presenting the results as four separate tanks. The industrial settings thus limit the possibilities for traditional scientific analyses. Therefore, statistical analyses were performed within groups among the tanks, and observations and discussions about group differences were based more on qualitative comparison than on statistical significance.

4.2 Discussion of Results

4.2.1 Ozone added and concentration of bromate

The results of this study confirmed a statistically significant positive correlation between the dosing of ozone and the following formation of bromate (BrO_3^-) in both RAS1 and RAS2 for G3, as indicated by the regression models (figure 3.2) with p-values of 0.0024 and 0.0015. This suggests a dose-dependent increase in bromate concentrations with increased ozone application. A study by Jung *et al.* (2014) injected ozone (dose rate of 1 mg/L per minute) into a 500 mL reactor and collected samples at a predetermined time to measure BrO_3^-. They also found a linear formation, with BrO_3^- increasing to 20 µg/L at an ozone dose of 10 mg/L per minute. If we convert this to mg/L bromate and ozone measured in mg/L per hour, we get 0.12 mg/L bromate per mg/L ozone added per hour. This result is much lower than the regression slopes presented in my study (0.6216 and 0.6640 mg/L bromate per mg/L of ozone added per hour). My data showed much higher sensitivity to changes in ozone levels compared to the study in the article. One difference in experimental setup is that the study had a salinity of 31.1‰, while the salinity in my study were 25‰. Other factors that may have affected the results could be measurement sensitivity, concentration of bromide in the saltwater, system size, the fact that they did not have fish in the system, water turnover rate or specific water chemistry.

The dynamics of bromate formation are also linked to the feeding practices. As feeding rates increase, the demand for ozone rises due to higher organic loads needing more intensive treatment. This study's findings suggest that increased ozonation, necessitated by higher feeding rates, directly correlates with higher bromate formation. The dilution effects due to water exchange played a critical role in modulating these concentrations. The continuous addition of make-up water at an average rate of 13% daily (table 3.1) helped maintain nitrate levels below 75 mg/L while also diluted bromate concentrations, complicating the straightforward assessment of ozone's impact on bromate levels. The calculated actual production of bromate (table 3.1), adjusted for dilution (using a factor of 1.13), provides a more accurate reflection of the system's internal dynamics than raw concentration measurements alone.

4.2.2 Condition factor, weight, and SGR

All tanks for both G1 and G3 showed an increase in the CF from Sampling 1 to 2. The first measurement was performed immediately after the fish were transferred to saltwater and had completed smoltification, meaning they were finished with seawater adaptation and could focus on growth (Handeland *et al.*, 2003). CF decreases during smoltification, is around 1 for smolts and increases with fish size in the saltwater (Noble *et al.*, 2018). Therefore, it is expected that the CF would increase after the initial adaptation period to saltwater. Following this initial increase, we observed that the CF stabilized, indicating that the fish had reached a stable state of health and growth under the existing aquaculture conditions.

The presence of significant difference in CF within G1 and their absence in G3 offers an interesting contrast, suggesting that while some tanks may provide a similar growth environment for the cohorts, others may diverge considerably. The factors contributing to this variation could range from feeding regimes, stock density, water quality parameters, or other unmeasured environmental conditions that require further investigation. Notably, the uniformity in G3 may indicate optimized conditions that consistently support a stable physiological state across the tanks. Which may also explain why G3 showed a lower mortality than G1.

The weight of all fish steadily increased from start to finish. The results showed significant differences in growth patterns among tanks within both G1 and G3 (figure 3.4). In G1, PS3

consistently showed lower weights compared to PS1 and PS2. There can be several reasons for this, such as differences in tank environment or social dynamics among the fish. Notably, no significant differences were found between the other tanks in G1, indicating that except for PS3, the environmental and genetic conditions across these tanks were likely similar. Likewise, G3 demonstrated a significant weight difference between PS4 and PS2, while no significant differences were found between the other tanks.

SGR observed in this study varied between the two groups studied. SGR gives an indication of increased daily growth. G1 showed a noteworthy increase in SGR from T1_T2 to T2_T3 (figure 3.5), suggesting a possible adaption to saltwater conditions, that might have allowed the fish to focus more on growth. G3 exhibited generally higher SGR than G1, this indicates that the fish in G3 grew faster than those in G1. Although there were no direct measurements of ozone levels for G1, certain indications suggest that ozonation was higher for G3. This is partly because ozonation had to be stopped on several occasions in G1 due to ozone in the air, as previously mentioned. The study conducted by Davidson *et al.* (2021), which suggests that Atlantic salmon post-smolt grow faster in ozonated RAS, provides useful context, even though direct comparisons are challenging. Their study compared ozonated water in freshwater systems with non-ozonated water, whereas in my experiment, both groups operated under ozonated conditions in saltwater.

Nevertheless, the fact that G1 is assumed to have had lower levels of ozonation and shows lower growth rates (SGR) supports the theory that higher ozonation can promote growth. This is further supported by measurements of turbidity; G1 had higher turbidity compared to G3, which may indicate lower ozonation and thus more particles in the water. These observations suggest that G3, with lower turbidity and presumed higher ozonation, could have better water quality that supports faster growth. Clearer water with decreased turbidity is said to enhance visibility and feeding efficiency, potentially leading to increased growth (Sigler *et al.*, 1984).

Bromate levels were somewhat higher for G3, which was expected given the higher degree of ozonation. Although the difference was not major, it was noticeable that G3 achieved higher bromate values earlier in the study period and maintained these values for a longer duration. Interestingly, there is no indication that the bromate levels had a negative impact on growth, as G3, despite higher bromate values, exhibited a higher SGR compared to G1. This may suggest that the bromate levels reached under these ozonation conditions do not exceed a

threshold harmful to growth. Further analysis would be necessary to explore the limits of bromates impact on growth in more detail, possibly by comparing with existing literature on the effect of bromate on aquatic organisms. A study by Hutchinson *et al.* (1997) looked at the effects of bromate on four species of marine phytoplankton, using cell division (growth) of the cultures as the test endpoint. In the case of the species *Glenodinium halli* and *Thalassiosira pseudonana*, cell growth was increased when exposed to 13.6 mg of BrO_3^- per liter, compared to control cultures (duration of exposure was not specified). This suggests that within this concentration range, bromate did not inhibit growth, instead, it enhanced it, which can be compared to the SGR-findings in this study.

Another possible reason for G3 having a higher SGR-value than G1 could possibly be because G3 started at a higher temperature. Water temperature influences the growth of Atlantic salmon as a crucial environmental factor (Volkoff and Rønnestad, 2020). A study by Handeland *et al*. (2008) using SGR ± Standard Error (SE) as a measure of growth indicated that the ideal temperature for growth for Atlantic Salmon post-smolt (170-300 grams) is approximately 14°C. In my study, G1 started at a water temperature of just under 10°C (figure 2.2), while G3 started at slightly above 12°C (figure 2.4). This difference places G3 closer to the optimal temperature of 14°C identified by Handeland *et al*. (2008), potentially predisposing G3 to better growth conditions. The temperature for G3 was adjusted to approximately 7°C to meet the actual sea temperatures upon transfer to the sea. This adjustment may explain why SGR for tanks PS3 and PS4 in G3 decreases from T1_T2 to T2_T3. By comparison, a similar trend would be expected in G1, however this is not the case. In G1, a notable increase in SGR is observed, even though the temperature here was also adjusted down from about 12°C to approximately 8.5°C. All groups had the same conditions regarding oxygen, salinity, and photoperiod, indicating that factors other than the controlled environmental variables play a role. Other physiological factors may also have contributed to the observed differences in SGR between the groups.

4.2.3 Fish welfare

Welfare scores revealed some differences between the groups throughout the experimental period. The results from the welfare tests were distributed as follows: G3 scored the worst on active damage on the dorsal fin and pectoral fin, G2 had the highest scores for caudal fin and

healed damage on the dorsal fin, while G1 had the highest scores for the pelvic fin, this result shows even variations within fin status among the groups. Generally, the fish in G3 showed a slightly worse condition on the operculum compared to those in G1 and G2. Scale loss was the welfare indicator that generally received the highest scores across all groups. Among the groups, it was G2 that had the highest scale loss, with considerable variation between the different scores: score 0 at 10.4%, score 1 at 34.3%, score 2 at 39.8%, and score 3 at 15.5%. The scale loss was consistent at the fish in G2, with an area on one side lacking scales, thus suggesting that something had rubbed against the fish. Therefore, the fish in the smolt department were also checked to see if the scale loss possibly occurred during transport to the post-smolt facility. The same trend of scale loss was also observed in the smolt department, indicating that it had occurred earlier, possibly while handling the fish during vaccination or similar procedures. The result was better in G3, so perhaps the potential issue was corrected, or it could also have been that the fish group itself in G2 was weaker and more vulnerable to scale loss. There were only 2 total cases of skeletal deformity (both cases a score 1), and both these fish belonged to G3, likewise, there were few cases where the fish had poor slime layers, these cases also concerned G3. Otherwise, G2 had the highest scores for wounds and cataracts, nonetheless, there were not much of this either.

G1 generally had better welfare, according to the results presented in Figures 3.7 to 3.11. Two important factors must be considered here: First, only 20 fish were scored in G1, which provides a narrower insight into the conditions compared with G2 and G3, where 50 and sometimes 60 fish were scored, respectively. Second, it appears that my assessment has become stricter over time. This is observed in that G1 consistently scores better, while G2 and G3 often have higher and more varied scores, with G3 showing higher total scores. This may indicate that I have become more observant to small deviations as I have scored more fish. Such experience and potential bias in scoring are important to consider when reading the results, as they can affect the reliability of the welfare results.

In a study conducted by Lazado *et al.* (2022) post-smolt fish from the Salmobreed strain (N = 200,000, average weight 233 grams) were assessed for welfare. They scored the welfare of 30 fish, which is comparable to my study's sample sizes. The fish weight in Lazado's study aligns best with my second measurements, where average weights were 200 grams for G1, 210 grams for G2, and 180 grams for G3. Lazado *et al.* reported a fish tank of 850 m^3 with a density of 49 kg/m^3. At Osan Settefisk, the tanks are 2250 m^3, housing 250,000 to 320,000

fish, with densities at the end of the study being 51.5 kg/m³ for G1, 39 kg/m³ for G2, and 38.2 kg/m³ for G3. Therefore, the densities at my second welfare measurement were likely lower than in Lazado's study.

Key welfare indicators showed notable differences. Caudal fin condition was remarkable better in my study (94% undamaged vs. 14% in Lazado's). For dorsal fins, Lazado *et al.* reported 16% undamaged, 80% with minor damage, and 4% with moderate damage, whereas my study found 43.7%, 40.7%, 12.5%, and 1.7% for scores 0 to 3, respectively. Pectoral fin conditions were 0% undamaged in Lazado's study, while my study reported 58.1% undamaged. Pelvic fins showed 40% undamaged in Lazado's study compared to 96% in mine. This difference in fin condition might be attributed to the larger tank sizes and lower densities in my study, which possibly reduce stress and physical interactions that can lead to damage. Overall, both studies indicate good fish welfare with minimal damage or stress, as most indicators scored 0 or 1.

CF (Figure 3.3) is relatively similar for both G1 and G3, however, G3 showed a somewhat higher CF than G1. This suggests that the welfare indicators have not had a major impact on the CF, since G1 generally had somewhat better results on the welfare scores than G3. Nevertheless, it is G3 that had the best CF. The same result also applied to mortality, weight and SGR, where G3 had the lowest mortality, grue faster and had a generally higher SGR than G1, even though G1 had better fish welfare according to the welfare measurements that were carried out.

However, looking at the measurements made for ozone and bromate in the water against the welfare tests, it seems that increased ozonation and extended bromate levels lead to poorer fish welfare. Although the difference was not huge, it was noticeable that G3 achieved higher bromate values earlier in the study period and maintained these values for a longer duration. This may have impacted the welfare, as it appears that the trend was that the welfare of the fish in G3 gradually worsened from measurement to measurement, while the welfare in G1 remained stable. However, it's important to remember that the welfare of all three groups followed in this study was good (scores of 0-1 for most indicators), and that the bromate level was far below the specified limit, which ideally should remain under 3 mg BrO_3^- per liter (limit set using a factor of 10 to protect against acute and potential chronic toxicity) for juvenile fish and fish larvae (Hutchinson *et al.,* 1997). Nevertheless, more research is needed

on this topic to see if bromate can have a direct impact on the fish and fish welfare. Particularly, it would be interesting to see if bromate in the water the fish live in could lead to bromate in the fish itself, especially considering that bromate is classified as a Group 2B carcinogen by the International Agency for Research on Cancer (Dong *et al.*, 2019). I contacted several laboratories and fish health personnel to inquire if they could conduct sampling on the fish to see if bromate accumulates in the fish. None of those I contacted had developed methods to conduct such analyses. Therefore, it is very necessary that funds are assigned for this type of research in the future. Especially now as the industry faces a technological shift where larger portions of fish production are moved to land-based facilities. If we are to continue ozonating the water, which we are likely to continue since it has proven to produce good results, solutions must be found to avoid bromate from accumulating over time, especially in cases with production of large fish on land. As this could involve many months of production where bromate reaches the toxic threshold value. To the best of the authors knowledge, there are no current studies that tell us what will happen if this occurs, will bromate begin to accumulate in the fish? Can bromate from the fish be transferred to the humans who eat the fish? Can such toxic values be reached that the fish die as a result? These are questions that need to be answered in future research.

5. Conclusion

In the study, three separate fish groups were monitored, all using ozonation as a method to clear the water, and water samples were sent to Eurofins Environmental Testing Bergen, a laboratory specializing in the analysis of nutrients in seawater, for bromate analysis. The main findings showed that the use of ozone led to the formation of bromate in all three cases, with bromate levels increasing in line with ozonation and production time. The statistically significant positive correlation between the dosing of ozone and the subsequent formation of bromate suggests a dose-dependent increase in bromate concentrations with increased ozone use. However, the values never exceeded 0.13 mg/L BrO_3^-, which is over twenty times lower than the recommended threshold value for aquatic organisms. We can assume that the degree of ozonation was higher in G3 compared to G1, still, G3 generally showed higher SGR, supporting previous research that says higher ozonation can promote growth. Despite higher bromate values in G3 compared to G1, there are no indications that bromate levels have had a negative impact on growth or mortality, which may suggest that bromate levels under these ozonation conditions do not exceed a harmful threshold.

All three groups showed good results in the welfare measurements, where they mostly scored 0 or 1, indicating good welfare. G1 generally had slightly better results on the welfare tests than G3, which may indicate that increased ozonation and prolonged bromate levels lead to poorer fish welfare, as it seems that the welfare of the fish in G3 gradually became a bit worse from measurement to measurement, while the welfare in G1 remained stable. However, the welfare of all three groups followed in this study was good. A key conclusion is that if ozonation is to be a successful strategy for clearing the saltwater in RAS, water quality must be closely monitored so that bromate values do not exceed the toxic limit, which was not a problem in this study.

The following conclusions about the formulated hypothesis were obtained:

(H_{A1}): Ozonation in saltwater RAS will lead to the formation of bromate in the water, **is accepted.** (H_{01}): Ozonation in saltwater RAS will not lead to the formation of bromate in the water, **is rejected.** (H_{A2}): The concentration of bromate will accumulate over the course of production, **is accepted.** (H_{02}): The concentration of bromate will not accumulate over the course of production, **is rejected.** (H_{A3}): Ozonation in saltwater RAS will reduce fish performance, **is rejected.** (H_{03}): Ozonation in saltwater RAS will not reduce fish performance, **is accepted.**

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7. Appendix

7.1 Table 1: Day-by-day table of ozonation for G3

Date	Kg ozone used per hour RAS1	Kg ozone used per hour RAS2	Mg/L ozone per hour RAS1	Mg/L ozone per hour RAS2
28.09.2023	0.200	0.000	0.018	0.000
29.09.2023	ND	ND	ND	ND
30.09.2023	0.141	0.059	0.013	0.005
01.10.2023	0.139	0.061	0.013	0.006
02.10.2023	0.139	0.061	0.013	0.006
03.10.2023	0.214	0.086	0.019	0.008
04.10.2023	0.182	0.118	0.017	0.011
05.10.2023	0.185	0.115	0.017	0.010
06.10.2023	0.180	0.120	0.016	0.011
07.10.2023	0.180	0.120	0.016	0.011
08.10.2023	0.180	0.120	0.016	0.011
09.10.2023	0.180	0.120	0.016	0.011
10.10.2023	0.180	0.120	0.016	0.011
11.10.2023	0.180	0.120	0.016	0.011
12.10.2023	0.240	0.160	0.022	0.015
13.10.2023	0.269	0.231	0.024	0.021
14.10.2023	0.338	0.262	0.031	0.024
15.10.2023	0.386	0.314	0.035	0.029
16.10.2023	0.386	0.314	0.035	0.029
17.10.2023	0.441	0.359	0.040	0.033
18.10.2023	0.441	0.359	0.040	0.033
19.10.2023	0.495	0.405	0.045	0.037
20.10.2023	ND	ND	ND	ND
21.10.2023	0.559	0.541	0.051	0.049
22.10.2023	0.582	0.518	0.053	0.047
23.10.2023	0.655	0.545	0.060	0.050

24.10.2023	0.730	0.570	0.066	0.052
25.10.2023	0.881	0.719	0.080	0.065
26.10.2023	0.815	0.785	0.074	0.071
27.10.2023	0.855	0.845	0.078	0.077
28.10.2023	0.850	0.850	0.077	0.077
29.10.2023	0.906	0.894	0.082	0.081
30.10.2023	0.906	0.894	0.082	0.081
31.10.2023	0.900	0.900	0.082	0.082
01.11.2023	0.950	0.950	0.086	0.086
02.11.2023	1.000	1.000	0.091	0.091
03.11.2023	0.987	1.013	0.090	0.092
04.11.2023	1.236	0.964	0.112	0.088
05.11.2023	1.236	0.964	0.112	0.088
06.11.2023	1.283	1.017	0.117	0.092
07.11.2023	1.280	1.020	0.116	0.093
08.11.2023	1.279	1.021	0.116	0.093
09.11.2023	1.391	1.109	0.126	0.101
10.11.2023	1.405	1.095	0.128	0.100
11.11.2023	1.450	1.150	0.132	0.105
12.11.2023	1.453	1.147	0.132	0.104
13.11.2023	1.830	0.970	0.166	0.088
14.11.2023	1.669	1.331	0.152	0.121
15.11.2023	1.655	1.345	0.150	0.122
16.11.2023	1.552	1.448	0.141	0.132
17.11.2023	1.552	1.448	0.141	0.132
18.11.2023	1.561	1.439	0.142	0.131
19.11.2023	1.574	1.426	0.143	0.130
20.11.2023	1.618	1.482	0.147	0.135
21.11.2023	1.670	1.530	0.152	0.139
22.11.2023	1.685	1.515	0.153	0.138
23.11.2023	1.695	1.505	0.154	0.137

24.11.2023	1.690	1.510	0.154	0.137
25.11.2023	1.675	1.525	0.152	0.139
26.11.2023	1.675	1.525	0.152	0.139
27.11.2023	1.675	1.525	0.152	0.139
28.11.2023	1.504	0.496	0.137	0.045
29.11.2023	0.902	0.598	0.082	0.054
30.11.2023	0.396	0.104	0.036	0.009
01.12.2023	0.000	0.000	0.000	0.000
02.12.2023	0.000	0.000	0.000	0.000
03.12.2023	0.000	0.000	0.000	0.000
04.12.2023	0.000	0.000	0.000	0.000
05.12.2023	0.000	0.000	0.000	0.000
06.12.2023	0.000	0.000	0.000	0.000

7.2 Table 2: Estimated daily water exchange rates for RAS1

Date	Dilution between the previous measured date and the current date (in %)	Estimated dilution per day (in %)	Dilution water (m^3) added between the previous measured date and the current date	Estimated dilution water (m^3) per day.
27.09.23	0.00%	0.00%	0	0
29.09.23	30.52%	15.26%	1 679	839.5
02.10.23	43.00%	14.33%	2 362	787.3
04.10.23	34.50%	17.25%	1 895	947.5
06.10.23	30.55%	15.28%	1 681	840.5
09.10.23	39.05%	13.02%	2 148	716
11.10.23	28.41%	14.21%	1 563	781.5
13.10.23	35.24%	17.62%	1 938	969
16.10.23	37.23%	12.41%	2 048	682.7
18.10.23	35.20%	17.60%	1 936	276.6
20.10.23	37.15%	18.58%	2 043	1 021.5
23.10.23	24.92%	8.31%	1 371	457
25.10.23	32.54%	16.27%	1 789	894.5
27.10.23	29.08%	14.52%	1 599	799.5
28.10.23	21.65%	21.65%	1 191	1 191
30.10.23	28.57%	14.29%	1 571	785.5
01.11.23	31.89%	15.95%	1 754	877

03.11.23	23.30%	11.65%	1 281	640.5
06.11.23	46.40%	15.47%	2 552	850.7
08.11.23	7.23%	3.62%	398	199
10.11.23	25.94%	12.97%	1 427	713.5
13.11.23	28.19%	9.40%	1 551	517
15.11.23	32.05%	16.03%	1 763	881.5
17.11.23	14.97%	7.49%	824	412
20.11.23	29.76%	9.92%	1 637	545.7
22.11.23	47.10%	23.55%	2 590	1 295
24.11.23	0.00%	0.00%	0	0
26.11.23	22.46%	11.23%	1 236	618
29.11.23	38.07%	12.69%	2 094	698
01.12.23	0.00%	0.00%	0	0
04.12.23	26.92%	8.97%	1 481	493.7
06.12.23	24.21%	12.11%	1 332	666

Table 3: Estimated daily water exchange rates for RAS2

Date	Dilution between the previous measured date and the current date (in %)	Estimated dilution per day (in %)	Dilution water (m^3) added between the previous measured date and the current date	Estimated dilution water (m^3) per day.
29.09.23	0.00%	0.00%	0	0
02.10.23	16.91%	5.64%	930	310
04.10.23	32.02%	16.01%	1761	880.5
06.10.23	35.20%	17.6%	1936	968
09.10.23	40.26%	13.42%	2 214	738
11.10.23	22.16%	11.08%	1 219	609.5
13.10.23	32.15%	16.08%	1 768	884
16.10.23	36.08%	12.03%	1984	661.3
18.10.23	49.93%	24.97%	2746	1373
20.10.23	17.33%	8.67%	953	476.5
23.10.23	37.86%	12.62%	2082	694
25.10.23	28.86%	14.43%	1 587	793.5
27.10.23	68.33%	34.165%	3758	1879
28.10.23	0.00%	0.00%	0	0
30.10.23	30.39%	15.20%	1 672	836
01.11.23	29.06%	14.53%	1 598	799
03.11.23	34.45%	17.26%	1 895	947.5
06.11.23	30.64%	10.21%	1685	842.5
08.11.23	20.03%	10.02%	1102	551
10.11.23	26.42%	13.21%	1452	726.5
13.11.23	26.06%	8.69%	1433	477.7

15.11.23	46.71%	23.26%	2569	1284.5
17.11.23	0.00%	0.00%	0	0
20.11.23	27.90%	9.30%	1 535	511.7
22.11.23	35.55%	17.76%	1955	977.5
24.11.23	13.45%	6.73%	740	370
27.11.23	25.04%	12.52%	1 377	459
29.11.23	15.17%	7.59%	834	417
01.12.23	18.7%	9.35%	1028	514
04.12.23	39.00%	13.00%	2145	715