Master thesis

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Growth performance and welfare of post-smolt (*Salmo salar L.*) reared in semi closed containment systems (S-CCS) – a comparative study

For the Fulfilment of the Master of science in Aquaculture and seafood

By

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Abstract

The salmon industry faces challenges related to sea lice infestations, escapees, diseases and environmental impact. Semi closed containment systems (S-CCS) have been proposed to abate these challenges. In the S-CCS, cultured fish are separated from the natural environment by a physical barrier. The use of these systems reduces the time fish spend living in open sea cages.

This study investigated and documented welfare and growth performance of Atlantic salmon through an acute challenge test experiment and a big-scale benchmark study.

The acute challenge test experiment was conducted using post-smolts raised in two large scale semi-closed system (S-CCS: Preline and Neptune), with reference groups raised in open sea cages. The post-smolt was stressed by confining them in a holding tank with reduced water level for a short period. Corresponding baseline sampling was done on unstressed fish for comparable measurements.

For the benchmark study, selected production data from six generations of salmon was used to compare growth and performance of fish raised in S-CCS (Preline) and in open sea cages (reference). The benchmark study was carried out in two phases. Phase one used post-smolts from approximately 100 g to 800 g in seawater, and fish in S-CCS were compared with a reference group from an open sea cage. The second, grow-out phase used salmon from approximately 800 g to 5000 g in open sea cages; here fish previously reared in S-CCS were compared with fish from a reference group.

Fish raised in the S-CCS showed lower concentration of plasma cortisol, magnesium and lactic acid at baseline levels, giving a stronger response to the acute stress challenge than fish from the reference group. The results suggest lower basal stress in the S-CCS group compared with the reference group in open sea cages, as well as a more balanced response to stress in the S-CCS fish.

The findings from the benchmark analyses showed a significantly lower infestation of sea lice in Preline fish during the post-smolt phase. Furthermore, in the grow-out phase the Preline group showed higher weight gain and higher final weight compared to the reference group in open pen (Weight at harvest: Spring transfer, Preline=4.65 kg *vs* reference group=3.79 kg, Fall transfer, Preline=4.87 kg *vs* reference group=4.03 kg). Finally, salmon raised in Preline showed significantly higher survival compared to the reference group, indicating increased robustness in fish raised in S-CCS when transferred to open net pens in sea.

As the results indicate reduced stress, lower sea lice infestations and greater weight gain, S-CCS appears to have advantages compared to traditional long exposure to the natural environment in open sea cages in Norway. However, to determine the real potential of S-CCS strategy, further research is needed.

Introduction

Atlantic Salmon (*Salmo salar*) – Life Cycle

In nature, the Atlantic salmon spawn and hatch in freshwater, where the juvenile stages are spent before undergoing a pre-adaptive preparatory transformation to a life in seawater (Figure I.1). This transformation is referred to as parr-smolt transformation or smoltification and is stimulated by external environmental cues like water temperature and photoperiod (Björnsson et al., 2011; Hoar, 1988; McCormick, 2013; Stefansson et al., 2008). The effect of photoperiod is translated via the light–brain–pituitary axis, involving several downstream endocrine factors such as cortisol, thyroid hormones, and growth hormone (Ebbesson et al., 2003). The effect of temperature is more direct, acting as a rate-controlling factor on the physiological responses to the seasonal changes in photoperiod (Hoar, 1988; Stefansson et al., 2008).

Parr-smolt transformation includes changes in morphology, physiology, and behavior (Heggberget et al., 1993; Stefansson et al., 2016), including development of dark fin margins and silvery scales (McCormick, 1993; Stefansson et al., 2003). The physiological preparation for life in a hyperosmotic environment (seawater) results in the development of increased drinking rate and absorption of water through the intestine. The expression of genes that regulates the development of seawater chloride cells (Na^+/K^+-ATPase) in gill tissue increases, allowing for an active excretion of monovalent ions (D'Cotta et al., 2000; Tipsmark et al., 2010). In nature, smoltification is accompanied by downstream migratory behavior. In the ocean, the post-smolt grow for two to three years before they become adult Atlantic salmon. The adult salmon usually return to their river of origin to reproduce (McCormick, 2013). The life cycle of the Atlantic salmon has led to the successful development of the Norwegian aquaculture industry.

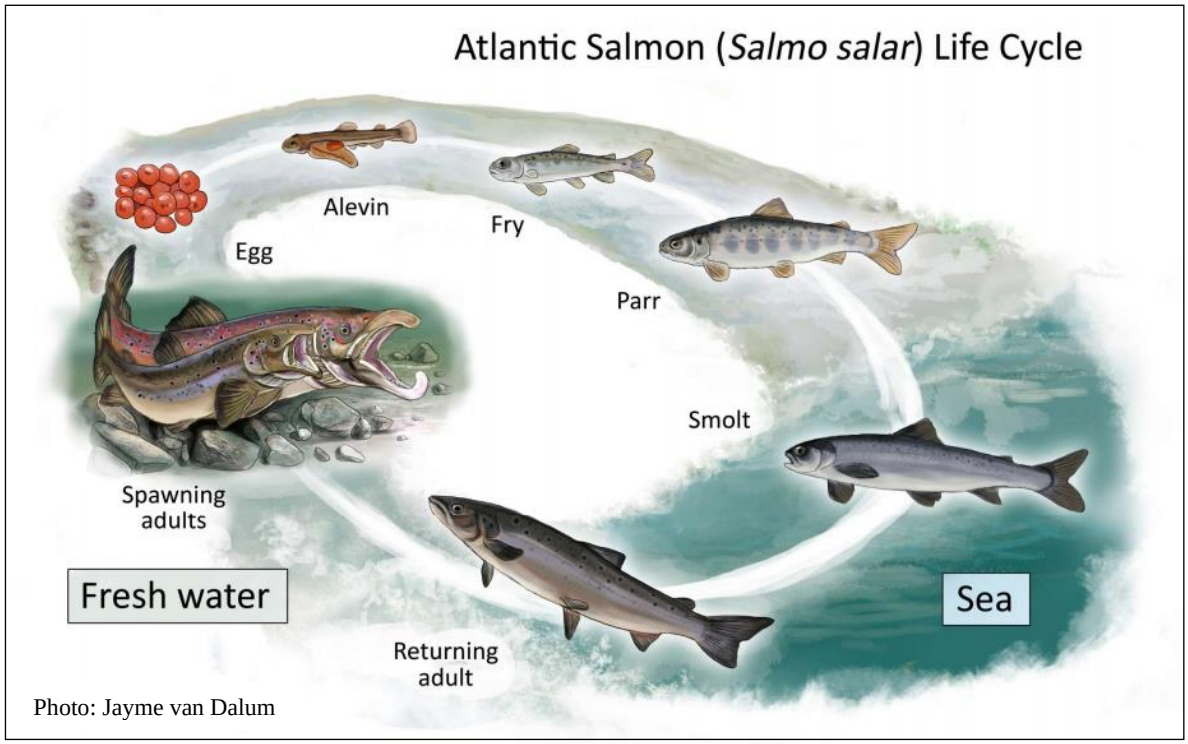

Figure I.1. Schematic overview of the wild Atlantic salmon life cycle, from eggs to adult in freshwater and seawater.

Aquaculture in Norway; challenges and potential

With a coastline of more than 102,936 km, including fjords and islands (Norwegian Mapping Authority, 2020), Norway is one of the leading nations regarding production through marine fisheries and aquaculture farming (FAO, 2013). Intensive cultivation of Atlantic salmon (*Salmo salar*) accounts for the majority of farmed volumes. In 2018, nearly 1.36 million tons of farmed fish were produced, with the production of Atlantic salmon accounting for 95% of the total aquaculture volume, making Norway the largest producer of Atlantic salmon in the world (Statistics Norway, 2019). However, the political ambition is to achieve a five-fold increase in aquaculture production by 2050, which means production of 5 million tons, presuming sustainable environmental growth (Olafsen et al., 2012).

The commercial launch of aquaculture equipment and farming of Atlantic salmon was introduced in the 1970s when the Grøntvedt brothers developed the octagonal floating sea cage (Berge, 2014). Today, nearly five decades later, the conventional open net cage has proven to

both be cost-effective and enable efficient utilization of the coastal sea area. In 2019, Norway had 862 active locations (Directorate of Fisheries, 2019) for production of salmonids (Figure I.2a) and the conditions along the coast facilitate increased production volume from aquaculture.

The salmon farming industry has failed to achieve such an increase in production. In fact, the produced volume has stagnated at around the 2012 level (approximately 1.2 million tons, Statistics Norway, 2019, Figure I.2b). This lack of increase is a consequence of challenges concerning pathogens and diseases (Rosten et al., 2011). In addition, escaped farmed Atlantic salmon pose a significant environmental challenge that has a negative impact on wild salmon (Glover et al., 2012). These challenges have created a temporary bottleneck for the expansion of salmon farming and require new sustainable production systems.

The sea lice (*L. salmonis*) are one of the major pathogens affecting the commercial culture of salmonids, both in Norway and the rest of the world (MacKinnon, 1998; Mustafa et al., 2000). The sea lice feed on mucus, skin, and blood of the host, and the impact on lice-infested fish varies from mild skin damage to more severe damage to individual fish (Bowers et al., 2000; Dawson et al., 1999). Other factors, such as growth rate, reduced appetite, and feed-conversion efficiency are also negatively affected (Dawson et al., 1999; Pike & Wadsworth, 1999). Consequently, salmon farmers are inflicted with vast costs in relation to preventive efforts and sea lice treatment. In addition, wild salmon populations are negatively affected by increasing incidences of sea lice (Anon, 2011; Costello, 2009a, 2009b)

To address this challenge, regulating authorities have recently implemented a system using traffic lights (green, yellow, and red) to control the increase in production of Atlantic salmon. (MTIF, 2017a). The Production Area Regulation divides the Norwegian coast into 13 production areas. In each area, the traffic light system controls the potential for growth or a reduction in potential production, based on the mortality risk from sea lice infestation for wild

salmon. The traffic light indicates three different levels, where green denotes low risk, yellow represents moderate risk, and red indicates high risk of sea-lice induced mortality (MTIF, 2017b). Hence, the traffic light system includes a forced reduction in production volume in locations with high sea lice pressure (Myksvoll et al., 2018; Vollset et al., 2017), and the aim is to protect wild salmon populations and improve salmon welfare (MTIF, 2015).

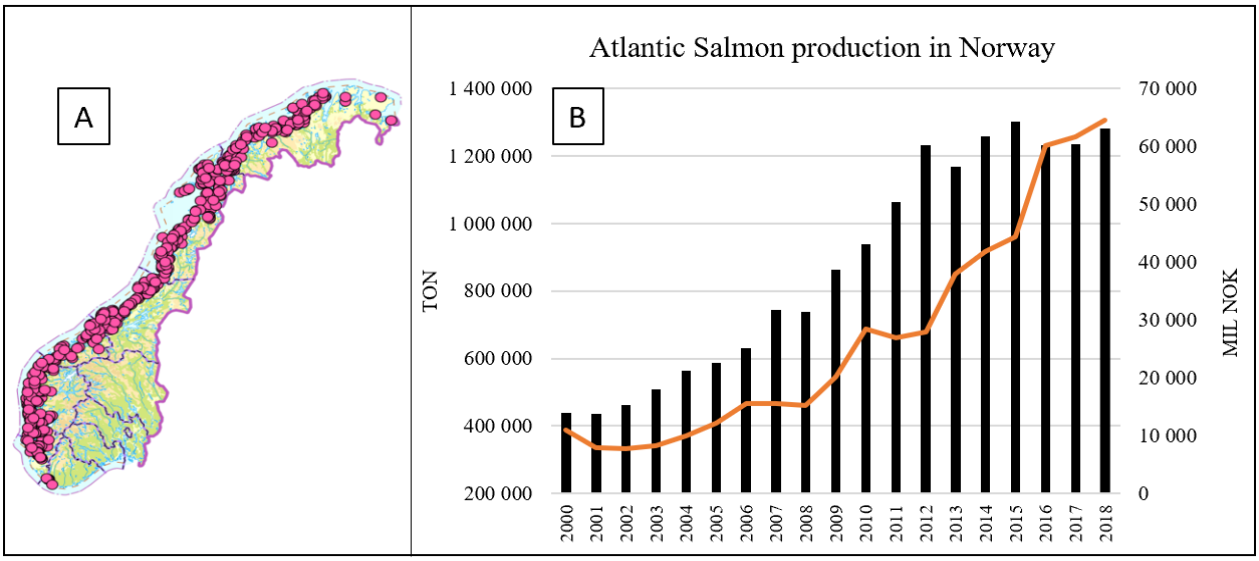

Figure I.2. Atlantic Salmon *(Salmo salar)* aquaculture production in Norway. Active aquaculture locations according to the Directorate of Fisheries, 2019 (A). Production and first-hand value of Atlantic salmon in the period 2000–2018. Source: Statistics Norway, 2019 (B).

To cope with the current challenges, the aquaculture industry demands new production regimes for farming of Atlantic salmon. A key factor in abating the current challenges of open sea cage farming is simply to reduce the open sea period for the fish. This reduction will reduce the exposure period to sea lice, diseases and possible upgrade the Atlantic salmon production cycle. In addition, findings have shown that larger smolts are more robust and capable of handling the transfer to open net-pens in seawater (Ytrestøyl et al., 2015).

Production of Atlantic Salmon; introduction of new technologies

In Norwegian aquaculture, the Atlantic salmon is hatched and raised in freshwater at land-based facilities. To stimulate growth and parr-smolt transformation in juvenile salmon, industrial manipulation of environmental parameters, such as photoperiod and temperature, is commonly used. In addition to accelerated growth, it has been reported that hatchery fish reared under intensive conditions develop faster and show typical smolt characteristics such as increased silvering, seawater tolerance, and increased gill NKA activity several months prior to the normal smolt season (Handeland & Stefansson, 2001). However, for the farmers, it is of great importance to conduct the transfer to seawater during a specific period called the 'smolt window'. If the transferred smolts fail to reach seawater during this critical 'smolt window,' the fish undergo desmoltification, a process which includes a loss of hypo-osmoregulatory abilities and metabolic adaptions (Stefansson et al., 2008). After achieving smolt-status, the fish is transferred to sea cages, where the predominant production of Atlantic salmon takes place (Oppedal et al., 2011). The Atlantic salmon is now referred to as post-smolt until it reaches a weight of 1 kg (Hjeltnes et al., 2017).

The post-smolt phase in open seawater is considered to be the most critical, due to physiological and environmental challenges such as sea lice, diseases and suboptimal water conditions. Consequently, up to 20% of the smolt transferred to sea cages can be lost before reaching harvest size (Bleie & Skrudland, 2014; Hjeltnes et al., 2017). These biological and environmental challenges have been suggested as being harmful to prospective growth of the industry (Gullestad et al., 2011). To mitigate this situation, it has been suggested that farmers should produce larger and more robust post-smolt as a preventive strategy to reduce production-related losses in open sea cages.

Hence, innovative technologies are emerging in the aquaculture industry, making it possible to move part of the post-smolt phase to land-based, closed, recirculating aquaculture

systems (RAS) or by using floating semi-closed containment systems (S-CCS) in the sea (Rosten et al., 2011; Thorarensen & Farrell, 2011). Examples of floating semi closed systems in sea are the Preline raceway platform (Preline Fishfarming system and Lerøy AS) and the Neptune tank (AquaFarm Equipment AS and Mowi AS). Introduction of these systems could prospectively have an impact on limiting the environmental challenges, which include sea lice infestations, outbreak of diseases, escapes, organic waste, and delousing agent pollution.

This study investigates the use of floating S-CCS (Preline and Neptune) in commercial post-smolt production. The research combines two different approaches: (1) Acute challenge test (ACT), as described in the "Stress response and allostasis" paragraph; and (2) the benchmark analysis, further described in the "Biological performance in S-CCS" paragraph. The first approach measures the biological response in fish exposed to an acute stressor, and the second one analyzes and benchmark the performance of fish reared in an S-CCS prior to grow-out in open sea cages.

Stress Response and Allostasis in fish

Stress can be defined as the summary of the physiological responses the fish use to maintain or restore normal metabolism after an environmental challenge (Iwama, 1997). Interruptions of the internal equilibrium (homeostasis) generated by internal or external stimuli are defined as a stressor (Selye, 1950; Wendelaar Bonga, 1997). According to the duration and magnitude of exposure to stressors, a stress response can be divided into primary, secondary, and tertiary responses (Figure I.3). In aquaculture, stress is related to conditions that negatively affect the fish immune system response capacity, resistance against infections, growth, and reproduction (Wendelaar Bonga, 1997).

When exposed to a primary response, stressors activate the hypothalamus–pituitary–interrenal (HPI) axis. Perception of a stressor by a fish initiates a rapid, neural stimulated release of stress hormones (catecholamines and cortisol) into the circulatory system. Catecholamines (specifically adrenaline and noradrenaline) are released from the chromaffin tissue situated in the head kidney of teleosts, and from the endings of adrenergic nerves (Randall & Ferry, 1992). Cortisol is released from the interrenal tissue, which is located in the head kidney, in response to pituitary hormones. In this process the adrenocorticotropic hormone (ACTH) is essential (Iwama, 2006; Wendelaar Bonga, 2011).

The secondary stress response has a mobilizing effect on the fish. If exposed to a challenge, the fish will increase the production of catecholamines and cortisol from the head kidney. This has a strong effect on metabolism toward increasing the availability of glucose. Consequently, less acute functions in the fish body, including digestion, reproduction, and growth, are not prioritized. The secondary response will also increase the heart rate and blood circulation to muscles nourished by glucose.

Repeated or long-term exposure to a stressor could lead to a tertiary stress response, resulting in a chronic stress state for the fish. In this state, the fish are not able to maintain or

retain homeostasis, reducing the ability for reproduction, growth, and survival (Schreck, 2010; Wendelaar Bonga, 1997). In aquaculture, farming salmon exposes the fish to challenging situations that could potentially lead to stressors. Such stressors could be suboptimal water conditions, diseases, transport, vaccination, and malnutrition (Chrousos, 1998; Madaro et al., 2015). In an S-CCS system, it is suggested that the magnitude of some of these stressors could be reduced within the system, in contrast to open sea cages (Rosten et al., 2011).

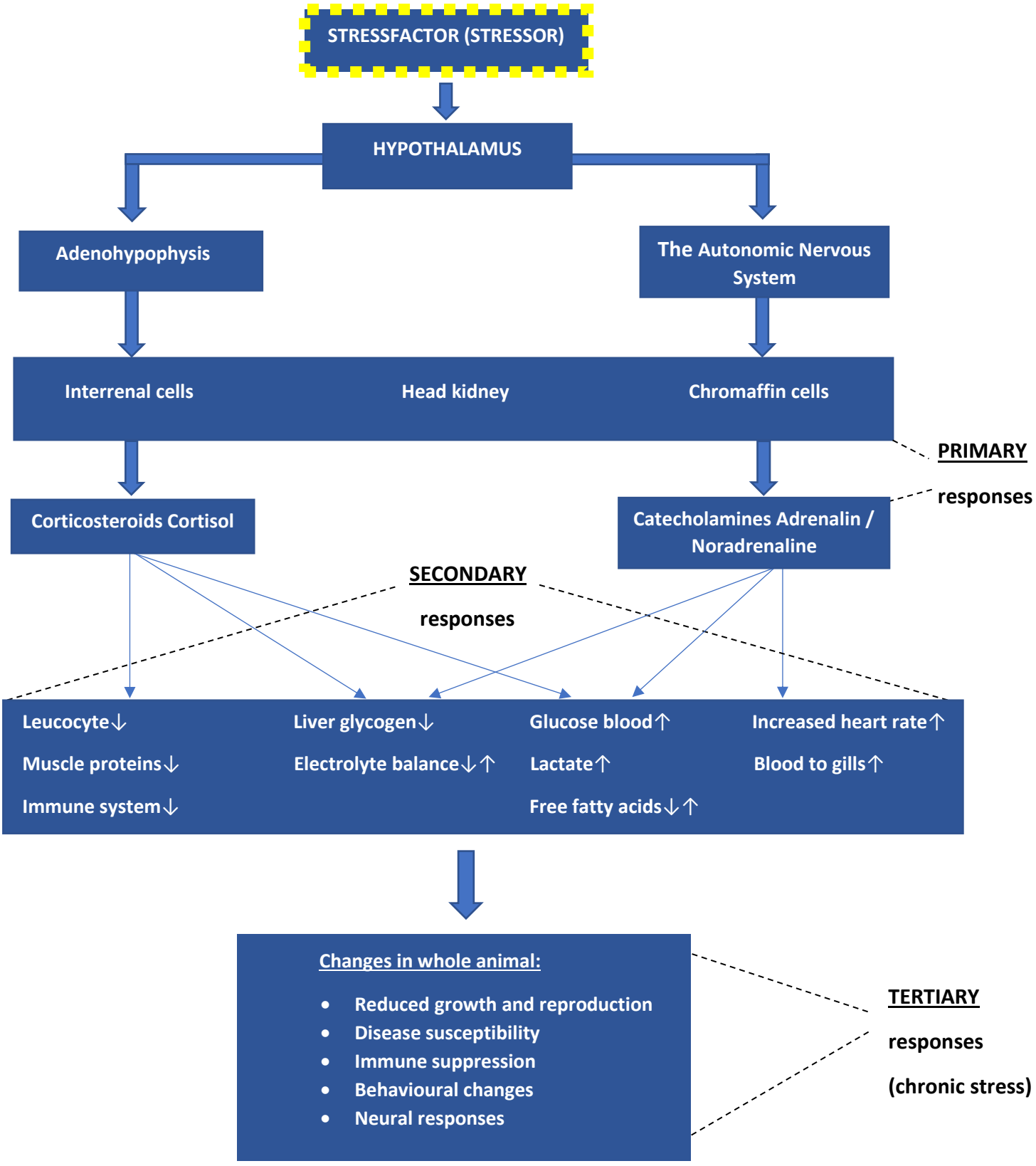

Figure I.3. Schematic representation of stress response in the teleost, including endocrine, metabolic and osmotic responses. An adaptive response will try to maintain homeostasis and increase individual survival. (Figure based on Wendelaar Bonga, 1997; Tort, 2011).

In fish stress physiology, the concept of welfare has gradually been more commonly presented in terms of allostasis, introducing a more dynamic and flexible view of the internal balance (McEwen, 1998; Sapolsky, 2004). This process suggests an inverted U-shaped relationship where both too little or too much stress leads to poor welfare (Korte et al., 2007; Sterling & Eyer, 1988). It is important to emphasize that stress in itself is not negative, and that the stress response is part of the normal physiology. Moreover, allostasis is the bodily process of attempting to achieve stability, i.e., homeostasis, by varying physiological and behavior operations (McEwen & Wingfield, 2003). When exposed to persistent and intense stressors, the organism goes into an allostatic state (McEwen & Wingfield, 2003).

The physiological cost of maintaining the allostasis is referred to as an allostatic load (Ramsay & Woods, 2014). An increase in allostatic load would demand more energy to maintain homeostasis (McEwen & Wingfield, 2003). Allostatic overload is reached if the energy demand for maintaining allostasis is greater or equal to the available energy, consequently forcing the organism to allocate energy from other biological functions, such as the immune system, reproduction, and growth (McEwen, 2002). In this state of allostatic overload, the fish will have a limited amount of energy to handle additional stressors (Korte et al., 2007). The allostatic overload is here likely to reach a chronic stress response, and in terms of welfare, this stage would also lead to an increased risk for pathologies and systemic failures (Korte et al., 2007; Ramsay & Woods, 2014).

It has been suggested that fish reared in open sea cages and exposed to suboptimal water quality might have a reduced capacity to handle an acute stressor (Rosten et al., 2011). Hence, in terms of the stress response in fish, the study in Chapter 1- “Acute challenge test in S-CCS” compares two semi closed containment systems (S-CCS: Preline and Neptune) implemented in the post-smolt phase of producing Atlantic salmon. The study in chapter 1 investigate the biological performance after assessing the fish with an acute stress test. The experiments aim

to address whether there is a different response to a stressor in the fish reared in S-CCS compared to fish in open net-pens during the post-smolt phase.

Benchmark of biological performance; S-CCS *versus* open sea cages

A variety of technologies have been deployed to handle the current challenges associated with the open sea cage culture of salmonids, such as farming fish in floating closed containment systems. A semi-closed aquaculture system (S-CCS) is defined as a fish-producing system that has an impenetrable, or close to an impenetrable barrier, between the fish and the surrounding environment (Iversen et al., 2013). Presently, floating concepts of S-CSS in the aquaculture industry differ in shape, size and volume. The construction material differs between more-rigid materials, such as concrete, steel, polyethene (PE) and fiberglass, to less-rigid materials, such as enclosed plastic bags (Iversen et al., 2013; Teknologirådet, 2013). In an S-CCS, it is expected that there will be more stable water quality and precise monitoring of the system, contrasting with the situation of an open sea cage, which is fully exposed to environmental fluctuations induced by changes in current regime, water stratification, weather conditions, and seasonal differences (Remen et al., 2013; Remen et al., 2016).

In an S-CCS system the water can be pumped from intermediate water layers to avoid areas in which sea lice are the most abundant (Rosten et al., 2011). In Norway, the temperature in the sea is dependent on depth and stratification (Figure I.4). The S-CCS generate water flow through an inlet at a depth of 20-30 m beneath the surface, while the open pen is exposed completely to the water stratification. Consequently, the temperature profile will differ between an S-CCS system and an open sea cage system during the season. The temperature in seawater (surface) during summer is higher in an open net-pen compared to S-CCS and is the opposite during winter, i.e., higher in the S-CCS system compared to open pen. This shows that S-CCS with inlet water at 30 m generates an “opposite season” temperature parameter compared to

open sea cages. These variations in the temperature will then affect growth and feed conversion in fish during seasons (Talbot, 1993).

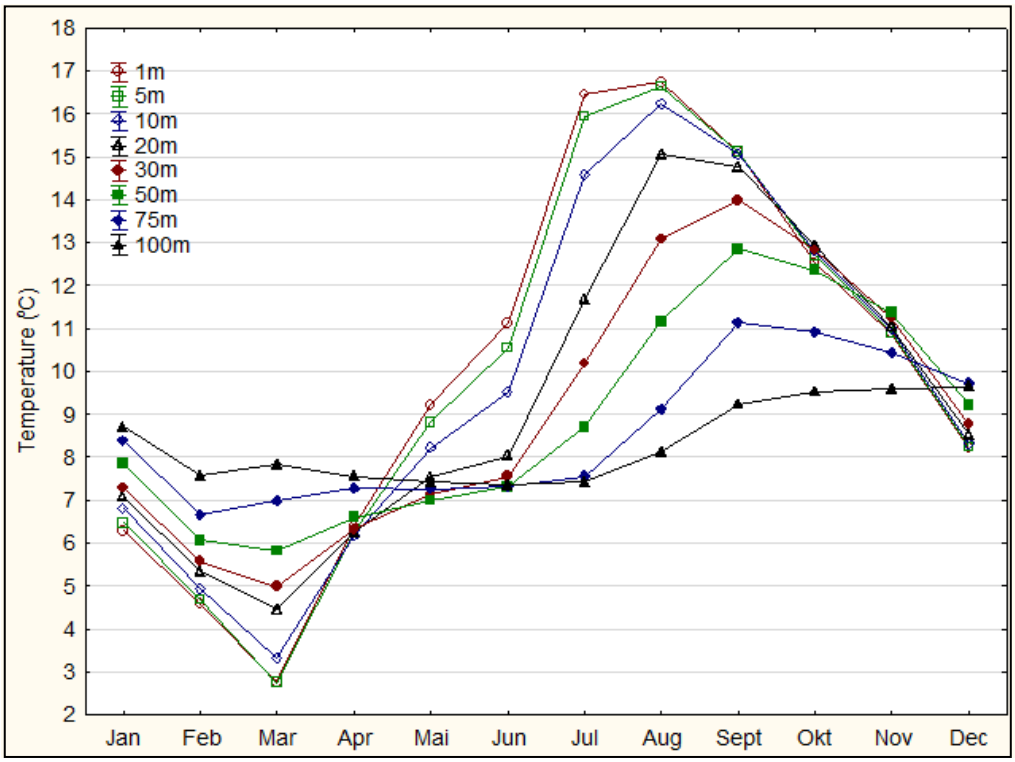

Figure I.4. Seasonal temperature profile of seawater in western Norway. Data are collected from Institute of Marine Research (IMR).

By utilizing water from low layers, it allows for more stable conditions (temperature and salinity) that might have a positive effect on the welfare and growth of the fish (Rosten et al., 2011). The S-CCS system may also reduce central environmental challenges, such as organic waste emissions, spreading of sea lice, and farmed escapees (Rosten et al., 2011).

Recent studies have shown a low mortality rate for post-smolt reared in closed containment systems with optimal density (Calabrese et al., 2017; Ytrestøyl et al., 2015). Further investigation of the biological performance in terms of growth, feed conversion, mortality and robustness of Atlantic post-smolt reared in S-CCS is required to assess the application of this technology.

Such an assessment is presented in Chapter 2; Benchmark analysis, where the aim of the study is to investigate and benchmark biological performance in fish reared in the Preline

system (S-CCS), and fish reared in a traditional open cage system. To achieve a broader understanding of the systems' performance independent of seasonal variations, the study was conducted from May 2015 to January 2019. The benchmark analysis was performed through two phases; post-smolt and grow-out phase, where both production stages are compared to fish reared in open net-pens and was followed until harvest for each generation.

Chapter 1 – Acute Challenge Test in S-CCS

Objectives

This study aims to compare biological performance in fish reared in two S-CCS (Neptune and Preline) and compare them to fish reared in traditional open cage system after an acute challenge test (ACT).

The experiment was based on the following hypotheses:

$H0_1$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic cortisol concentration after an acute challenge test (ACT).

$H1_1$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic cortisol concentration after an acute challenge test (ACT).

$H0_2$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic chloride concentration after an acute challenge test (ACT).

$H1_2$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic chloride concentration after an acute challenge test (ACT).

$H0_3$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic sodium concentration after an acute challenge test (ACT).

$H1_3$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic sodium concentration after an acute challenge test (ACT).

$H0_4$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic calcium concentration after an acute challenge test (ACT).

$H1_4$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic calcium concentration after an acute challenge test (ACT).

$H0_5$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic magnesium concentration after an acute challenge test (ACT).

$H1_5$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic magnesium concentration after an acute challenge test (ACT).

$H0_6$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic glucose concentration after an acute challenge test (ACT).

$H1_6$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic glucose concentration after an acute challenge test (ACT).

$H0_7$: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic lactic acid concentration after an acute challenge test (ACT).

$H1_7$: Post-smolt rearing methods (S-CCS or open reference cage) have a significant effect on plasmatic lactic acid concentration after an acute challenge test (ACT).

Materials and Method

Fish Material and Rearing Conditions

The fish (n = 240) used in (Experiment 1 – Preline and Experiment 2 – Neptune) originated from Lerøy Vest AS and Mowi AS. Fish in the Preline experiment (n = 120) originated from the Salmobreed strain that had been reared at Sjøtroll Havbruk AS (Kjærelva, Fitjarstern Norway) from hatching to the smolt stage. Fish in the Neptune experiment (n = 120) were of the Mowistrain, reared at Vågafossen Settefisk AS (Imsland, Vindafjord Norway) from hatching to smolt stage.

All fish used in the experiments were part of the respective commercial production lines and followed a standard production protocol, according to Lerøy Vest AS and Mowi AS.

Experimental Facilities

The acute challenge test (ACT) conducted in this study includes two experiments, experiment (1); Preline and reference, and experiment (2); Neptune and reference.

Experiment 1- Acute challenge test: Preline

Experiment 1 was conducted at the Lerøy Vest AS facilities at Sagen (60° 20.903 N' 5° 38.640 E') in the Trengereidfjord, Samnanger in Hordaland (Preline) with Bognøy facility in the Radfjord (60° 36.235 N' 5° 04.633 E'), Radøy in Nordhordaland as Preline reference. The S-CCS Preline included a 50 m-long raceway (PE) platform and has an elliptical cross-section (Figure 1.1). The Preline platform has a rearing volume of 2,000 m^3 with a max water flow of 400 m^3/min. The inlet water was pumped from a depth of 30 m (total depth 100 m). At each end of the system, propellers create a continuous water flow through the raceway and the water exchange rate was approximately 4–5 min (current 12–15 cm/s, Vector 3D acoustic Velocimeter, Nortek AS, Norway). Oxygen concentrations, temperature, and feeding were controlled by automatic systems and all data were registered daily (OxyGuard, Sterner). Daily water measurements were taken in the inlet and outlet drain, and commercial dry diets (Ewos

raid air) were fed from automatic feeders. The pellet was designed to have a longer retention time. All husbandry practices, including lice count, were conducted following the standard protocol for salmon rearing for Lerøy Vest AS.

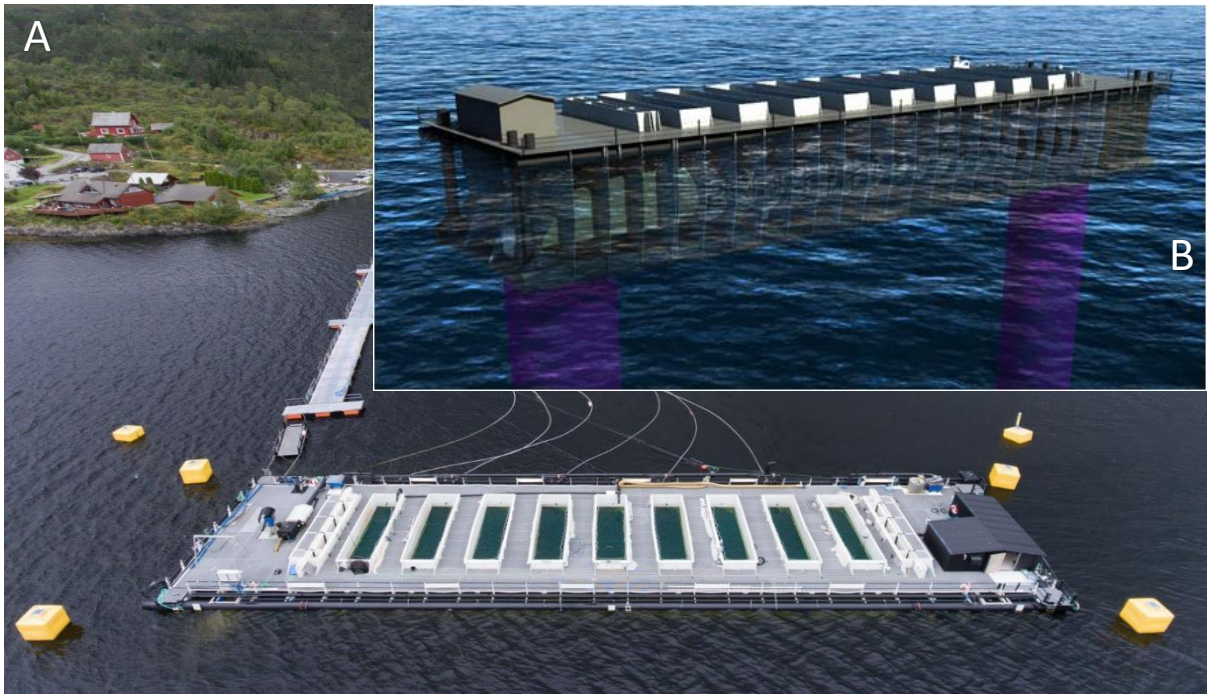

Figure 1.1. The Preline system placed at the Sagen location (A) and a 3D model (B) of the platform. Photo: (A) Lerøy Vest AS, (B) Preline Fishfarming System AS.

The reference group were reared in an open 160 m conical circular (*Spissnot* in Norwegian) sea cage (Bognøy, Radfjorden). Fish from the reference group followed the same feeding regime as in the Preline group and were fed with standard commercial pellets (EWOS) throughout the whole experimental period. Employees conducted daily measurements of water parameters (temperature and oxygen saturation) in both groups.

Experiment 2 – Acute challenge test: Neptune

Experiment 2 was conducted at the Mowi AS facilities Molnes (59° 43.195 N' 5° 51.475 E') in Skånevik, Hordaland (Neptune) and Munkholmen (59° 17.130 N' 5° 37.882 E'), situated in Hervikfjorden in Tysvær, Rogaland, as Neptune reference. The Neptune facility (Figure 1.2), is formed as a circular fiberglass tank with a circumference of 126 m made of glass-fiber reinforced plastic (GRP). The sidewalls are coated with Norpol gel and topcoat (Reichold, Durham, NC, USA). The bottoms are coated with a Büfa standard gel and topcoat (Büfa, Rastede, Germany). The intake depth is fixed at 25 meters and the system has a rearing volume of 21,000 m^3. Dry diets (MH Transfer STG, made by Mowi) were fed from automatic feeders throughout the Neptune period.

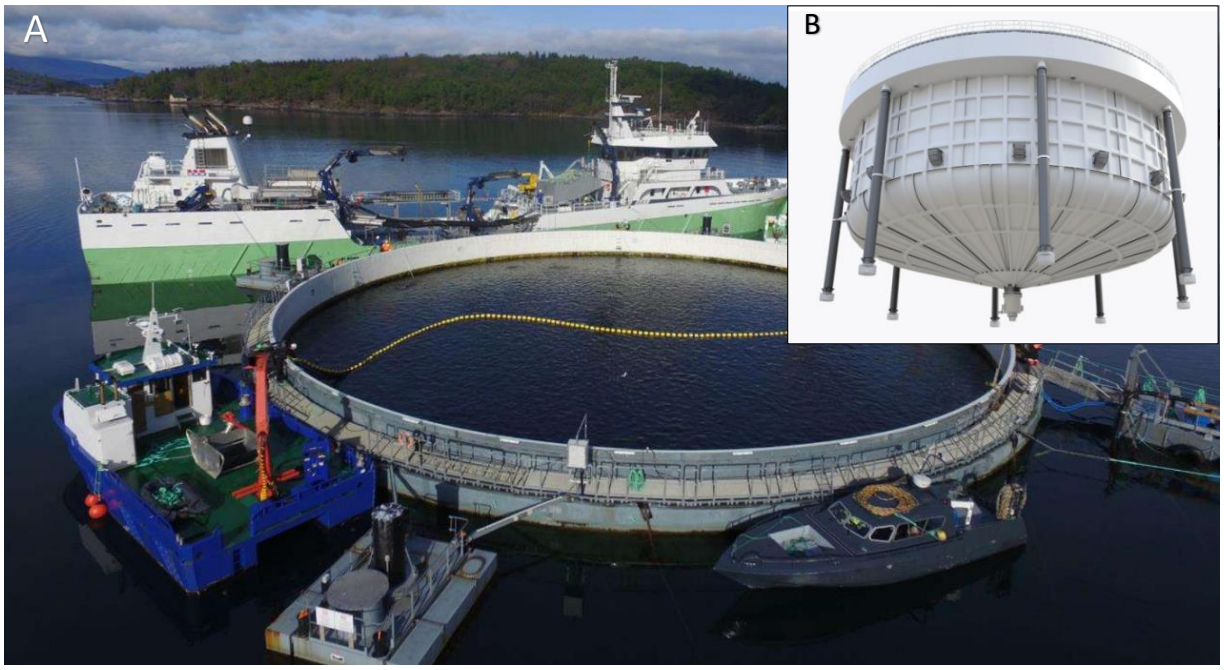

Figure 1.2. Experimental facility for Neptune (A), Molnes (Mowi ASA/ Design by AquaFarm Equipment) and (B) a 3D model of the Neptune system. Photo: (A) Mowi AS and (B) AquaFarm Equipment AS.

The reference group fish (Munkholmen, Hervikfjorden) were stocked in a traditional open sea cage. Fish from the reference group followed the same feeding regime as the Neptune

group throughout the whole experimental period. Daily husbandry was conducted by employees at Mowi AS in both groups.

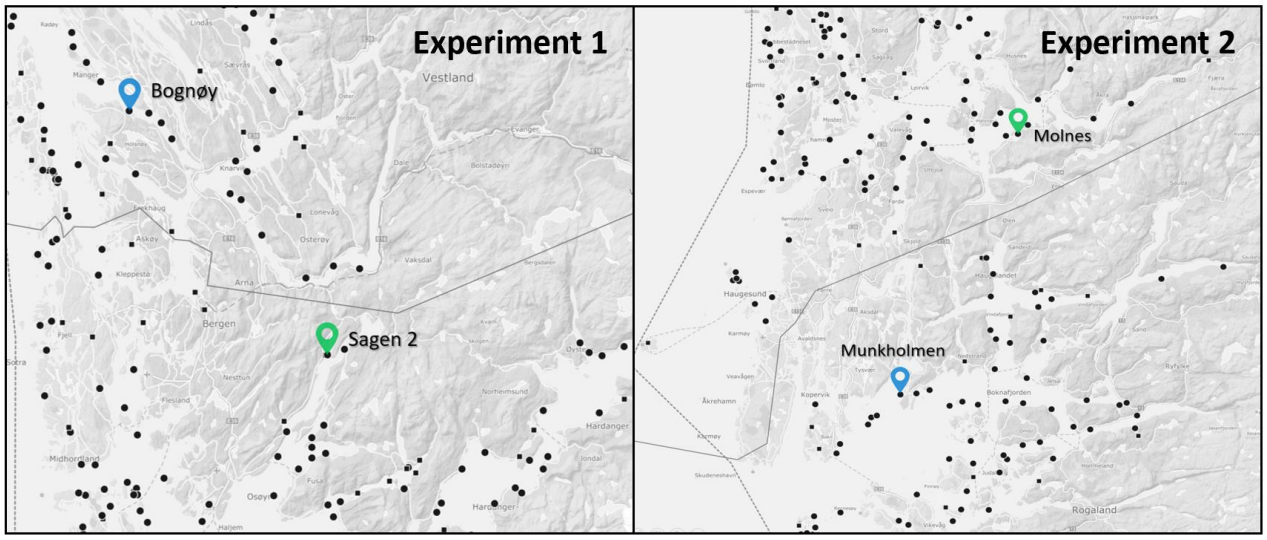

Figure 1.3. Overview of the facilities used in experiments 1 and 2. The S-CCS facilities; Sagen (Preline) and Molnes (Neptune), are plotted with the green marker. The reference group; Bognøy (experiment 1) and Munkholmen (experiment 2) are plotted with a blue marker. Black dots represent other open pen farms in the area.

Experimental Design; Experiment 1 and 2

Fish from Lerøy Vest AS, Kjærelva was divided into Preline (S-CCS group) and Bognøy (reference) and fish from Mowi AS, Vågafossen, was divided into Neptune (S-CCS group) and Munkholmen (reference), respectively. A total of 120 fish were part of each experiment and included subsets of fish (n = 30) that were selected from four different groups (Table 1.1 and Table 1.2). All the locations for the different groups are indicated in Figure 1.3

Sampling of the groups consisted of two treatments; first baseline sampling, second the acute challenge test (ACT). A schematic representation of the experimental protocol used at Preline, Bognøy, Neptune and Munkholmen is depicted in Figure 1.4

Table 1.1 *Overview of treatments group in experiment 1 – Preline System*

Date	Location	Fish group	Treatment	Fish N
01.03.18	Sagen	Preline ACT	Acute challenge test (ACT)	30
01.03.18	Sagen	Preline Baseline	Baseline	30
12.03.18	Bognøy	Preline Ref ACT	Acute challenge test (ACT)	30
12.03.18	Bognøy	Preline Ref Baseline	Baseline	30

Table 1.2 *Overview of treatments group in experiment 2 – Neptune System*

Date	Location	Fish group	Treatment	Fish N
05.04.18	Molnes	Neptune ACT	Acute challenge test (ACT)	30
05.04.18	Molnes	Neptune Baseline	Baseline	30
06.04.18	Munkholmen	Neptune Ref ACT	Acute challenge test (ACT)	30
06.04.18	Munkholmen	Neptune Ref Baseline	Baseline	30

Experimental Procedure

Baseline sampling; Baseline samplings were conducted on site in order to perform comparable measurements of the treatments in the experiments. Fish was collected by using a special net (*orkast* in Norwegian) which was lowered down in the systems (open net pens and S-CCS). Thereafter, feed was thrown over the net to attract fish and was then quickly raised to collect the fish. The baseline sampling was conducted directly after collection of the fish (Table 1.1 and 1.2).

Acute challenge test (ACT); To determine the stress response in fish, an acute challenge test (ACT) was performed after the baseline sampling (Figure 1.5). The ACT was performed by netting the fish per group (n = 30) and then confining them in a 200 L holding tank (supplied with water from the original system). The water level was then reduced with 80% in the tank for 15 min. Fresh water from the system was constantly supplied during the stress test at the

20% water level. After 15 min, the water level was increased to normal level and allowed the fish a 45 min recovery period before sampling (Table 1.1 and 1.2).

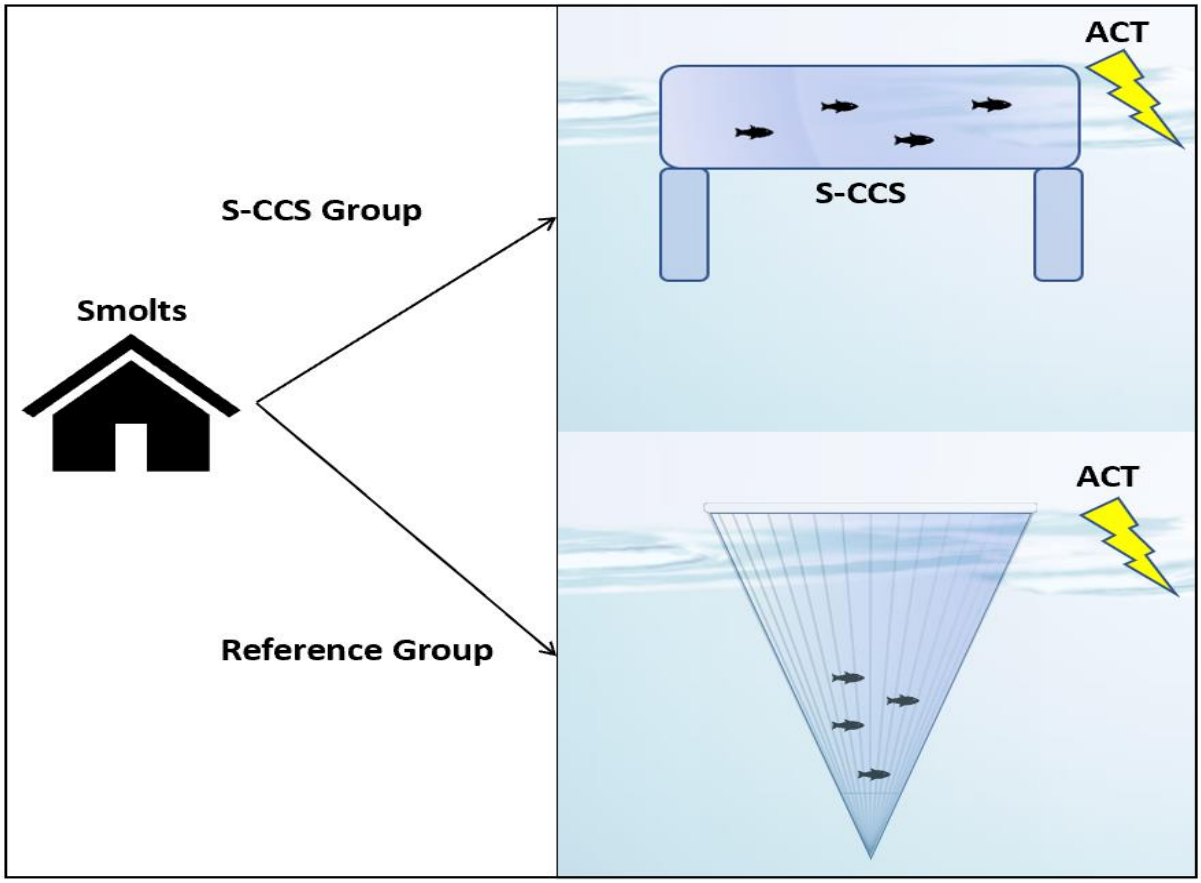

Figure 1.4. Schematic setup of the experimental protocol, post-smolt reared in S-CCS (Preline and Neptune) and reference group in open net-pens. The S-CCS figure is schematized by the Preline system, but the same setup was conducted for the Neptune system. The post-smolt group was reared in S-CCS for approximately 3–4 months prior sampling.

Figure 1.5. Equipment used for the ACT treatment. The confinement tank where the post-smolt were reared during the ACT (15 min) and the 45 min recovery period.

Sampling Protocol

Randomly chosen fish were rapidly netted and humanely euthanized with a lethal dose (200 mg/L) of Benzocaine. To avoid coagulation, heparinized 23G needle 2 mL syringes were used to collect fish blood from the caudal vein. Gathering of blood samples was prioritized, and all fish were sampled within 10 min of netting. Plasma was separated from the blood`s cellular fraction in Eppendorf tubes by centrifugation (3 min at 5,000 rpm) and frozen immediately after collection in dry ice, then transported to Høyteknologisenteret, Bergen and stored at -80^{o}C until further analysis could take place. This sampling was conducted by Prof. Sigurd Handeland (UiB), Senior researcher Pablo Balseiro (Norce) and PhD student Patrik Tang (UiB).

ELISA Cortisol Concentration

The Cortisol ELISA (enzyme-linked immunosorbent assay) Kit (Demeditec, Kiel) is based on the principle of competitive binding.

The samples were measured in triplicate, and every plate measured included standard and two control samples of a known concentration, in addition to the samples. The microtiter wells were coated with an anti-cortisol antibody. An unknown amount of cortisol present in the sample competes with a known concentration of cortisol horseradish peroxidase conjugate for binding to the well-coating antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase-conjugate is inversely proportional to the concentration of cortisol in the sample. The color developed by TMB (3.3'.5,5'-Tetramethylbenzidine) is measured at 450 nm in a Tecan Spark® multimode microplate reader and compared with known concentration standards. The intensity of color developed is compared with known standards using 4 Parameters Marquardt logistic regression with an extrapolation factor of 1 in the SparkControl Magellan v2.2.10 software.

Blood Chemistry

The Pentra c400 with Ion-Selective Electrode (ISE) module clinical chemistry analyzer (HORIBA, Kyoto Prefecture, Japan) was used to measure sodium and chloride concentration in the plasma samples by potentiometry. The ISE module was calibrated using the ABX Pentra Standard 1, ABX Pentra Standard 2, and ABX Pentra Reference. The samples (>180 µl of plasma was subjected to blood chemistry analysis) were measured using specific electrodes in line with a special membrane and a solution with a known concentration of the ions. The analyzed ion creates a difference of potential across the electrode membrane that is compared to one of the reference electrodes (Buck, 1981). The rest of the analyses performed on the clinical chemistry analyzer were analyzed by colorimetric spectrophotometry determination (calcium, magnesium, glucose, lactic acid) and used the ABX Pentra Multical for calibration of the reagents, followed by a quality control using the ABX Pentra N and P controls as indicated in the manufacturer protocol.

A method based on metallochromogen Arsenazo III using the ABX Pentra Calcium AS CP reagent (HORIBA) was used to measure calcium concentration. In the reaction, calcium ions (Ca^{2+}) reacted with Arsenazo III (2.2'-[1.8-Dihydroxy-3.6-disulphonapthyylene-2.7-bisarzo]-bisbenzenearsonic acid), forming an intense purple-colored chromophore at pH 6.75. The sample (5 µl), distilled water (10 µl), and the reagent (300 µl) were mixed, and absorbance of the Ca-arsenazo III complex was measured bichromatically at 660/700 nm (Michaylova & Ilkova, 1971). The calcium concentration was directly proportional to the increase in absorbance of the reaction mixture. The Arsenazo III has a high affinity for calcium ions (K d = 1 x 10^{-7}), and other cations normally present in the plasma did not show interference with the method, according to manufacturer protocol.

$$Ca^{++} + \text{Arsenazo III} \xleftrightarrow{pH\ 6.75} \text{Ca-Arsenazo III complex (purple)}$$

ABX Pentra Magnesium RTU reagent (HORIBA) was used for the quantitative in vitro diagnostic determination of magnesium. In an alkaline solution, magnesium ions form a purple-colored complex with xylidyl blue. The reagent included GEDTA, which forms complexes with calcium ions and makes the reaction specific. The sample (2.5 µl), distilled water (10 µl), and the reagent (250 µl) were mixed for each analysis. The absorption of the complex was measured at 520/700 nm in a photometric test (Burcar et al., 1964). The magnesium concentration was proportional to the intensity of the purple color of the magnesium-xylidyl blue complex measured in the test, according to manufacturer protocol.

ABX Pentra Glucose HK CP reagent (HORIBA) was used for the quantitative in vitro diagnostic determination of glucose. Glucose was determined using the hexoquinase method, which couples the production of the phosphorylated Glucose-6-phosphate with the posterior production of D-gluconate-6-phosphate and reduction of NAD+. The increase in NADH concentration is proportional to the glucose concentration and can be measured spectrophotometrically at 340/380 nm (Burrin & Price, 1985).

$$Glucose + O_2 \xrightarrow{Glucose\ oxidase} Glucose\ acid + H_2O_2$$

$$2H_2O_2 + Phenol + 4AAP \xrightarrow{Peroxidase} Quinoneimine + 4H_2O$$

ABX Pentra Lactic Acid reagent (HORIBA) was used for the quantitative in vitro diagnostic determination of lactic acid. The release of hydrogen peroxide is triggered by lactate oxidase. Hydrogen peroxide then reacts with 4 – aminoantipyrine and ESPAS (*N-ethyl-N-sulfopropyl-m-anisidine*) to a colored complex in the presence of peroxidase that is measured bichromatically at 550/700 nm. Lactate concentration present in the sample was proportional to the intensity of the coloring (Trinder, 1969).

$$Lactate + O_2 \xrightarrow{Lactate\ oxidase} Pyruvate + H_2O_2$$

$$2H_2O_2 + 4AAP + ESPAS \xrightarrow{Peroxidase} Quinoneimine + 4H_2O$$

(4 AAP = 4-aminoantipyrine, ESPAS = N-ethyl-N-sulfopropyl-m-anisidine)

Statistical Analyses

All statistical analyses and figures were generated using RStudio (Version 1.2.500, Rstudio, Inc, Boston, MA, USA) and R (Version 3.6.1, R core team, Vienna, Austria), including the following packages; Rcompanion (Mangiafico, 2018), car (Fox & Weisberg, 2011), dplyr (Wickham et al., 2018), ggplot2 (Wickham, 2016), tidyr (Wickham & Henry, 2019), gridExtra (Augie, 2017), plyr (Wickham, 2011), ggpubr (Kassambara, 2018), reshape 2 (Wickham, 2007), and multcomp (Hothorn et al., 2008).

Outliers, with values greater than 1.5 times the interquartile range, were excluded using the Tukey fences method in Excel (Microsoft, Redmond, Washington, USA), although the outliers are retained in the figures. The datasets were checked for normality and homogeneity of variance assumptions using the Shapiro-Wilk test and the Levene test, respectively. To determine the degree of significance of differences in blood chemistry parameter concentrations, a one-way ANOVA with a Tukey post-hoc test was conducted. If the requirements for normality or homogeneity of variances were not met, a data transformation was conducted using the Tukey Ladder of Powers transformation (Mangiafico, 2016). If the transformation still failed to satisfy the assumptions, a non-parametric Kruskal–Wallis test was conducted. The Tukey HSD and Mann–Whitney–Wilcoxon post-hoc comparison tests were used for the Kruskal–Wallis models. The degree of significance between the groups in this study was considered as significant when p-value < 0.05 and flagged with one star (*). If the p-value is less than 0.01, it is flagged with two stars (**). If a p-value is less than 0.001, it is

flagged with three stars (***) and if a p-value is less than 0.0001, it is flagged with (****). All the statistical results in this study are reported in the Appendix II.

Results

Plasmatic Cortisol Concentration

A significant increase in the cortisol level (Figure 1.6A) was observed for Preline ACT compared to Preline Baseline ($p < 0.0001$, Wilcoxon). Further, a significant increase was observed for the Preline ACT compared to Preline Ref ACT ($p < 0.0001$, Wilcoxon). No difference was observed between the Preline Ref ACT and Preline Ref Baseline. For the Preline Baseline and Preline Ref Baseline, a significant increase in cortisol level was observed in Preline Ref Baseline ($p < 0.0001$, Wilcoxon)

A significant increase (Figure 1.6B) in cortisol level was observed in Neptune ACT compared to Neptune Baseline ($p < 0.001$, Wilcoxon). Further, between Neptune ACT and Neptune Ref ACT, a significantly increase was observed for Neptune ACT ($p < 0.01$, Wilcoxon). In addition, significant increase was observed for the Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.01$, Wilcoxon). Between Neptune Baseline and Neptune Ref Baseline, no difference was observed in the cortisol level.

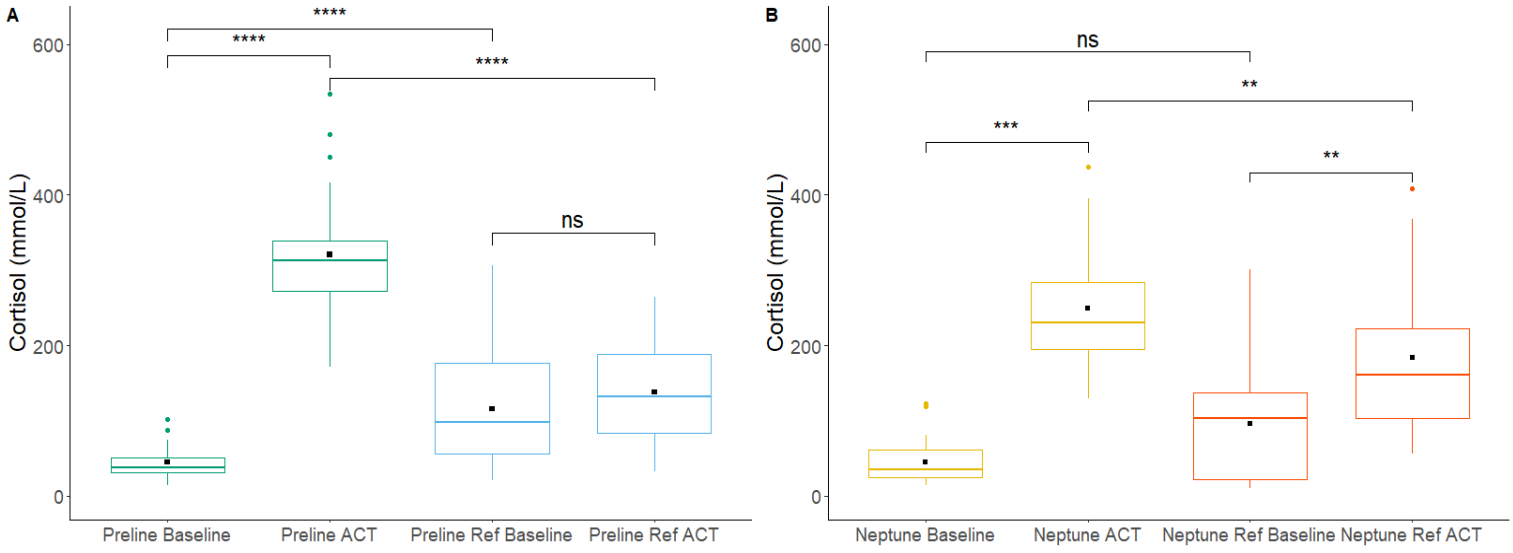

Figure 1.6. Plasma cortisol concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: median, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), $p<0.0001$(****), ns = no significance, as assessed by the Wilcoxon post-hoc test.

Plasmatic Chloride Concentration

Between Preline ACT and Preline Baseline (Figure 1.7A), a significantly higher increase in the chloride concentration was observed for the Preline ACT ($p < 0.001$, Tukey). No difference in chloride level was observed between the Preline ACT and Preline Ref ACT. In addition, significant increase was observed in Preline Ref ACT compared to Preline Ref Baseline ($p < 0.001$, Tukey). In the Preline Ref Baseline, a significantly higher chloride level was observed compared to Preline Baseline ($p < 0.05$, Tukey).

A significant increase in chloride level (Figure 1.7B) was observed in Neptune ACT compared Neptune Baseline ($p < 0.001$, Tukey). Further, between Neptune ACT and Neptune Ref ACT, no difference was observed in chloride level. A significant increase in chloride level was observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.01$, Tukey). Also, for the Neptune Baseline and Neptune Ref Baseline, no difference was observed in chloride concentration.

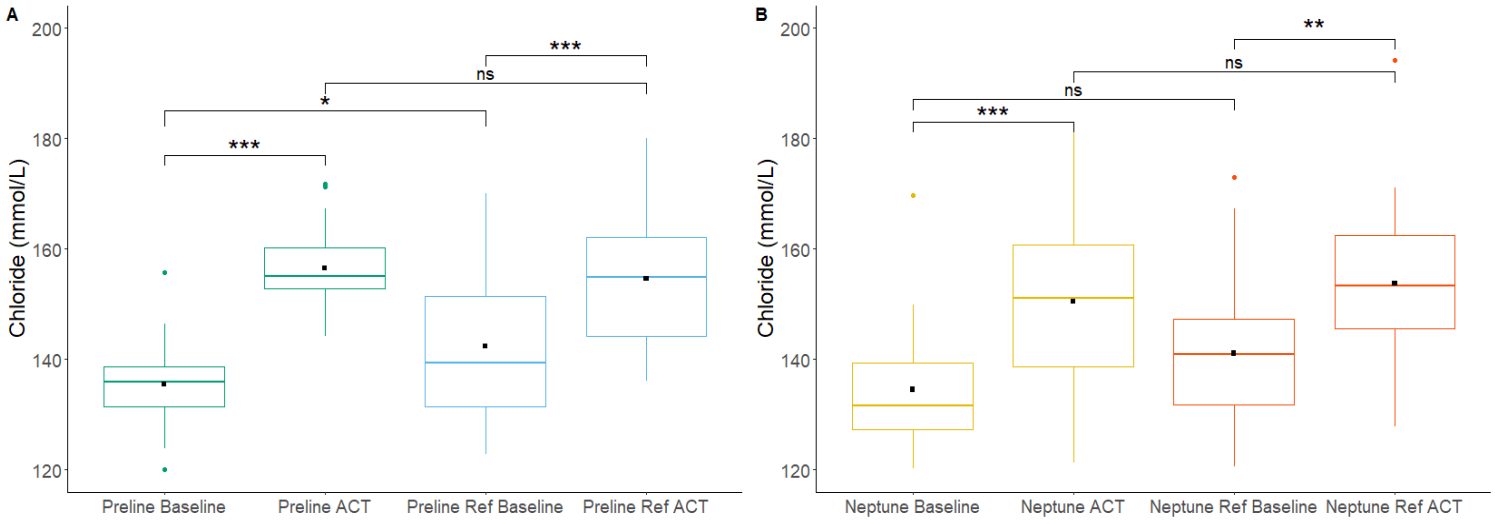

Figure 1.7. Plasma chloride concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: median, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), ns = no significance, as assessed by the Tukey's post-hoc test.

Plasmatic Sodium Concentration

Between Preline ACT and Preline Baseline (Figure 1.8A), a significantly higher increase in the sodium concentration was observed for Preline ACT ($p < 0.0001$, Wilcoxon). No difference was observed between Preline ACT and Preline Ref ACT. In addition, significant increase was observed in Preline Ref ACT compared to Preline Ref Baseline ($p < 0.001$, Wilcoxon). For the Preline Baseline and Preline Ref Baseline, a significant increase in sodium level was observed in Preline Ref Baseline ($p < 0.001$, Wilcoxon).

A significant increase in sodium level (Figure 1.8B) was observed in the Neptune ACT compared to Neptune Baseline ($p < 0.001$, Tukey). Further, between Neptune ACT and Neptune Ref ACT, no difference was observed in sodium level. A significant increase in sodium level was observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.001$, Tukey). For the Neptune Baseline and Neptune Ref Baseline, a significant increase was observed in Neptune Ref Baseline ($p < 0.001$, Tukey).

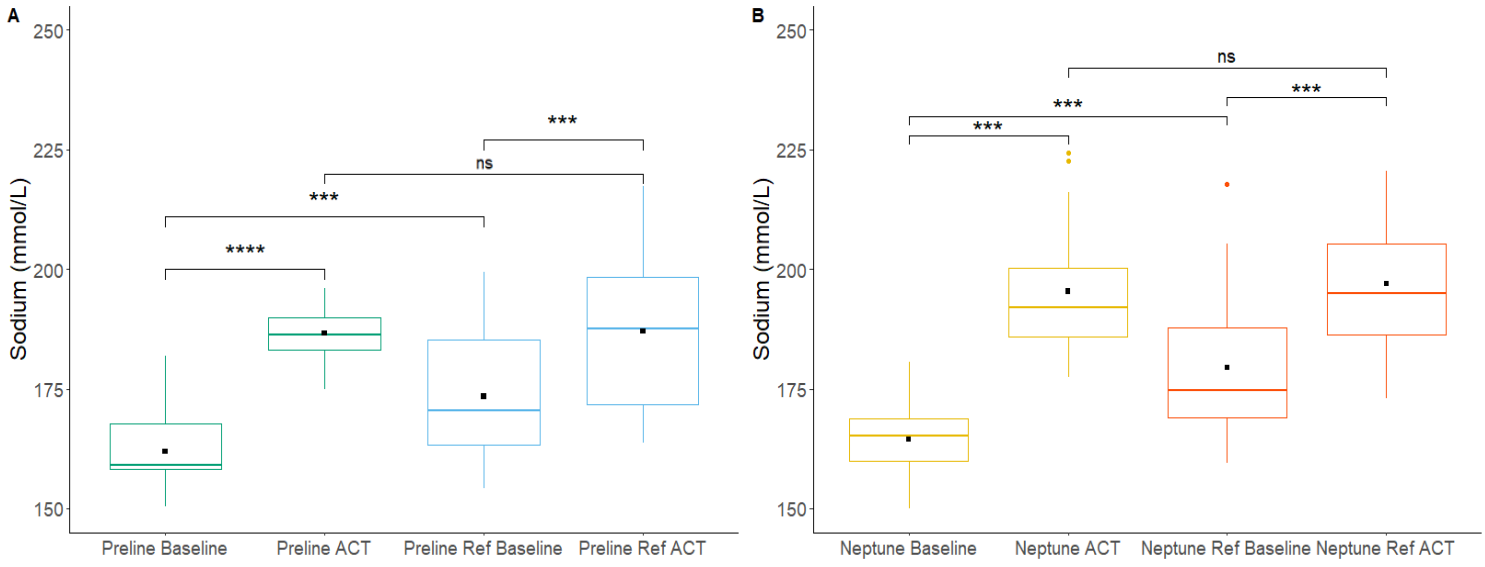

Figure 1.8. Plasma sodium concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: medians, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****), ns = no significance as assessed by the Wilcoxon post-hoc test for the Preline groups and Tukey's post-hoc test for the Neptune groups.

Plasmatic Calcium Concentration

Between Preline ACT and Preline Baseline (Figure 1.9A), a significant increase in the calcium concentration was observed for Preline ACT ($p < 0.0001$, Wilcoxon). No difference was observed between Preline ACT and Preline Ref ACT. In addition, significant increase was observed for the Preline Ref ACT compared to Preline Ref Baseline ($p < 0.001$, Wilcoxon). For the Preline Baseline and Preline Ref Baseline group, a significant increase in calcium level was observed in Preline Ref Baseline ($p < 0.01$, Wilcoxon).

A significant increase in calcium level (Figure 1.9B) was observed in Neptune ACT compared to Neptune Baseline ($p < 0.001$, Tukey). Further, between Neptune ACT and Neptune Ref ACT, no difference was observed in calcium level. A significantly higher calcium level was observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.001$, Tukey). A significantly higher calcium concentration was observed in Neptune Ref Baseline than in Neptune Baseline ($p < 0.05$, Tukey).

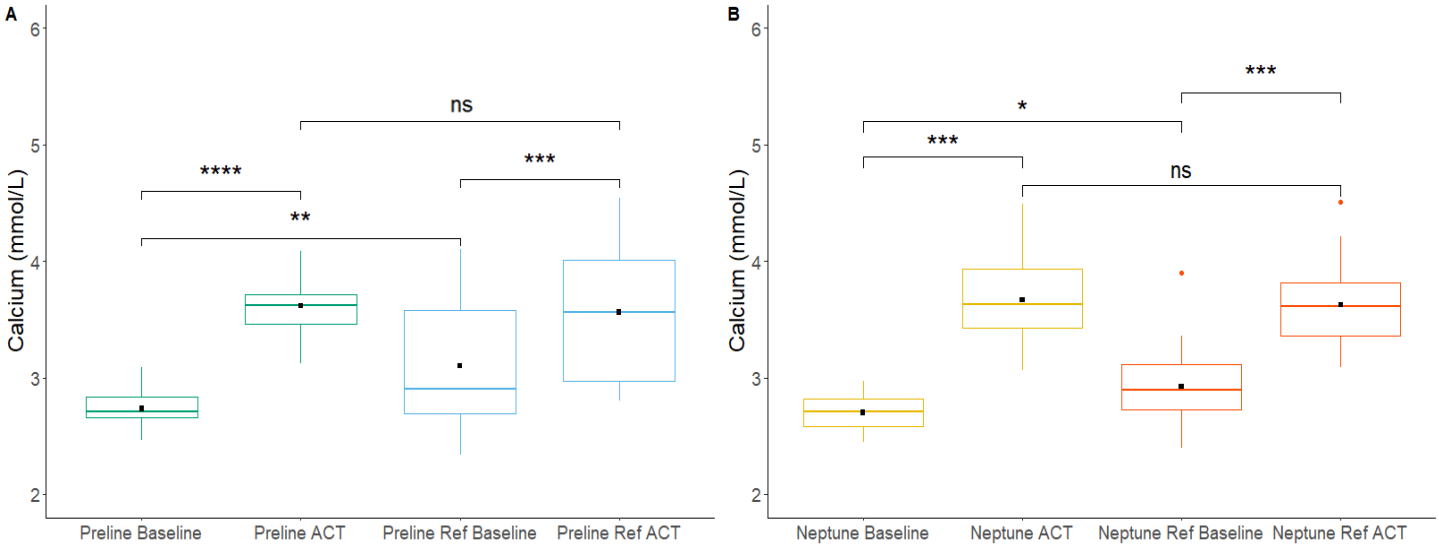

Figure 1.9. Plasma calcium concentration in Preline (A) and Neptune (B) S-CCS, both in the Baselines for each system and after Acute Test Challenge (ACT). Comparative Reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: medians, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), $p<0.0001$(****), ns = no significance as assessed by Wilcoxon post-hoc test for the Preline groups and Tukey's post-hoc test for the Neptune groups.

Plasmatic Magnesium Concentration

A significant increase in the magnesium (Figure 1.10A) concentration was observed in Preline ACT compared to Preline Baseline ($p < 0.0001$, Wilcoxon). No difference in magnesium was observed between Preline ACT and Preline Ref ACT. In addition, significantly higher magnesium concentration was observed in the Preline Ref ACT compared to Preline Ref Baseline ($p < 0.001$, Wilcoxon). Between Preline Baseline and Ref Baseline group, no difference in magnesium level was observed.

A significant increase (Figure 1.10B) in magnesium level was observed in Neptune ACT compared to Neptune Baseline ($p < 0.001$, Tukey). Further, in Neptune Ref ACT a significant increase was observed compared to Neptune ACT ($p < 0.01$, Tukey). A significant increase in magnesium was also observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.001$, Tukey). For the Neptune Baseline and Neptune Ref Baseline, significant increase in magnesium concentration was observed in Neptune Ref Baseline ($p < 0.01$, Tukey).

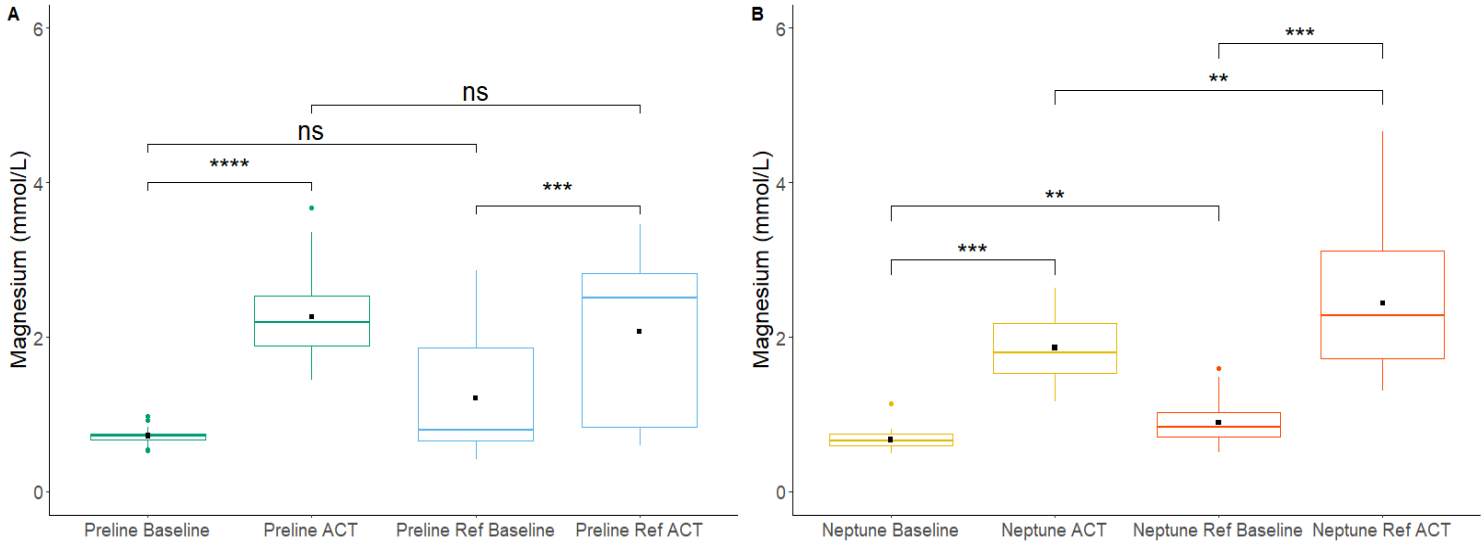

Figure 1.10. Plasma magnesium concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: medians, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), $p<0.0001$(****), ns = no significance as assessed by the Wilcoxon post-hoc test for the Preline groups and Tukey's post-hoc test for the Neptune groups.

Plasmatic Glucose Concentration

In the comparisons between (Figure 1.11A), Preline ACT – Preline Baseline, Preline ACT – Preline Ref ACT, Preline Ref ACT – Preline Ref Baseline, no difference was observed among the groups. For Preline Baseline and Preline Ref Baseline, a significantly lower glucose level was observed in Preline Baseline compared to Preline Ref Baseline ($p < 0.05$, Tukey).

A significant increase in glucose level (Figure 1.11B) was observed in Neptune ACT compared to Neptune Baseline ($p < 0.05$, Tukey). Further, in Neptune Ref ACT, a significantly higher glucose level was observed compared to Neptune ACT ($p < 0.001$, Tukey). A significant increase in glucose level was observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.05$, Tukey). For the Neptune Baseline and Neptune Ref Baseline, significant increase was observed in Neptune Ref Baseline ($p < 0.001$, Tukey).

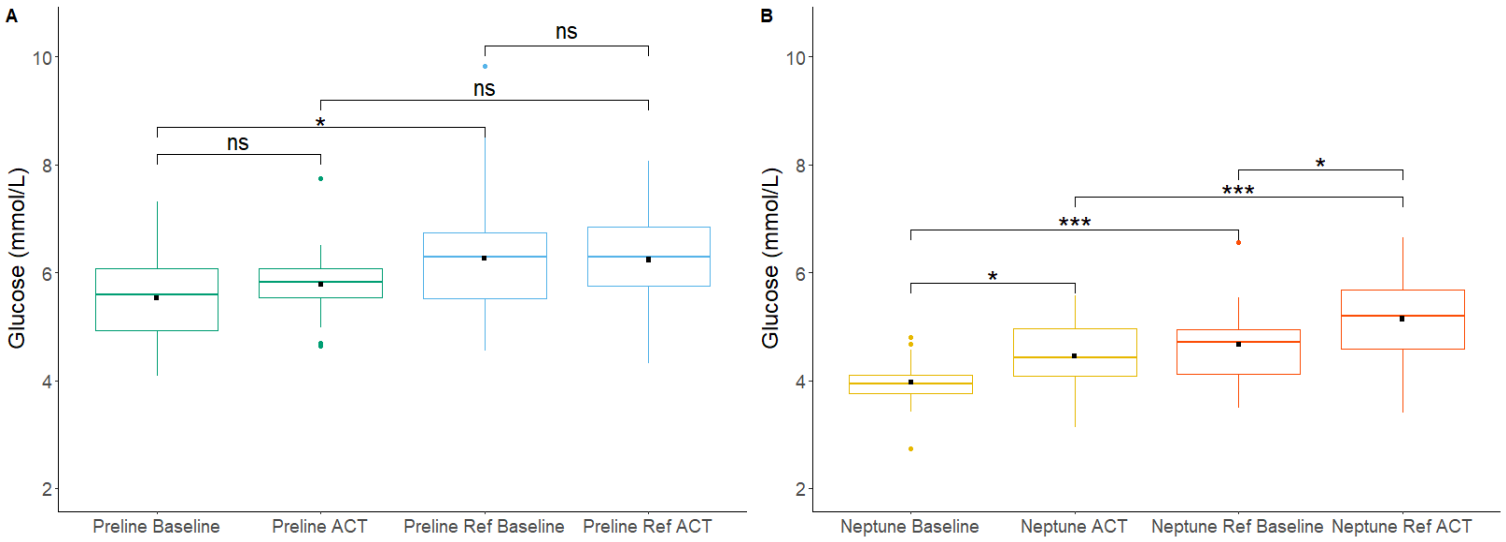

Figure 1.11. Plasma glucose concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: medians, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), ns = no significance as assessed by Tukey`s post-hoc test for Preline and Neptune groups.

Plasmatic Lactic Acid Concentration

A significant increase (Figure 1.12A) in the lactic acid concentration was observed in Preline ACT compared to Preline Baseline ($p < 0.0001$, Wilcoxon). No difference was observed between Preline ACT and Preline Ref ACT. In addition, significant increase was observed in Preline Ref ACT compared to Preline Ref Baseline ($p < 0.05$, Wilcoxon). Between the Preline Baseline and Preline Ref Baseline group, no difference was observed in lactic acid level.

Between Neptune ACT and Neptune Baseline (Figure 1.12B), a significant increase in lactic acid level was observed for Neptune ACT ($p < 0.001$, Tukey). However, between Neptune ACT and Neptune Ref ACT, no difference was observed. A significant increase in lactic acid level was observed in Neptune Ref ACT compared to Neptune Ref Baseline ($p < 0.001$, Tukey). Significant lower lactic acid concentration was observed for the Neptune Baseline compared to the Neptune Ref Baseline ($p < 0.001$, Tukey)

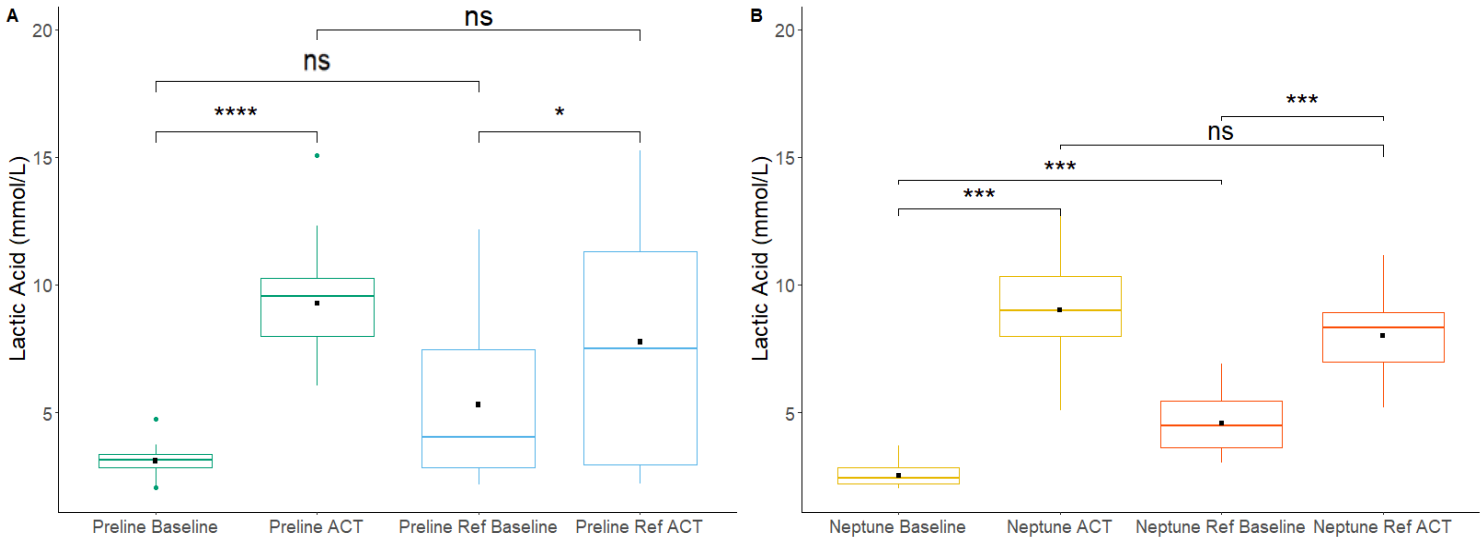

Figure 1.12. Plasmatic lactic acid concentration in Preline (A) and Neptune (B) S-CCS, both in the baselines for each system and after the Acute Test Challenge (ACT). Comparative reference groups are also included. In the boxplot, the upper line represents the 75% quantile, middle line: medians, 50% quantile, and lower line: 25% quantile. The black (square) dot represents the mean and the colored dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***), $p<0.0001$(****), ns = no significance as assessed by the Wilcoxon post-hoc test for the Preline groups and Tukey's post-hoc test for the Neptune groups.

Discussion of Methodology

Discussion of Methods: Chapter 1 – Acute Challenge Test in Two S-CCS

When comparing different rearing conditions (Preline and Neptune *versus* their Reference), a factorial design including replicates of this experiment would be preferred. This implies that all fish in the experiment originated from the same genetic strain and were reared in the same conditions during the freshwater and seawater stage.

The samplings of the ACT and baseline treatment was a one-time event, and no replicates were conducted. Ideally, all the systems should have replicates, and the physical environmental parameters should be controlled. In order to achieve a good comparison, sampling from two S-CCS and reference groups was conducted. The experiment was divided into two similar experiments; experiment 1 (Preline – Preline Reference) and experiment 2 (Neptune – Neptune Reference). Hence, the fish in the Preline and Neptune groups experienced similar rearing conditions during the experimental period. However, in a large-scale production study, identical environmental conditions between the experimental groups are impossible to achieve, in contrast to small-scale lab experiments. Despite these factors and variations, the present results from both the S-CCS systems clearly shows a similar impact and effect on post-smolt reared within these systems.

The experiment consisted of two treatments (Baseline and ACT), logistics and long distances between the locations made it impossible to conduct the samples in both groups on the same day. This applied to the samplings in both experiments. In experiment 1 (Preline system) the samplings for both groups were conducted within a period of 10 days. In experiment 2, (Neptune system) the samplings were conducted within two days. The baseline and ACT sampling at each location were conducted the same day, which was important since some of the measured parameters are influenced by feeding time, daylight, temperature and other factors. For instance, cortisol follows the circadian rhythm that releases varied levels of cortisol

during the day (trigged by temperature, photoperiod, and feeding time) in the fish (Barton, 2002; Mommsen et al., 1999; Wendelaar Bonga, 2011).

In the two S-CCS (Preline and Neptune), the water parameters in the systems were quite stable during the experimental period, as a result of the fixed water inlet being at a depth of 25–30 m. For the fish in the reference groups reared in the open sea cages, the locations were exposed to different water masses during the experimental period due to stratification. In addition, the fish swam freely and distributed themselves over various depths, making it hard to determine precisely which parameter the fish were exposed to, in contrast to fish reared in the S-CCS. Consequently, this swimming behavior might cause variety in the fish material that were part of the experiments.

Discussion of Results

Cortisol

The main glucocorticoid produced by fish is cortisol, which, besides its central role in the stress response and stress-related homeostasis, influences many other processes, such as behavior, growth, reproduction, and osmoregulation (Wendelaar Bonga, 1997; Mommsen et al, 1999). Cortisol is also central for its involvement in the "fight-or-flight" response and temporary increase in energy production, at the expense of processes that are not required for immediate survival (Cannon, 1915). Thus, plasma cortisol is a widely used indicator of stress in fish (Wendelaar Bonga, 1997). Cortisol syntheses and its release from internal cells have a delay of several minutes, making it possible to measure the resting level of this hormone in fish. Fish that are in a state of good welfare increase cortisol levels to react to an acute challenge according to the concept of allostasis (Korte et al., 2007). This contrasts with fish that are exposed to chronic stress, where plasma cortisol falls back to resting levels, even though the fish may be responding to the stressor (Vijayan & Leatherland, 1990).

In the present study, both groups reared in S-CCS (Preline and Neptune) showed an acute increase of cortisol levels 1 hour after exposure to an ACT, which could be an indication of a good state of welfare for the fish. Further, studies have showed that salmonids exposed to a chronic stress situation have a reduced cortisol response to an acute stressor (Grassie et al., 2013; Madaro et al., 2016). In this experiment, both reference groups showed a lower cortisol response to ACT compared to the S-CCS groups. This might imply that long-term exposure of a suboptimal environment has an impact on reduced cortisol response for the reference groups in open pens. The baseline groups in both S-CCS showed lower cortisol levels compared to the reference baseline group. Baseline levels of plasma cortisol could give information about whether the fish are experiencing chronic stressors (lice pressure, diseases, density, feed,

environmental conditions, maturation status). They may even indicate future survival, given that chronic stress also affects the immune response in fish (Iversen & Eliassen, 2014). The findings in this study suggest that fish reared in S-CCS show a lower stress response compared to the reference groups in open pen. However, other influencing factors cannot be disregarded as a possible cause of this difference (Barton, 2002). Knowledge of normal plasmatic cortisol concentration baseline range in Atlantic salmon cultured in Norway across multiple situations is scarce and could help with the interpretation of future results.

Chloride and Sodium

For the ions, an acute stressor acts on the tight junctions of the epithelium, with induced leakage of chloride and sodium as a consequence (McDonald & Milligan, 1997). Nevertheless, plasma ions as measurements for chronic stress or long-term exposure to a stressor are difficult to interpret since the ion concentration is context-specific and is affected by internal and external factors (McDonald & Milligan, 1997). In the present study plasma chloride and sodium concentrations showed an increase in all the ACT groups. The increment of ions could be related to increment of ions leaked from the surrounding environment or to water loss, causing dehydration and increasing the ion concentration (differences in potassium and phosphorus represented in appendix I supports that this was not related to dehydration). The ACT groups showed no difference in the comparison between both S-CCS and reference groups. This indicates that when exposed to an acute stressor, the leaking of ions is similar in both the S-CCS and in the reference systems. These findings are similar to the study done by Einarsdòttir and Nilssen (1996), where chloride concentrations after a stress challenge, showed similar response between the experimental groups. One reason for this could be that the plasma ions did not manage to reach their maximum within the sampling period (>1 hour), and the maximal level of ion leakage over time is probably dependent on the environment and salinity concentrations in the systems. Also, another explanation could be that the plasma ions reached

a maximum and therefore no difference between was determined for ACT in the S-CCS groups and reference.

For the baseline groups, there was a clear difference in the plasma sodium concentration, indicating that fish reared in S-CCS have a lower level of sodium than in the reference group. Further, the plasma chloride concentration for baseline in the Preline system was lower than the Reference Baseline. This may be a result of exposure to water with different salinities (Preline has an inlet from 30 m depth) and ion concentrations. In the open sea, salinity is high and stable (35‰) in contrast to the fjords where salinity is affected by seasonal changes and present a higher stratification (Rinde et al., 2014). For the Neptune system and the reference group, the plasma chloride concentration was close to similar, indicating that the chloride level in the groups is not affected by the rearing system.

Calcium

Several different hormones and receptors interact in maintaining extracellular Ca^{2+} levels, which need to be tightly regulated. Prolactin and growth hormone are hypercalcemic (Pang et al., 1971), while calcitonin and stanniocalcin are hypocalcemic (Copp et al., 1962; Hirsch et al., 1964). Moreover, cortisol has been shown to stimulate calcium uptake through the gills in salmonids (Flik & Perry, 1989) and to influence intracellular calcium concentrations (Mommsen et al., 1999).

The present findings showed an increase in calcium concentration in all the ACT groups, suggesting an increase of ion leakage as a consequence of either the ACT-test or dehydration. The ACT groups showed no significance in the comparison between S-CCS and reference. This indicates that when exposed to an acute stressor, the leaking of ions from the fish is similar in both the S-CCS and reference systems. As for the other ions, it should be noted that the plasma calcium may not manage to reach its maximum within the sampling period (>1 hour). For the baseline groups, there was a difference in the plasma calcium concentration, indicating that fish

reared in S-CCS have a lower level of calcium than in the reference, which suggests that plasma calcium concentration is affected by the rearing system and is related to the environment.

Magnesium

Changes in magnesium balance are a good indicator of acute stress. Studies have shown that there is a high correlation between increased plasma magnesium and mortality after undergoing a stressor (Iversen & Eliassen, 2009; Liebert & Schreck, 2006). In addition, plasma magnesium as a measurement for chronic stress, may be difficult to interpret since the plasma magnesium is context-specific and is affected by internal and external factors (McDonald & Milligan, 1997). An increase of plasma magnesium levels was observed for ACT groups in both the S-CCS, and the baseline response showed lower individual variability compared to reference groups. This could be a result of water intake from a deeper layer in the S-CCS, as explained earlier. Further, for the Preline Reference group, significantly higher concentration was observed in the ACT than in the baseline. Moreover, in the Preline reference groups there was high individual variability compared to the Preline group.

For the Neptune system, the reference ACT group showed a higher individual response compared to the Neptune ACT. These results imply that the low variability of magnesium concentration in Neptune system are affected by rearing conditions. In contrast to the Preline groups, where no difference in magnesium concentration was found between the ACT and baseline groups.

Glucose

Glucose is a central and fundamental energy substrate in all vertebrate metabolism (Mergenthaler et al., 2013). The glucose can be absorbed through the gut during digestion or produced endogenously by the kidney and the liver, through either the breakdown of glycogen (glycogenolysis) or synthesis (gluconeogenesis) from amino acids and/or glycerol. Glucose is also produced through the Cori cycle, where lactate produced by anaerobic glycolysis in

muscles is transported to the liver and converted to glucose (Lehninger et al., 2005; Mommsen et al., 1999). The regulation of glucose availability and storage is tissue-specific, and both glucocorticoids and catecholamines play a central role in glucose regulation during stress in fish (Faught & Vijayan, 2016; Mommsen et al., 1999). An increase in plasma cortisol stimulates the glycogenolysis and starts the conversion of glycogen stored in the tissue in glucose. The glucose is then released into the bloodstream (Barton & Iwama, 1991). In the present study, the concentration of cortisol levels was similar for both the Preline and Neptune system. These findings correspond to the study of Einarsdottir and Nilssen (1996) showing no significant changes in plasma glucose in Atlantic salmon exposed to an acute stressor. Moreover, increased levels of plasma glucose could be used for investigation after having undergone an acute stress experience but should then be compared to a baseline group since glucose levels are also dependent on nutrient types, diet type, and other factors (Mommsen et al., 1999).

The plasma glucose level in the Preline group was similar for the ACT and reference groups. The increase in plasma glucose is a relatively slow response to stress and tends to reach a maximum after approximately 3–6 hours in Atlantic salmon (Olsen et al., 2002). This could explain that the groups did not manage to reach its maximum level of plasma glucose within the sampling period (>1 hour). Further, the overall values of plasma glucose in the Preline and reference fish were higher compared to the Neptune groups. The observed difference among these systems should be seen in context because glucose concentration is influenced by external and internal factors (Mommsen et al., 1999). For instance, the Preline and Neptune groups originate from different strains and had different diets during this study and the sampling was not conducted the same day.

In the Neptune groups, difference in plasmatic glucose concentration was observed between ACT and baseline, suggesting that sampling within 1 h was timely enough to observe differences between these systems, in contrast to the observations in Preline.

Lactic Acid

Lactate is the product of the anaerobe glycolysis in the cells, which is a result of an insufficient amount of oxygen available for the aerobe cell metabolism. This could be achieved when low oxygen levels are reached in the water (Remen et al., 2012) or by hard physical activity (Milligan & Girard, 1993). Lactate is mainly an indication of high muscle activity that often relates to an exposure of a stressor for fish (Iversen et al., 2003).

In the Preline system, an increase in lactic acid concentration is observed for the ACT groups, while the corresponding baseline showed a low response. This might be explained by a high muscle activity for fish reared in the Preline system.

Further, for the Preline Reference group, although some difference ($p < 0.05$) was shown between ACT and baseline, the high individual variability in these groups could mask the increment of lactic acid concentration as a result of ACT. Therefore, the reduced ability to increase lactic acid after being exposed to an acute stressor for the Preline reference group, suggest a higher stress level in the fish from this group. Moreover, the same trend is shown for the Neptune system, an increase in lactic acid concentration is observed for the ACT group, while the corresponding baseline showed a low and concentrated response, again, indicating high muscle activity for fish reared in the Neptune system. This probably relates to the exercise achieved in the Preline and Neptune system.

Conclusion

The ACT experiments determined that post-smolt reared in S-CCS show a general stronger response to an acute stress test and had a lower baseline value at rest. The stable water conditions and exposure to continuous water current generated by the two S-CCS have a positive impact on the fish based on several of the parameters measured in this study.

- **$H0_1$**: Post-smolt rearing methods (S-CCS or cage) have no significant effect on plasmatic cortisol concentration after an acute challenge test (ACT), is rejected for fish reared in S-CCS, and thereby **$H1_1$ is accepted**. The post-smolt rearing method (S-CCS or sea cage) have a significantly effect on plasmatic cortisol concentration after an ACT.
- **$H0_2$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic chloride concentration after an acute challenge test (ACT) **is accepted**. No difference was observed in plasmatic chloride between the ACT groups in S-CCS and open reference cage in the experiments.
- **$H0_3$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic sodium concentration after an acute challenge test (ACT) **is accepted**. No difference was observed in plasmatic sodium between the ACT groups in S-CCS and open reference cage in the experiments.
- **$H0_4$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic calcium concentration after an acute challenge test (ACT) **is accepted**. No difference was observed in plasmatic calcium between the ACT groups in S-CCS and open reference cage in the experiments.
- **$H0_5$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic magnesium concentration after an acute challenge test (ACT) **is rejected**. Difference in magnesium level for the ACT was observed

between the rearing methods. In Preline groups, no difference in ACT was found but was evident for the Neptune system, therefore **$H1_5$ is accepted**. The post-smolt rearing method (S-CCS or sea cage) have a significant effect on plasmatic magnesium concentration after an ACT.

- **$H0_6$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic glucose concentration after an acute challenge test (ACT) is rejected. For the Preline system no difference was observed in glucose level, in contrast to the Neptune system were a difference was shown in glucose level for ACT, and consequently, **$H1_6$ is accepted**. The post-smolt rearing method (S-CCS or sea cage) have a significant effect on plasmatic glucose concentration after an ACT.
- **$H0_7$**: Post-smolt rearing methods (S-CCS or open reference cage) have no significant effect on plasmatic lactic acid concentration after an acute challenge test (ACT) **is accepted**. No difference was observed in lactic acid between the ACT groups in S-CCS and open reference cage in the experiments.

Chapter 2 – Benchmark Analysis of Six Generations Reared in the Preline and Reference Groups

Objectives

This study aims to benchmark biological performance (growth, feed conversion ratio, mortality, and sea lice infestation) in fish reared in the Preline system (S-CCS), and fish reared in a traditional open cage system. The study follows the fish from stocking in seawater until harvest throughout two phases; phase 1 – post-smolt and phase 2 – grow-out. The experiment was based on several hypotheses in both phases.

Hypotheses for phase 1 (post-smolt, a):

$H0_{1a}$: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on growth compared to the reference group.

$H1_{1a}$: Rearing Atlantic salmon post-smolt in Preline system has a significant effect on growth compared to the reference group.

$H0_{2a}$: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on feed conversion ratio compared to the reference group.

$H1_{2a}$: Rearing Atlantic salmon post-smolt in Preline system has a significant effect on feed conversion ratio compared to the reference group.

$H0_{3a}$: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on mortality compared to the reference group.

$H1_{3a}$: Rearing Atlantic salmon post-smolt in Preline system has a significant effect on mortality compared to the reference group.

$H0_{4a}$: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on sea lice infestations compared to the reference group.

$H1_{4a}$: Rearing Atlantic salmon post-smolt in Preline system has a significant effect on sea lice infestations compared to the reference group.

Hypotheses for phase 2 (grow-out in open sea cages, b):

$H0_{1b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on growth during the grow-out phase compared to the reference group.

$H1_{1b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on growth during the grow-out phase compared to the reference group.

$H0_{2b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on feed conversion ratio.

$H1_{2b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on feed conversion ratio.

$H0_{3b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on mortality.

$H1_{3b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on mortality.

$H0_{4b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on sea lice infestations.

$H1_{4b}$: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on sea lice infestations

Materials and Methods

Fish Material and Rearing Conditions

In all generations (Table 2.1), the eggs were incubated in water (approximately 8–10ºC). The alevins were first fed approximately 390-degree days (dC) post-hatching, in 6 m tanks (circular, green, fiberglass, rearing volume 75 m^3) at constant light and in heated water (approximately 13–14 ºC). The light was provided by a fluorescent tube mounted under the tank cover (light intensity 50 lux at 0.5 m depth). When the fish reached a size of approximately 6–8 g, they were transferred from 6 m tanks to 8 m (circular, green fiberglass, volume 90 m^3), reared at constant light and further fed a standard dry diet (Ewos, Norway), according to temperature and fish size (Austreng et al., 1987). Finally, the fish were vaccinated at a size of 50–60 g and were then transferred to 15 m tanks (circular, green, fiberglass, volume 150 m^3) where they were supplied with ambient temperature freshwater and reared as described above. In all tanks, the oxygen content in outlet water was measured every day and was kept above 80%. During the experimental period, the 0+ fish experienced a freshwater temperature ranging from 12 to 20 ºC, while the 1+ smolts experienced a temperature varying from 6 to 18ºC.

To stimulate parr-smolt transition, a traditional photoperiod regime was conducted for all generations (Handeland & Stefansson, 2001). The treatment included a decrease in day-length from LD24:0 to LD12:12 for 5 weeks, followed by another 4 weeks on LD24:0. At the end of photoperiod treatment, fish in all generations showed typical morphological and physiological changes characteristics of smolting, including dark fin margins and silvery scales, and high gill NKA activity (McCormick, 1993; Stefansson et al., 2003). When the fish had completed the parr-smolt transformation, the group was split into two equal-sized groups (Preline and reference) and transferred to seawater by a well boat within three weeks.

Table 2.1: *Summary of rearing conditions in freshwater stage (generation, strain) and production data (smolts, incubation temperature, degree days; from fertilization to first feeding, first feeding date, rearing temperature).*

Generation	Smolts (0+,1+)	Incubation Temperature	Degree days	First feeding Date	Rearing Temperature	Strain
Gen 1	1+	7.4 °C	824	11.05.14	10.2 °C	Salmobreed QTL duo
Gen 2	0+	7.5 °C	914	14.02.15	15.1 °C	Salmobreed QTL duo
Gen 3	1+	7.4 °C	810	08.05.15	10.1 °C	Salmobreed QTL duo
Gen 4	0+	7.4 °C	902	18.02.16	15.0°C	Salmobreed QTL duo
Gen 5	1+	7.5 °C	826	13.05.16	10.2 °C	Salmobreed QTL duo
Gen 6	0+	7.5 °C	915	09.02.17	15.1 °C	Salmobreed QTL duo

Experimental Design

The fish used in this study were 0+ and 1+ Atlantic salmon smolts of the Salmobreed strain that had been reared at Sjøtroll Havbruk AS (Kjærelva, Fitjarstern Norway) from hatching to the smolt stage. A total of six generations were part of the experiment Generation 1 (n = 348,661), Generation 2 (n = 350,501), Generation 3 (n = 320,559), Generation 4 (n = 255,033), Generation 5 (n = 364,701) and Generation 6 (n = 464,540).

Before seawater transfer, each generation was divided into two separate groups; Preline (S-CCS) and reference group. The groups were then followed through two experimental phases;

Phase 1. Post-smolt in seawater (S-CCS): In the period from May 2015 to February 2019, a total of six generations of salmon post-smolts were transferred from freshwater (Storelva, Fitjar) to seawater by well boat (Preline and reference). Generation 1, 3 and 5 were stocked during spring, and generation 2, 4, and 6 were stocked during fall.

In phase 1 post-smolt, the Preline group fish were reared 4-6 months from approximately 100 to 284–844 g (rearing conditions presented in Table 2.3).

Phase 2. Grow-out phase in seawater: After undergoing post-smolt phase (4–6 months), the Preline fish were transferred by a well boat to a new location that was equipped with traditional sea cages for a further grow-out phase (Figure 2.3), where they grew 10-12 months from approximately 284–844 g (final weight phase 1) to 3,360–5,700 g (final weight phase 2). The grow-out experiment lasted until the first of the two groups (Preline and reference) were slaughtered (rearing conditions presented in Table 2.4).

A schematic representation of the experimental protocol is depicted in Figure 2.1 and overview over the facilities in Figure 2.2 and Table 2.2.

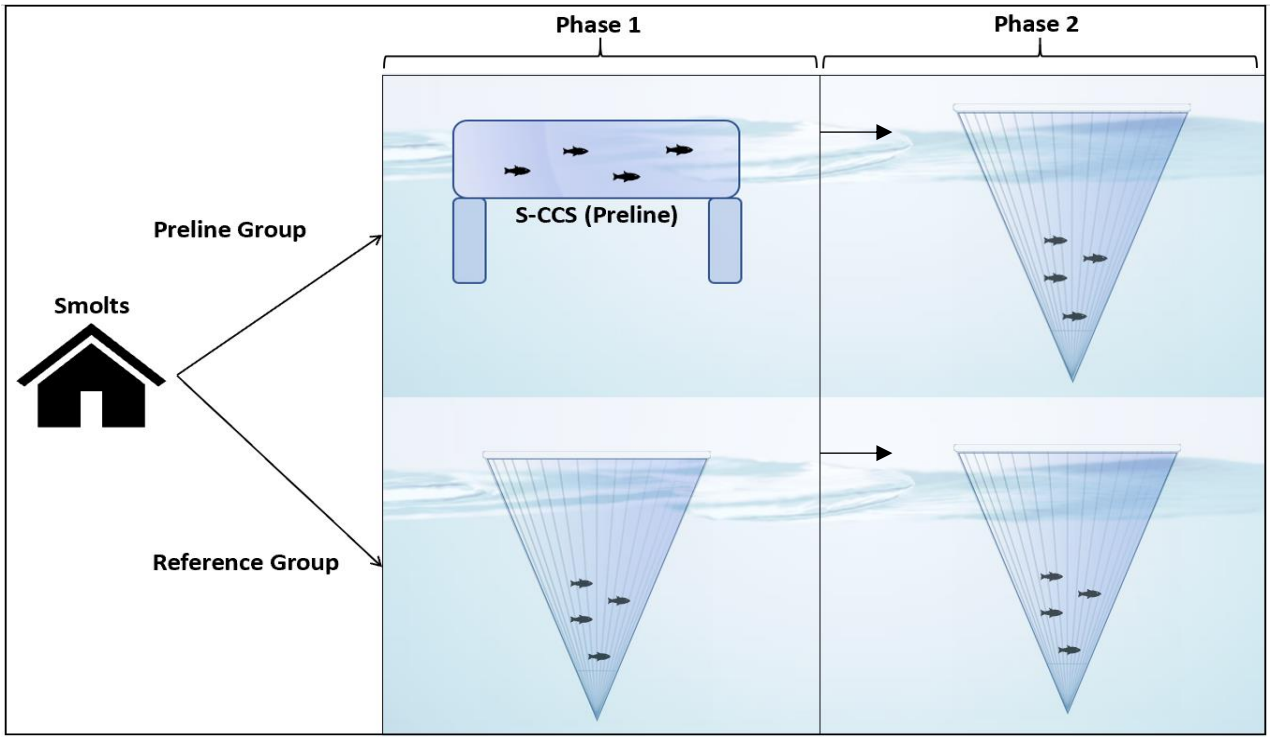

Figure 2.1. Schematic setup of the experimental protocol; Phase 1. Post-smolt in seawater (Preline and reference). Phase 2. Grow-out phase in seawater (Preline and reference).

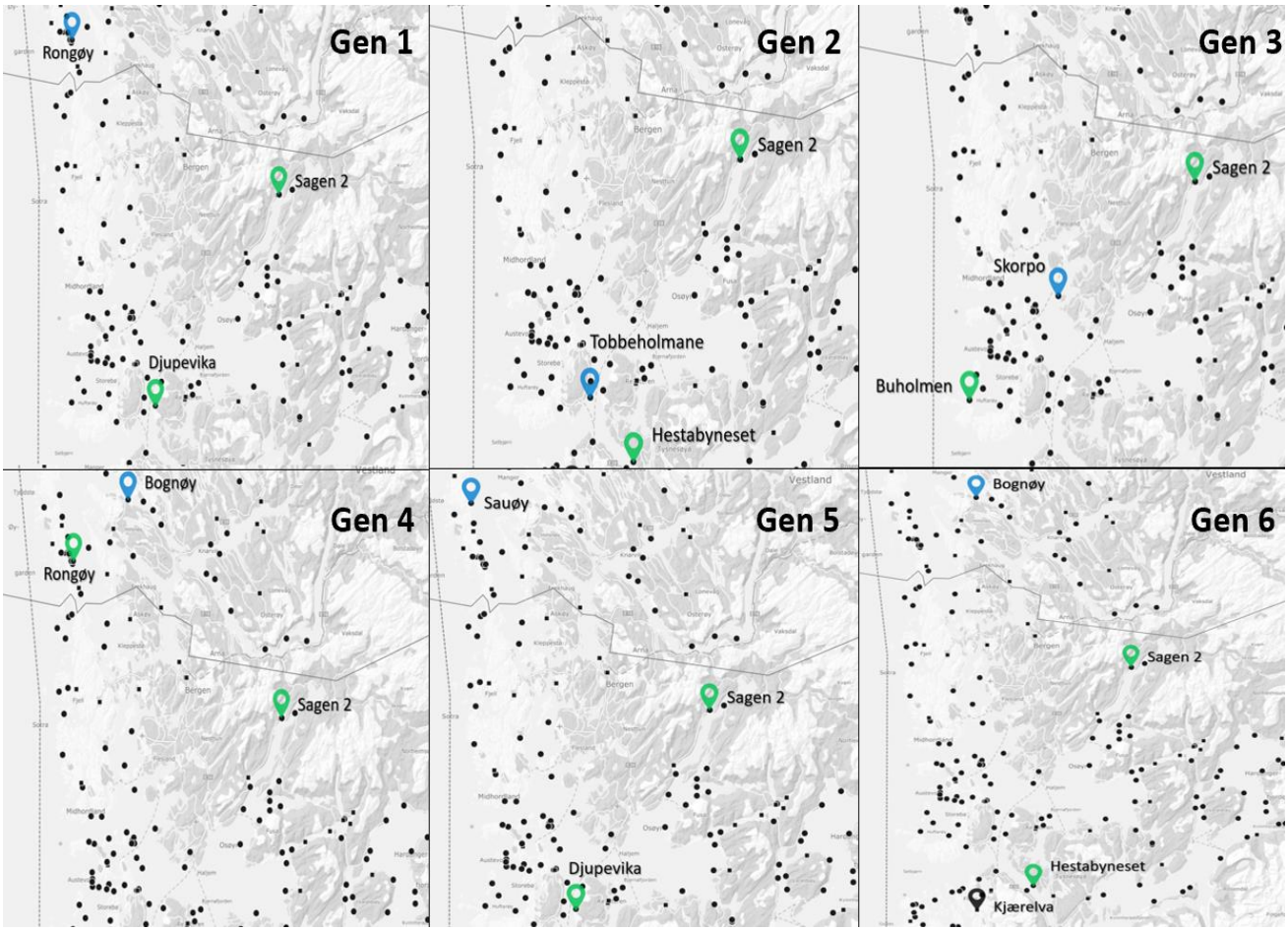

Figure 2.2. Overview over the facilities used in the experiment for six generations. Preline fish were transferred from Sagen to open net-pen facility, marked with a green marker. The reference group was reared at the same open net facility throughout phase 1 and 2 and are marked with a blue marker. Black dots represent open pen farms.

Table. 2.2. *Location, capacity, area and coordinates of the facilities*

Location	Capacity (tons)	Location number	Municipality	Coordinates
Bognøy	1560	13209	Alver	60° 37.9230 N' 5 29.7310 E'
Buholmen	5460	11543	Austevoll	60° 11.2550 N' 5 .4 8919 E'
Djupevika	3900	20455	Kvinnherad	60° 2.45700 N' 6 .5620 E'
Hestabyneset	3120	18015	Tysnes	59° 57.2180 N' 5 27.0840 E'
Rongøy	4680	29276	Øygarden	60° 30.5600 N' 4 55.9020 E'
Sagen	780	32137	Samnanger	60° 20.9030 N' 5 38.6420 E'
Sauøy	3120	11758	Øygarden	60° 35.6370 N' 4 51.6750 E'
Skorpo	3120	32877	Bjørnafjorden	60° 10.3990 N' 5 17.6210 E'
Tobbholmane	3120	100054	Austevoll	60° 1.4590 N' 5 18.4890 E'

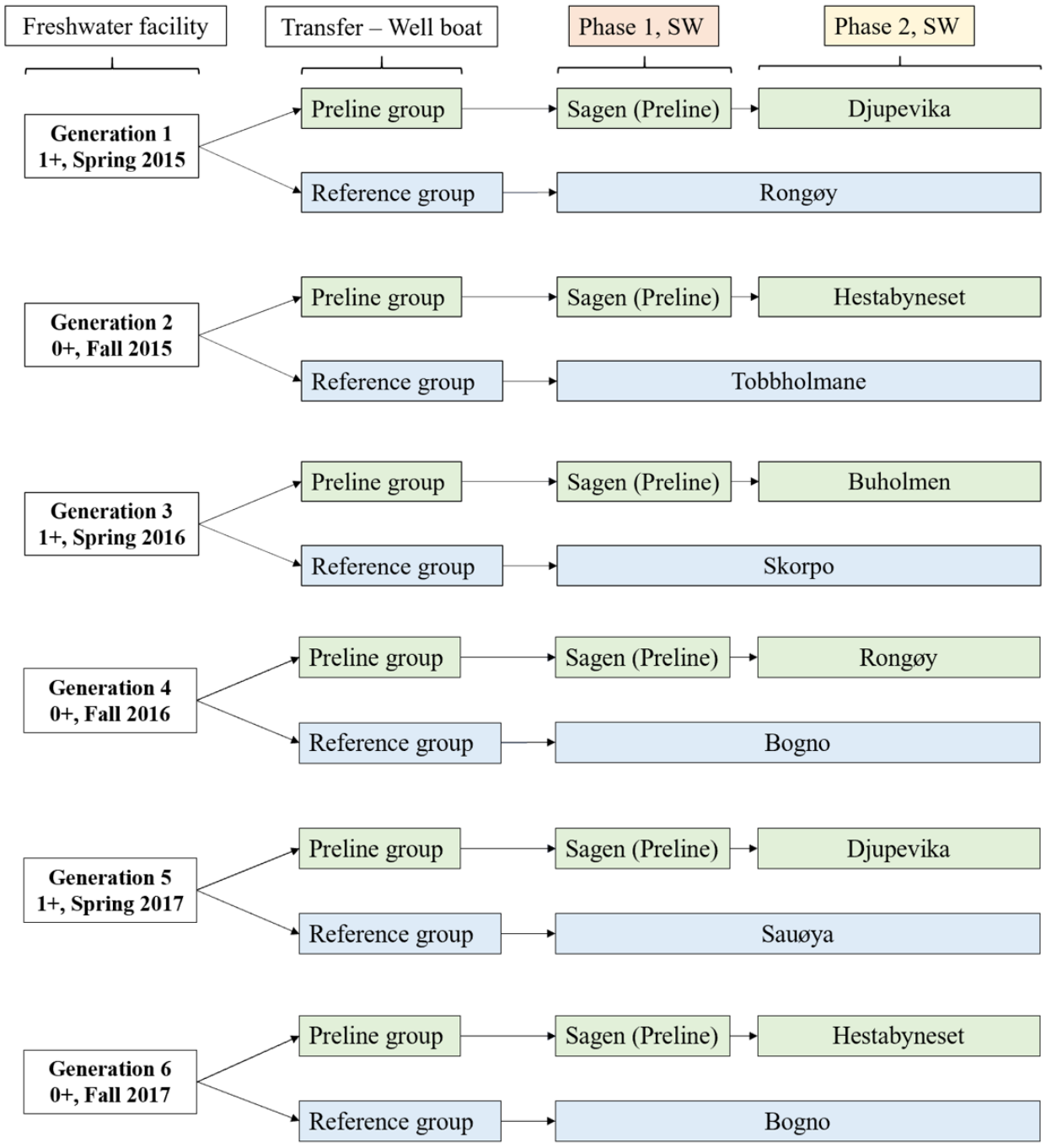

Figure 2.3. Review of the experimental locations. Preline fish were transferred from Sagen (Preline) to open net-pen facilities (after 4–6 months), and the reference group was reared at the same open net facility throughout phase 1 and 2, for each generation (0+,1+ indicates smolts type). See Figure 2.2 and Table 2.2 for location details.

Table 2.3: *Summary of rearing conditions. Production groups (date, generation, location) and production data (number of fish, temperature and oxygen) in six generations of post-smolt in phase 1.*

Period of deployment	Generation	Location	Fish N	Temperature (°C)				Oxygen saturation (%)		
				Sum	Mean	Min	Max	Mean	Min	Max
May 2015	Generation 1 **Reference**	Rongøy	191,378	1,263	11.9	7.4	16	83.2	66	96.5
May 2015	Generation 1 **Preline**	Sagen	157,283	1,033	9.7	8.3	14.2	90.6	75	100
Oct 2015	Generation 2 **Reference**	Tobbholmane	191,740	1,063	7.9	5.1	12.1	92	85	105
Oct 2015	Generation 2 **Preline**	Sagen	158,761	1,399	10.4	6.9	12.5	81	71	93
May 2016	Generation 3 **Reference**	Skorpo	164,286	1,819	14.8	8.7	21.7	96.4	74	105
May 2016	Generation 3 **Preline**	Sagen	156,273	1,185	9.6	7.5	16	95.5	75.3	97
Nov 2016	Generation 4 **Reference**	Bogno	162,390	863.8	7.9	5.4	11.8	100	75	105
Nov 2016	Generation 4 **Preline**	Sagen	92,643	1,064	9.8	6.4	11	79.3	73	97
Apr 2017	Generation 5 **Reference**	Sauøya	146,338	804.2	10.2	6.9	14.5	98.4	86.4	105
Apr 2017	Generation 5 **Preline**	Sagen	218,363	806.9	10.2	8.2	13.5	96	86.5	105.5
Oct 2017	Generation 6 **Reference**	Bogno	177,105	877	7.9	3.4	12.2	98	76.2	105.7
Oct 2017	Generation 6 **Preline**	Sagen	287,435	919.9	8.2	5	10.6	87	78.5	95

Table 2.4: *Summary of rearing conditions. Production groups (date, generation, location) and production data (number of fish, temperature and oxygen) in six generations during phase 2 – grow-out. Preline fish are now transferred to open net-pen facilities.*

Period of transfer	Generation	Location	Fish N	Temperature (°C)				Oxygen saturation (%)		
				Sum	Mean	Min	Max	Mean	Min	Max
Aug 2015	Generation 1 **Reference**	Rongøy	147,383	2,591.4	9.4	4.56	15.1	83.2	66.7	100.9
Aug 2015	Generation 1 **Preline**	Djupevika	139,182	2,553.8	9.2	5.2	15.4	90.6	74	105
Mar 2016	Generation 2 **Reference**	Tobbholmane	178,886	3,051.6	11.6	5	17	92	95	105.8
Mar 2016	Generation 2 **Preline**	Hestabyneset	142,703	3,058.4	11.7	5.2	17.1	81	83.3	93
Sep 2016	Generation 3 **Reference**	Skorpo	119,118	3,181.2	9.7	3.9	17.2	96.4	74.1	105
Sep 2016	Generation 3 **Preline**	Buholmen	139,924	3,246.4	9.8	4.9	16.9	95.5	75.3	105
Feb 2017	Generation 4 **Reference**	Bogno	152,635	2,884.6	11.2	5.9	17.4	100.5	75	105.8
Feb 2017	Generation 4 **Preline**	Rongøy	77,465	3,033.6	11.7	5.9	16.2	79.2	73	97
July 2017	Generation 5 **Reference**	Sauøya	111,177	2,929.4	9.5	2.8	15.9	98.4	86.4	105
July 2017	Generation 5 **Preline**	Djupevika	208,944	2,766.8	9.0	2.7	16.3	96.1	86.5	105.5
Feb 2018	Generation 6 **Reference**	Bogno	133,304	3,439.5	9.79	2.9	17.3	98	76.2	105.7
Feb 2018	Generation 6 **Preline**	Hestabyneset	119,033	3,628.8	10.34	4.9	17.8	87	78.5	95

Experimental Facilities: S-CCS System (Preline)

The Preline groups were reared in the Preline platform during the post-smolt phase; more information regarding this system is depicted in Chapter 1.

Experimental Facilities: Conventionally Open Sea Cages

The reference groups were reared in open 160 m conical circular sea cages (Figure 2.4). Each fish farm consisted of six to ten circular cages with a rearing capacity of up to 200,000 Atlantic salmon, and an operating feeding station. All the open net facilities in this experiment are located along the western Norwegian coast and are summarized in Figure 2.3 and Table 2.2; a timeline of the stocking period is presented in Figure 2.5.

Figure 2.4. Atlantic salmon aquaculture farm (A) and (B) a schematic overview of the conical circular cage (*Spissnot*).

During the experimental periods, all husbandry practices, including sea lice counting, were conducted in accordance with standard Atlantic salmon production protocol for Lerøy Vest AS. The fish were fed a standard dry diet (Ewos, Norway) in relation to environmental temperature and fish size (Austreng et al., 1987). To avoid early maturation, all groups were exposed to artificial led-light (35 W/m^2, submerged) from mid-December to the end of June.

Temperature and oxygen (Table 2.3 and Table 2.4) were measured daily at -3 m by automatic sensors (OxyGuard, Sterner), and all environmental data was registered in Fishtalk (AkvaGroup, Bryne).

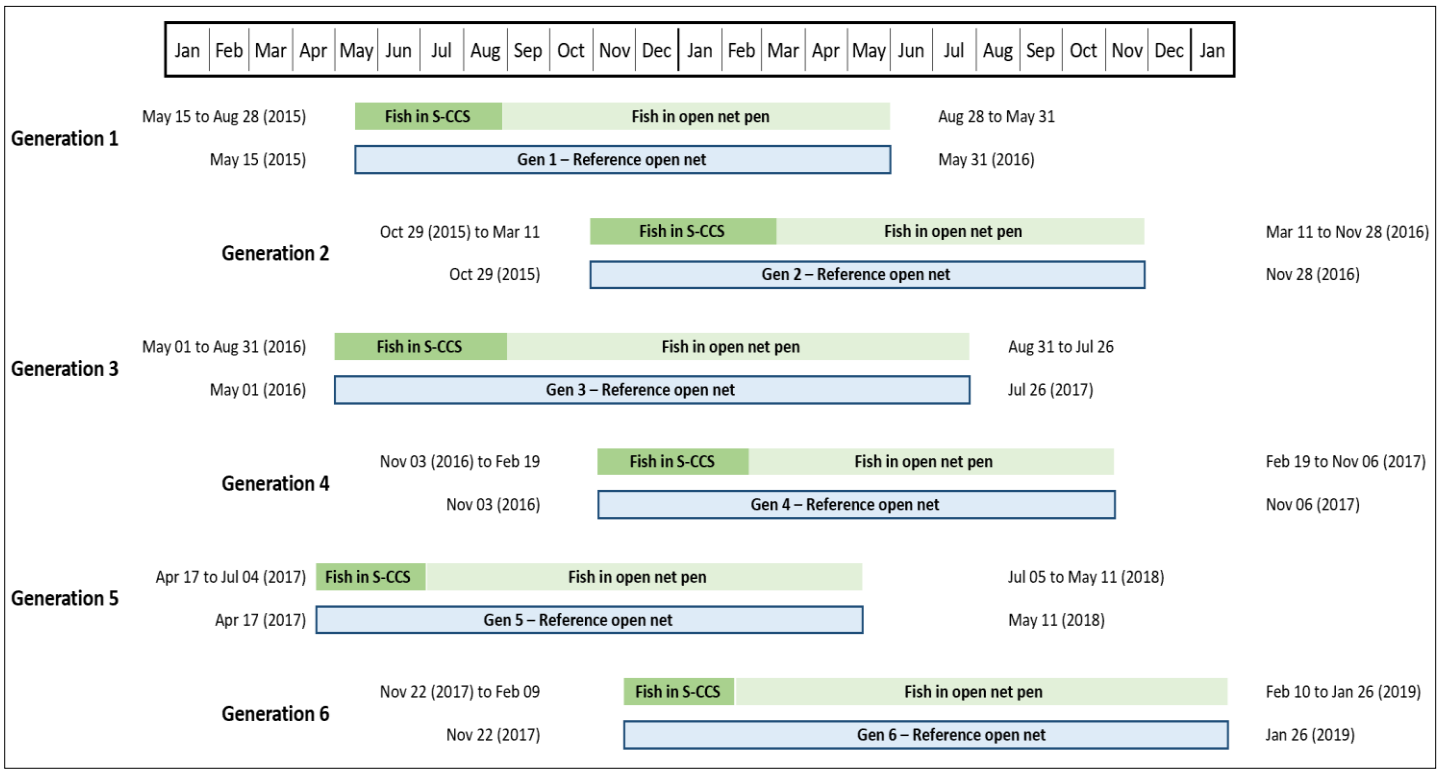

Figure 2.5. Period of stocking for the six generations in the experimental period from May 2015 to January 2019. The dark-green rubric represents fish reared in the Preline system (Generation 1, 3 and 5 represents spring stocking and Generation 2, 4, and 6 represents fall stocking), and the light-green rubric represents the following grow-out phase in open sea cage until harvested. The blue rubric represents the reference group reared in a conventional open sea cage over the same periods in each generation.

Data Collection

Biological production data and environmental data were collected regularly (daily and weekly) from May 2015 to February 2019 and included six generations of Atlantic salmon, from smolt to slaughter (Preline and reference**)**. Parameters included and investigated in this study are; 1. Growth (daily), 2. Feed conversion (daily), 3. Mortality (daily), 4. Sea lice infestations (weekly), and 5. Biomass estimation. All the measurements were conducted according to regulations and standard production protocol for Norwegian aquaculture.

1. Growth: Daily calculations on weight gain based on feed output was completed by Fishtalk (Feed conversion ratio; FCR: 1.1). Minor dips in the weight curve figures are caused by short periods of malfunctioning feeding equipment. Since the Preline and reference facilities were located in different places, which varied in surrounding seawater temperature, a weight model incorporating growth rate per day dependent on the daily temperature was employed (Thermal-unit Growth Coefficient, TGC). This model takes into account the optimal season temperature for fish growth (Iwama & Tautz, 2011). The following equation was used:

$$TGC = (Final\ weight^{1/3} - Start\ weight^{1/3}) \times 1000 / sum\ of\ daily\ temperature\ (^{\circ}C)$$

The specific growth rate (SGR) for weight was calculated (time interval is total number of days from initial weight to final weight) according to the formula:

$$SGR\ (\%\ body\ weight\ gain\ (\%/day)) =$$

$$[ln(Final\ weight(g)) - ln(Initial\ weight(g)) / (Time\ interval(days))] \times 100$$

2. Feed conversion: The daily feed ratio (kg or tons) was registered in each location and the feed conversion (FC) factor was calculated using the following equation:

$$FC = (feed\ provided / biomass\ increase).$$

3. Mortality was counted daily in each group by employees at Lerøy Vest AS.

4. Infestation of sea lice was counted every week by employees at Lerøy Vest AS, according to standard procedures by Mattilsynet (MTIF, 2017b).

5. Biomass estimations in phase 1 and 2 was calculated by the values (estimated final weight and observed mortality) from spring and fall stockings (n = 3) in both phases. Assumption for the estimation, fish N = 200,000. These calculations were used:

$$Fish_N = Fish_{200,000} \times [(Mortality\%) / (100)]$$

$$Fish_{Estimated} = Fish_{200,000} - Fish_N$$

$$Estimated\ biomass = Fish_{Estimated} \times Final\ weight_{Estimated}$$

Statistical Analysis

All statistical analyses were generated (and figures) using RStudio (Version 1.2.500, RStudio, Inc, Boston, MA, USA) and R (Version 3.6.1, R core team, Vienna, Austria), including following packages; Rcompanion (Magiafico, 2018), car (Fox & Weisberg, 2011). The datasets were checked for normality and homogeneity of variance assumptions using the Shapiro-Wilk test and the Levene's test, respectively. To determine the degree of significance, a two-way ANOVA was conducted followed by Tukey post-hoc test. The degree of significance in this study was considered as significant when p-value < 0.05 (*). If the p-value is less than 0.01, it is flagged with two stars (**). If a p-value is less than 0.001, it is flagged with three stars (***). If a p-value is less than 0.0001, it is flagged with four stars (****). The bar plots are plotted with ± standard error (SE). All the statistical results in this study are reported in the Appendix III.

Results

The growth within the two groups (Preline and reference) varied for all the six generations in phase 1 and phase 2, respectively. These growth performances are based on empirical production data (mean registered weight plotted against time), and for this dataset (n = 1), a statistical analysis could not be performed. Consequently, this is a descriptive presentation of the data. More information is depicted in Figure 2.5 and Table 2.5.

Growth in Phase 1: Post-smolt

Estimated weight; Generation 1, May 2015

The two post-smolt groups in generation 1 were transferred to their respective experimental facilities in mid-May 2015 (Figure 2.6). Following transfer to sea, the Preline group consisted of 157,283 fish, while the open net-pen reference group (Rongøy) consisted of 191,378 fish. The initial mean weight was 124.8 g (density 9.8 kg/m^3) in the Preline group and 120.4 g (density 0.8 kg/m^3) in the reference group. The final weight at the end of the experimental phase (28 August, 106 days) was 539.7 g (density 42 kg/m^3) in Preline group and 648.8 g (density 4.5 kg/m^3) in the reference group.

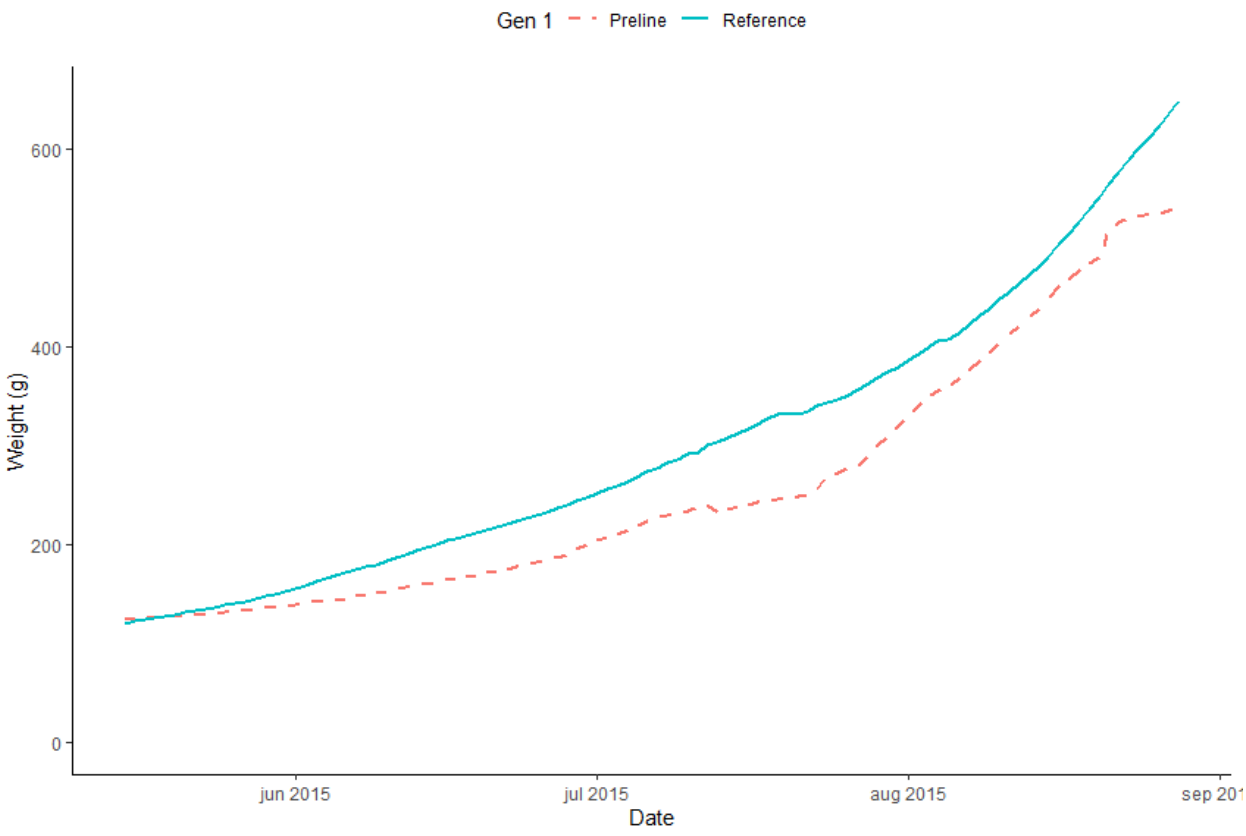

Figure 2.6. Registered growth during phase 1 for the Preline and reference group in generation 1 from 15 May to 28 August in 2015. The red dashed line represents the Preline group, whereas the turquoise line represents the reference group; this pattern applies to all graphs in this chapter.

Estimated weight; Generation 2, October 2015

At the end of October 2015, the two post-smolt groups in generation 2 were transferred to their respective experimental facilities (Figure 2.7). When relocated to sea, the Preline group consisted of 157,761 fish, and the open net-pen reference group (Tobbholmane) consisted of 191,740 fish. At stocking, the initial mean weight was 98.4 g in the Preline (density 7.85 kg/m^3) group and 98.8 g (density 0.7 kg/m^3) in the reference group. At the end of the experimental phase (11 March, 136 days), the final weight was 720 g (density 55.6 kg/m^3) in the Preline group and 577 g (density 3.9 kg/m^3) in the reference group.

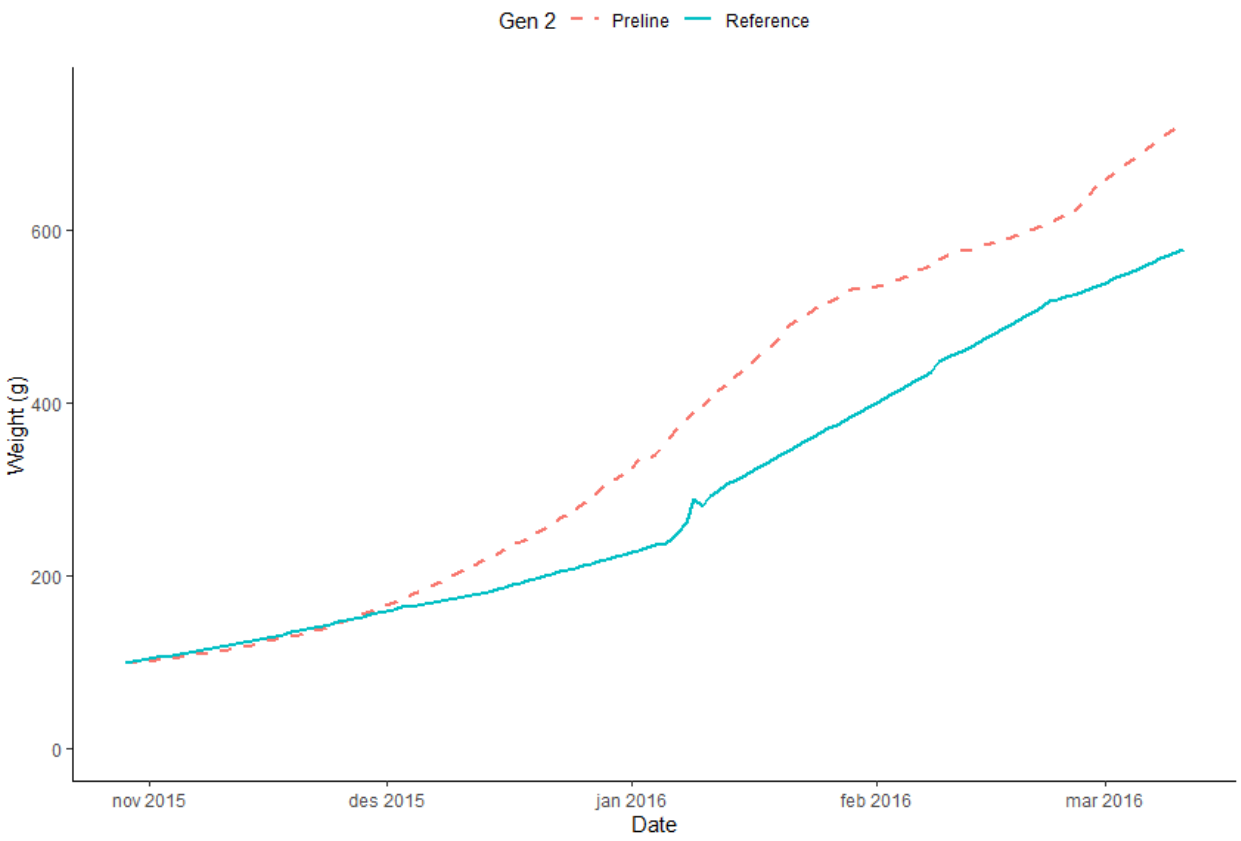

Figure 2.7. Registered growth during phase 1 for Preline and reference group in generation 2 from 29 October 2015 to 11 March 2016.

Estimated weight; Generation 3, May 2016

In generation 3, the two post-smolt groups were transferred to their experimental facilities in early May 2016 (Figure 2.8). Following relocation to sea, the Preline group consisted of 156,273 fish, while the open net-pen reference group (Skorpo) consisted of 164,286 fish. The initial weight in the two groups was 113 g (density 8.8 kg/m^3) in Preline and 109.4 g (density 0.6 kg/m^3) in the reference group. The final weight at the end of the experimental phase (31 August, 123 days) was 487 g (density 37.7 kg/m^3) in the Preline group and 844 g (density 5.0 kg/m^3) in the reference group.

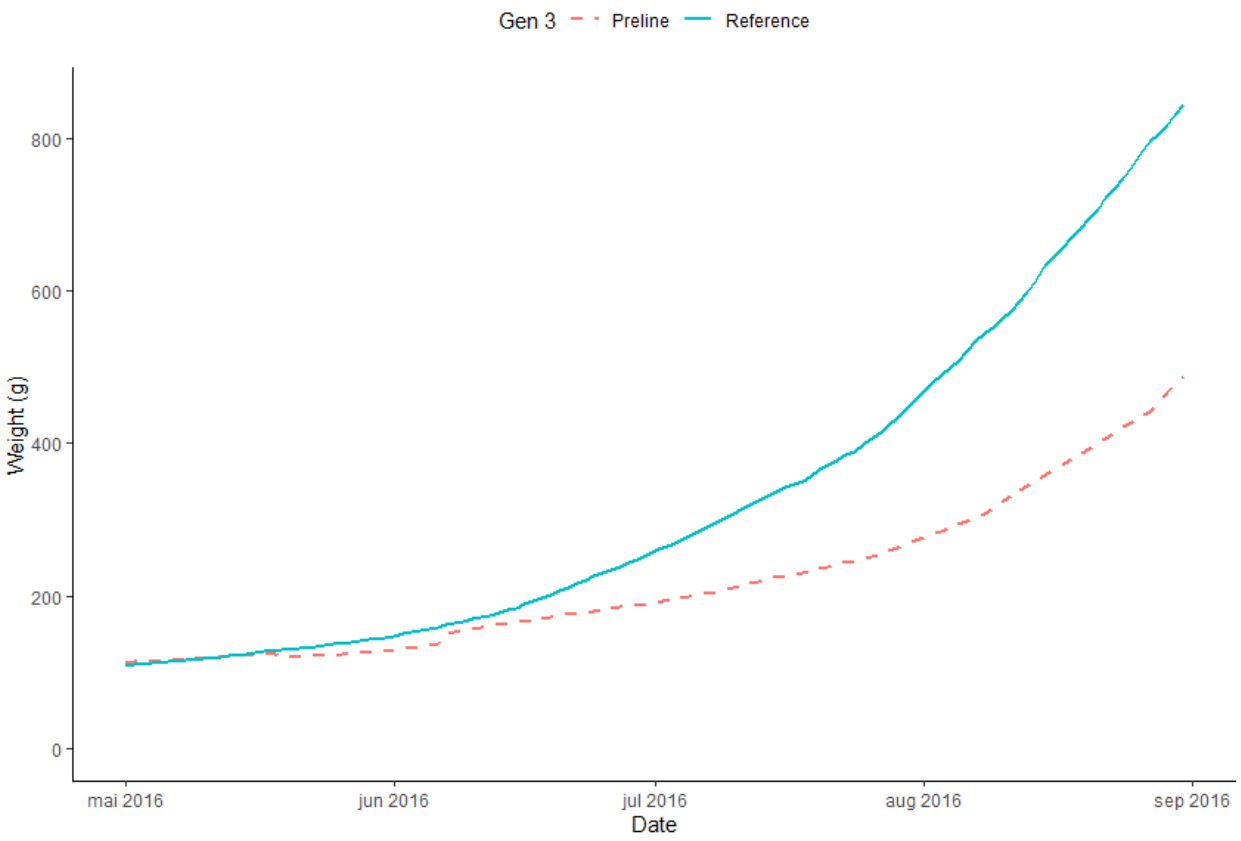

Figure 2.8. Growth during phase 1 for Preline and reference group in generation 3 from 1 May to the end of August 2016.

Estimated weight; Generation 4, November 2016

In November 2016, the two post-smolt groups in generation 4 were transferred to their respective experimental facilities (Figure 2.9). The Preline group consisted of 92,643 fish, and the reference group (Bogno) in the open net-pen consisted of 162,390 fish when transferred to sea. The initial mean weight was 111.3 g (density 5.15 kg/m^3) in the Preline group and 115 g (density 0.7 kg/m^3) in the reference group. At the end of the experimental phase (19 February, 109 days) the final weight was 450 g (density 20.7 kg/m^3) in the Preline group and 365 g (density 2.1 kg/m^3) in the reference group.

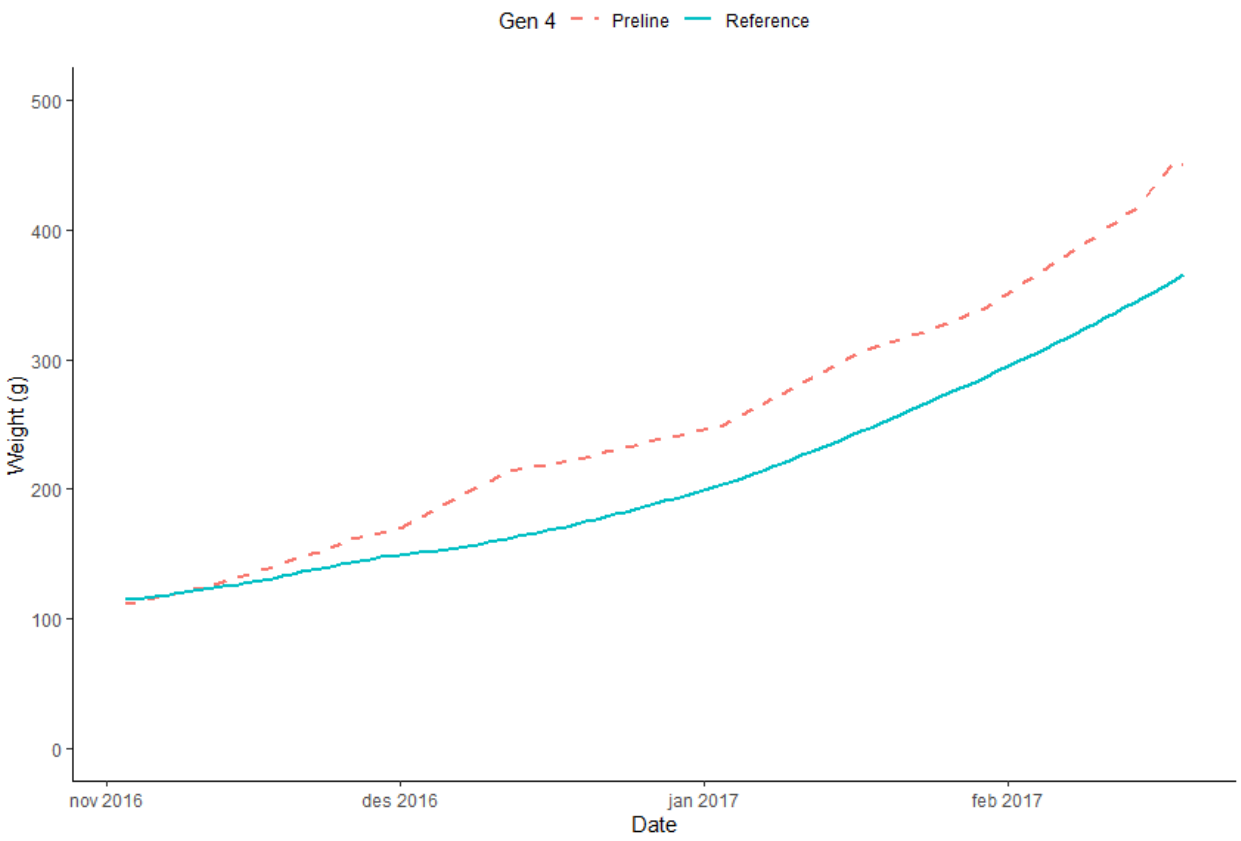

Figure 2.9. Growth during phase 1 for Preline and reference group in generation 4 from 3 November to 19 of February 2017.

Estimated weight; Generation 5, April 2017

In mid-April 2017, the two post-smolt in groups in generation 5 were transferred to their experimental facilities (Figure 2.10). When relocated to sea, the Preline group consisted of 218,363 fish, and the open net-pen reference group (Sauøya) consisted of 146,338 fish. At stocking, the initial mean weight was 133.6 g (density 14.5 kg/m^3) in the Preline group and 146.3 g (density 0.8 kg/m^3) in the reference group. The final weight at the end of the experimental phase (4 July, 79 days) was 553.8 g (density 59 kg/m^3) in the Preline group and 419 g (density 2.2 kg/m^3) in the reference group.

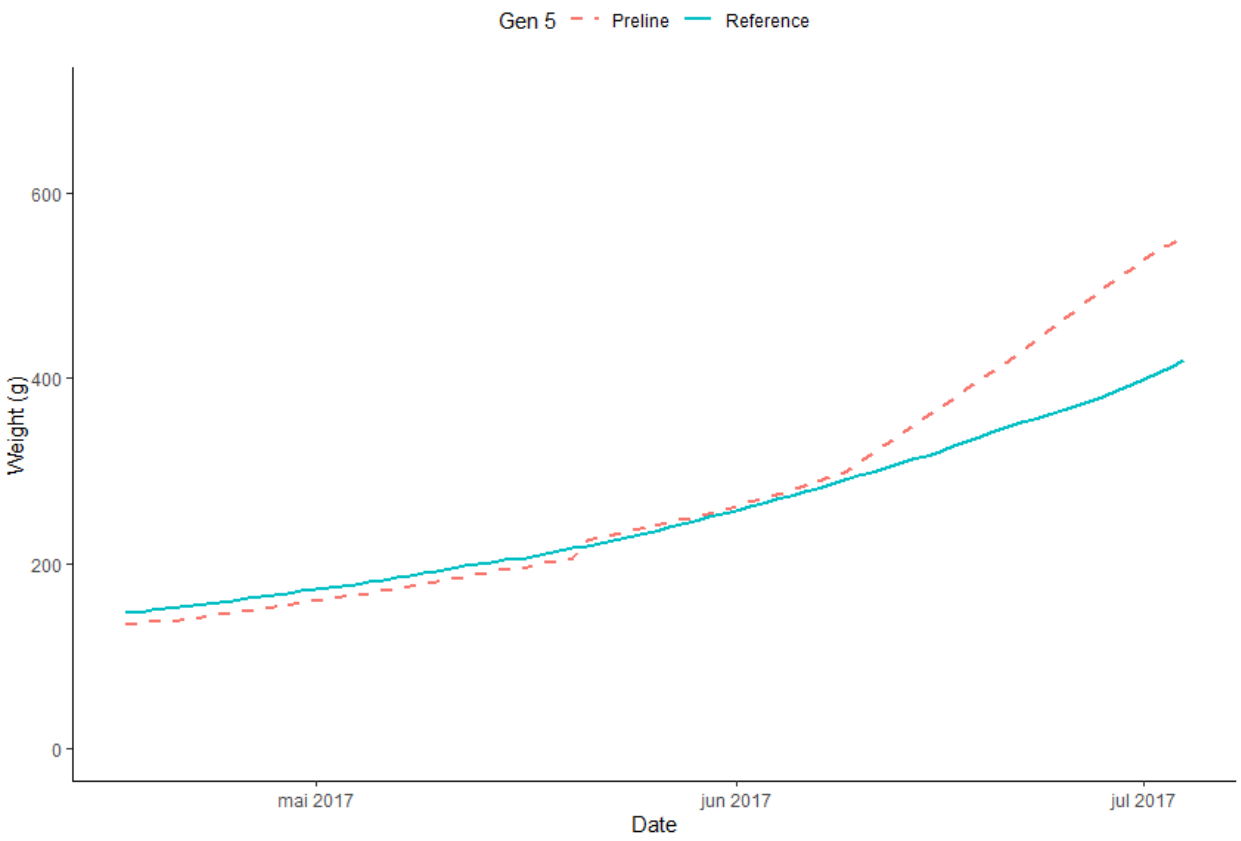

Figure 2.10. Growth during phase 1 for Preline and reference group in generation 5 from 17 April to 14 July 2017.

Estimated weight; Generation 6, October 2017

The two post-smolt groups in generation 6 were transferred to their respective experimental facilities in October 2017 (Figure 2.11). Following transfer to sea, the Preline group consisted of 287,435 fish, while the open net-pen reference group (Bogno) consisted of 177,105 fish. The initial mean weight was 110.8 g (density 15.9 kg/m^3) in Preline group and 114 g (density 0.7 kg/m^3) in the reference group. The final weight at the end of the experimental phase (9 February, 111 days) was 284 g (density 35.8 kg/m^3) in the Preline group and 366 g (density 2.4 kg/m^3) in the reference group.

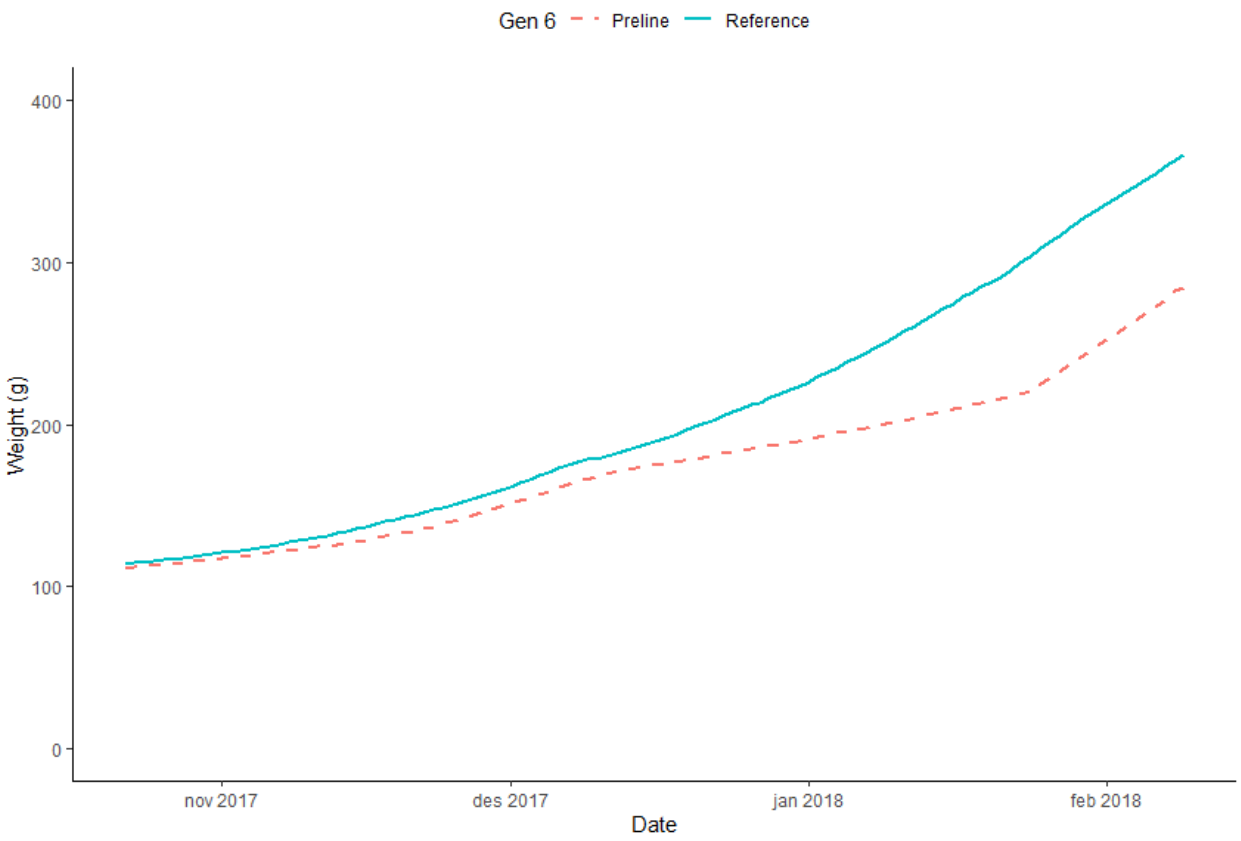

Figure 2.11. Growth during phase 1 for Preline and reference group in generation from 22 October to 9 February 2018.

Phase 1 - (Post-Smolt): Feed Conversion ratio, Mortality, Sea Lice Infestation and Growth performance.

Overall, a lower feed conversion was registered in the Preline system during spring in the comparison between the reference groups (Figure 2.12 A). In the fall stockings, no difference in feed conversion was observed. In the spring stockings, a lower mortality was observed in the Preline group. Whereas for the fall stockings, lower mortality was observed in the reference group (Figure 2.12 B), however no significant difference was observed for mortality. The sea lice infestations (Figure 2.12 C) for fish reared in the Preline system were significantly lower in spring and fall compared to reference group ($p < 0.05$, two-way Anova). For the initial weight, a significant difference was observed between seasons (0+ and 1+ smolts) for Preline and reference groups ($p < 0.05$, two-way Anova). The final weight in spring was higher in reference group in contrast to the fall stockings, where final weight was higher in Preline group (Figure 2. 12 D). Further, specific growth rate (SGR) and thermal growth coefficient (TGC) showed no significant difference between seasons and rearing systems.

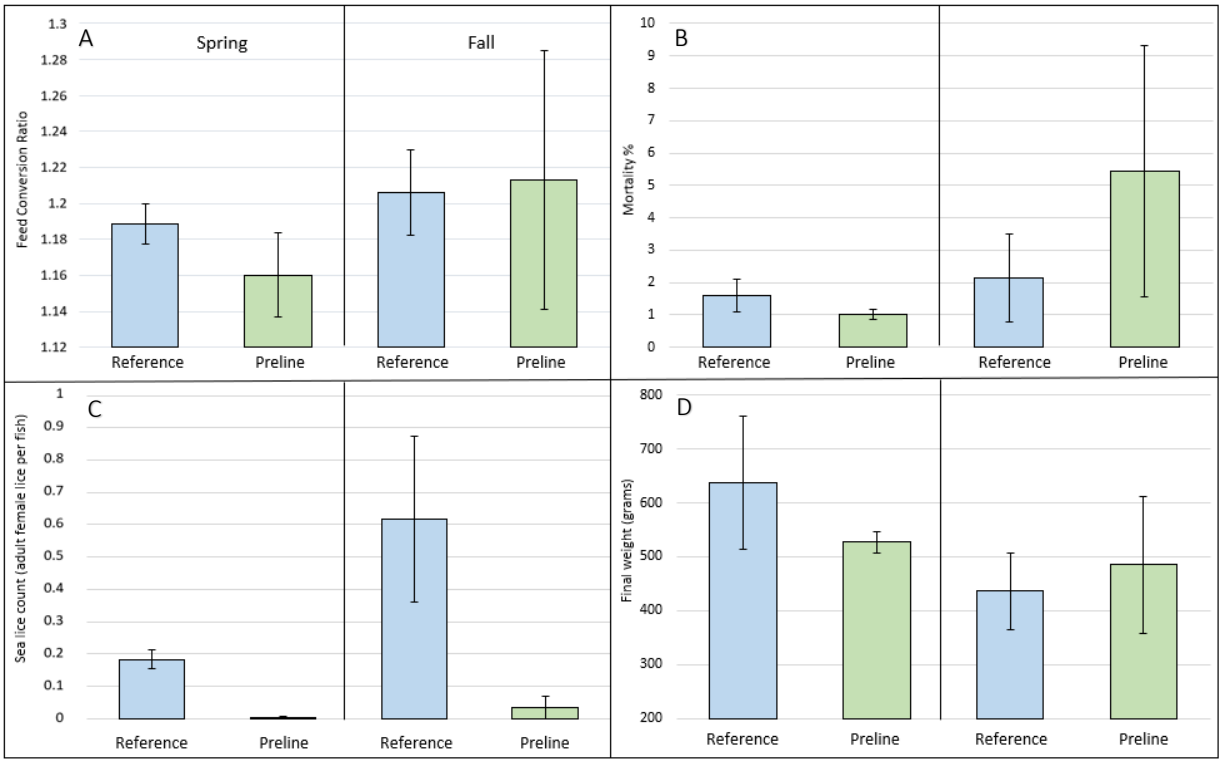

Figure 2.12. Registered feed conversion (A), mortality (B), sea lice infestations (C) and final weight (D) in phase 1 for spring (left) and fall (left) generations, mean ± SE (n = 3).

Growth in Phase 2: Grow-out

Estimated weight; Generation 1, August 2015

In August 2015, fish from the Preline system were transferred to an open net-pen facility for grow-out phase (Figure 2.13). Following the transfer, the Preline group (Djupevika) consisted of 155,989 fish and the open net-pen reference group (Rongøy) consisted of 188,420 fish. The initial mean weight was 544 g (density 3.1 kg/m^3) in the Preline group and 663.2 g (density 4.6 kg/m^3) in the reference group. At the end of the experiment (31 May, 277 days), the final mean weight was 4,271.8 g (density 21.8 kg/m^3) in the Preline group and 4,493.6 g (density 24.3 kg/m^3) in the reference group.

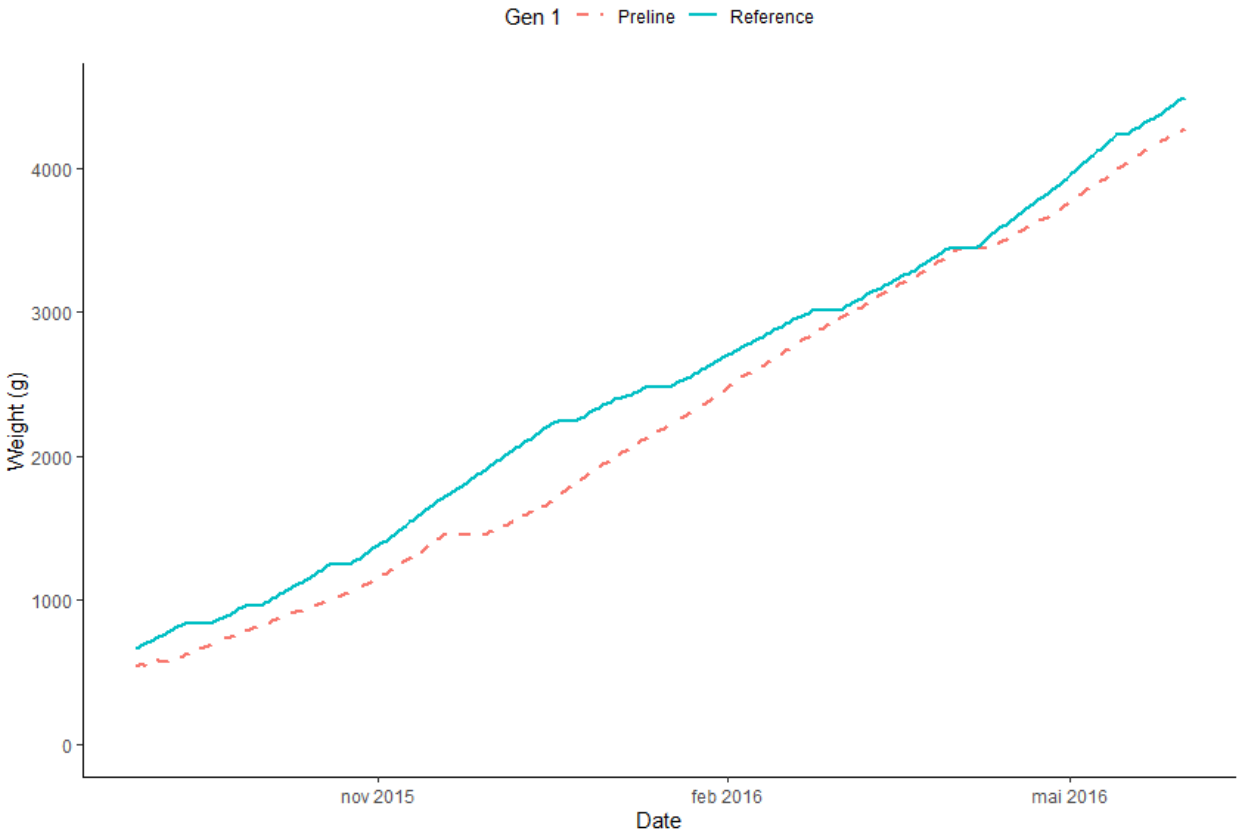

Figure 2.13. Growth during phase 2 in open net-pens for Preline group and reference group in generation 1 from 29 August 2015 to 31 May 2016.

Estimated weight; Generation 2, March 2016

Fish from the Preline system were transferred to an open net-pen facility in March 2016 for the grow-out phase. When relocated to the open net facility, the Preline group (Hestabyneset) consisted of 154,397 fish and the open net-pen reference group (Tobbholmane) consisted of 184,155 fish (Figure 2.14). The initial mean weight was 720.6 g (density 4 kg/m^3) in the Preline group and 577.2 g (density 3.9 kg/m^3) in the reference group. The final weight at the end of the experiment (28 November, 262 days) was 4,457.3 g (density 23.4 kg/m^3) in the Preline group and 3,358.8 g (density 22.1 kg/m^3) in the reference group.

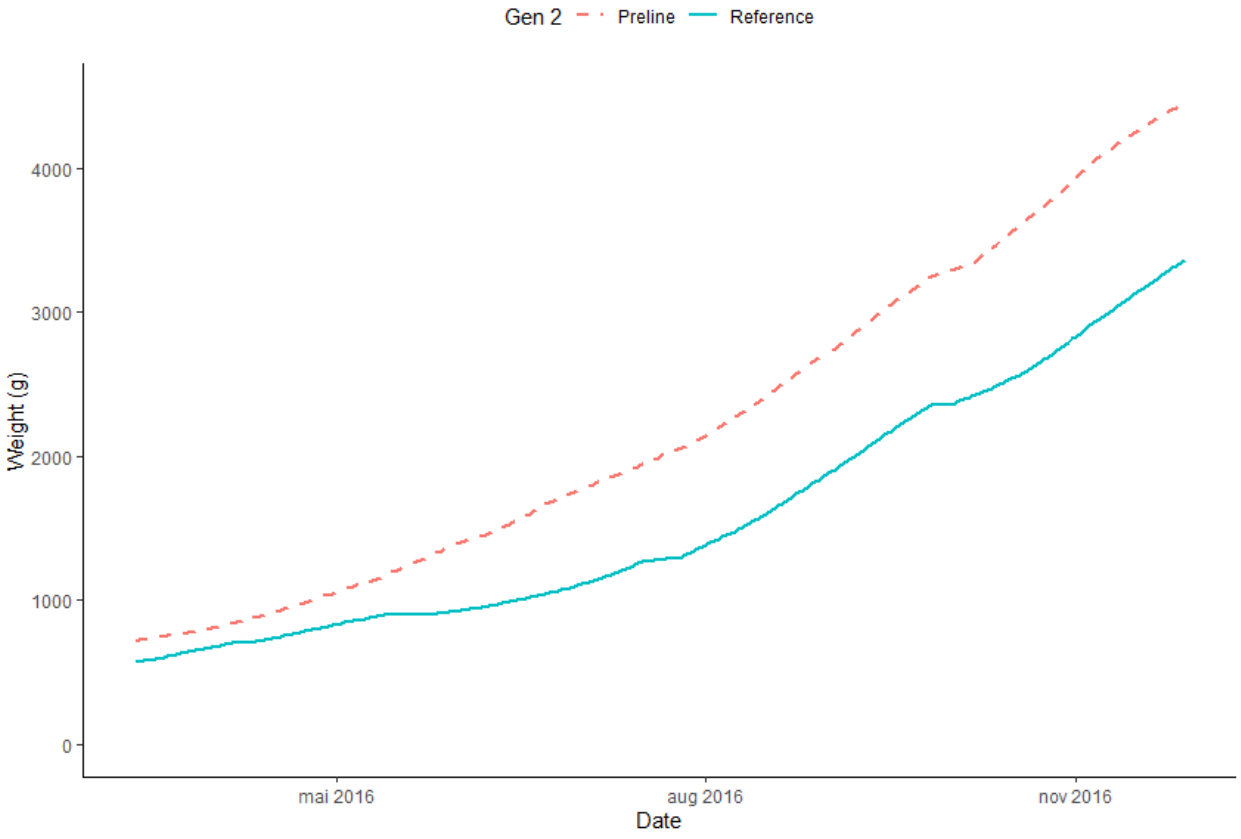

Figure 2.14. Growth during phase 2 in open net-pens for Preline group and reference group in generation 2 from 12 March to 28 November 2016.

Estimated weight; Generation 3, September 2016

In September 2016, fish from the Preline system was transferred to an open net-pen facility for grow-out phase (Figure 2.15). When relocated to the open net-pen, the Preline group (Buholmen) consisted of 154,940 fish, and the open net-pen reference group (Skorpo) consisted of 162,340 fish. The initial mean weight was 492.9 g (density 2.8 kg/m^3) in the Preline group and 856 g (density 5.1 kg/m^3) in the reference group. The final weight at the end of the experiment (26th of July, 329 days) was 4,626.2 g (density 23.8 kg/m^3) in the Preline group and 4,293.2 g (density 19 kg/m^3) in the reference group.

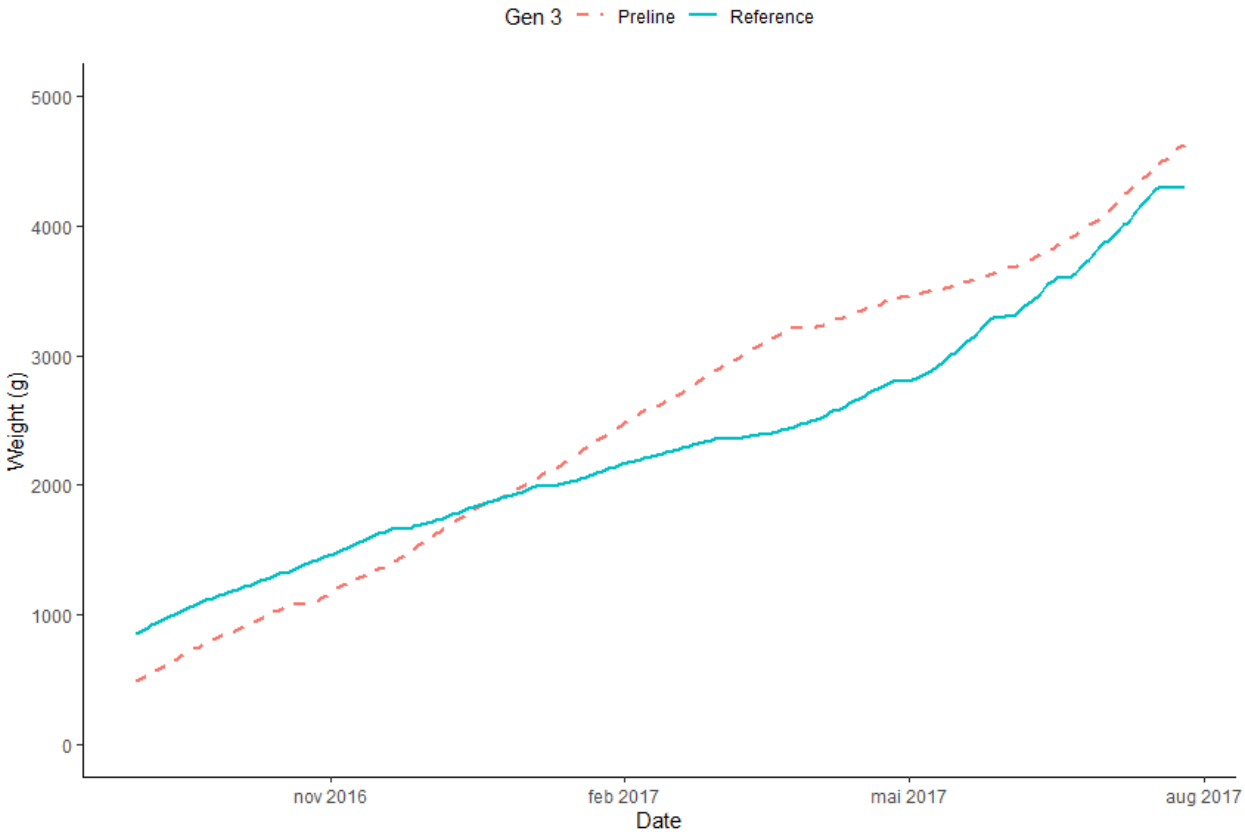

Figure 2.15. Growth during phase 2 in open net-pens for Preline group and reference group in generation 3 from 1 September 2016 to 26 July 2017.

Estimated weight; Generation 4, February 2017

In February 2017, fish in the Preline group were transferred to an open net-pen facility for the grow-out phase (Rongøy). When relocated to open net-pen, the Preline group consisted of 92,156 fish, and the open net-pen reference group (Bogno) consisted of 161,321 fish. The initial mean weight was 450 g (density 1.5 kg/m^3) in the Preline group and 369.4 g (density 2.1 kg/m^3) in the reference group (Figure 2.16). At the end of the experiment (6 November, 258 days), the final mean weight was 4,800.8 g (density 13.6 kg/m^3) in the Preline group and 3,991.3 g (density 22.4 kg/m^3) in the reference group.

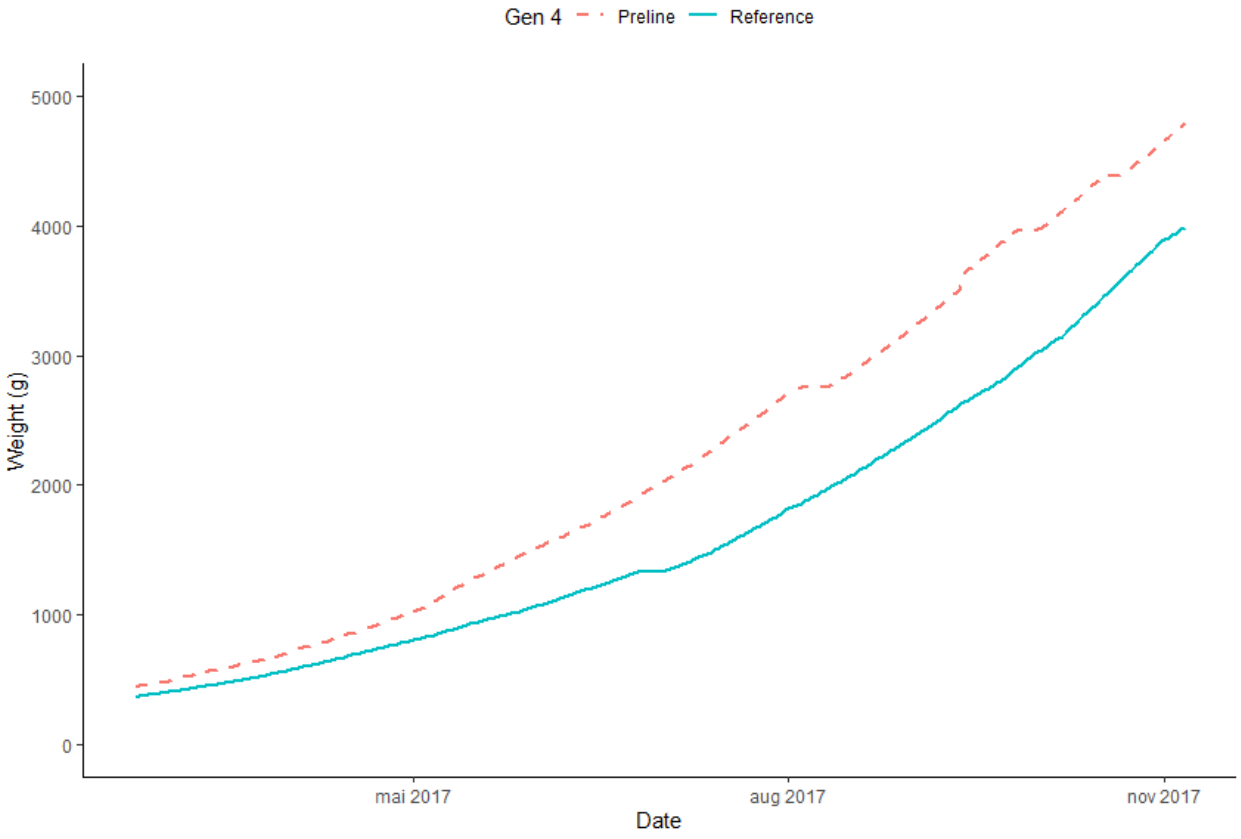

Figure 2.16. Growth during phase 2 in open net-pens for Preline group and reference group in generation 4 from 22 February to 6 November 2017.

Estimated weight; Generation 5, July 2017

In July 2017, fish from the Preline system were relocated to an open net-pen facility for the grow-out phase (Figure 2.17). When transferred to the open net facility (Djupevika), the Preline group consisted of 216,277 fish and the reference group (Sauøya) in open sea cages consisted of 144,840 fish. The initial mean weight was 560.8 g (density 4.4 kg/m^3) in the Preline group and 427.3 g (density 2.2 kg/m^3) in the reference group. The final weight at the end of the experiment (11 May, 307 days) was 5,718.4 g (density 43.9 kg/m^3) in the Preline group and 3,305.5 g (density 13.5 kg/m^3) in the reference group.

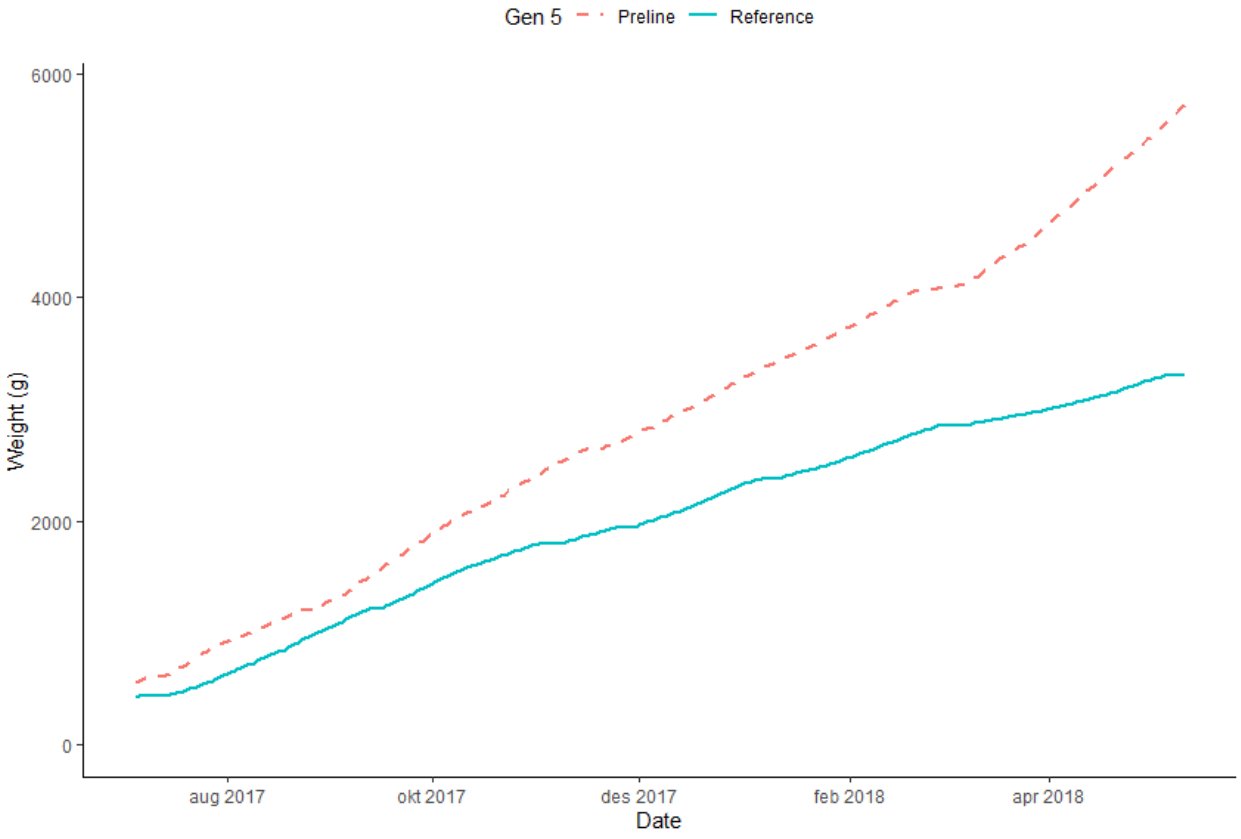

Figure 2.17. Growth during phase 2 in open net-pens for Preline group and reference group in generation 5 from 5 July to 11 May 2018.

Estimated weight; Generation 6, February 2018

Fish from the Preline group were transferred to an open net-pen facility in mid-February 2018 (Figure 2.18). The Preline group (Hestabyneset) consisted of 146,713 fish, and the reference group (Bogno) consisted of 146,211 fish. The initial mean weight in the groups was 284.1 g (density 1.5 kg/m^3) in the Preline group and 366.2 g (density 1.9 kg/m^3) in the reference group. At the end of the experiment (26 January, 351 days), the final mean weight was 4,704.9 g (density 20.6 kg/m^3) in the Preline group and 4,042.4 g (density 19.8 kg/m^3) in the reference group.

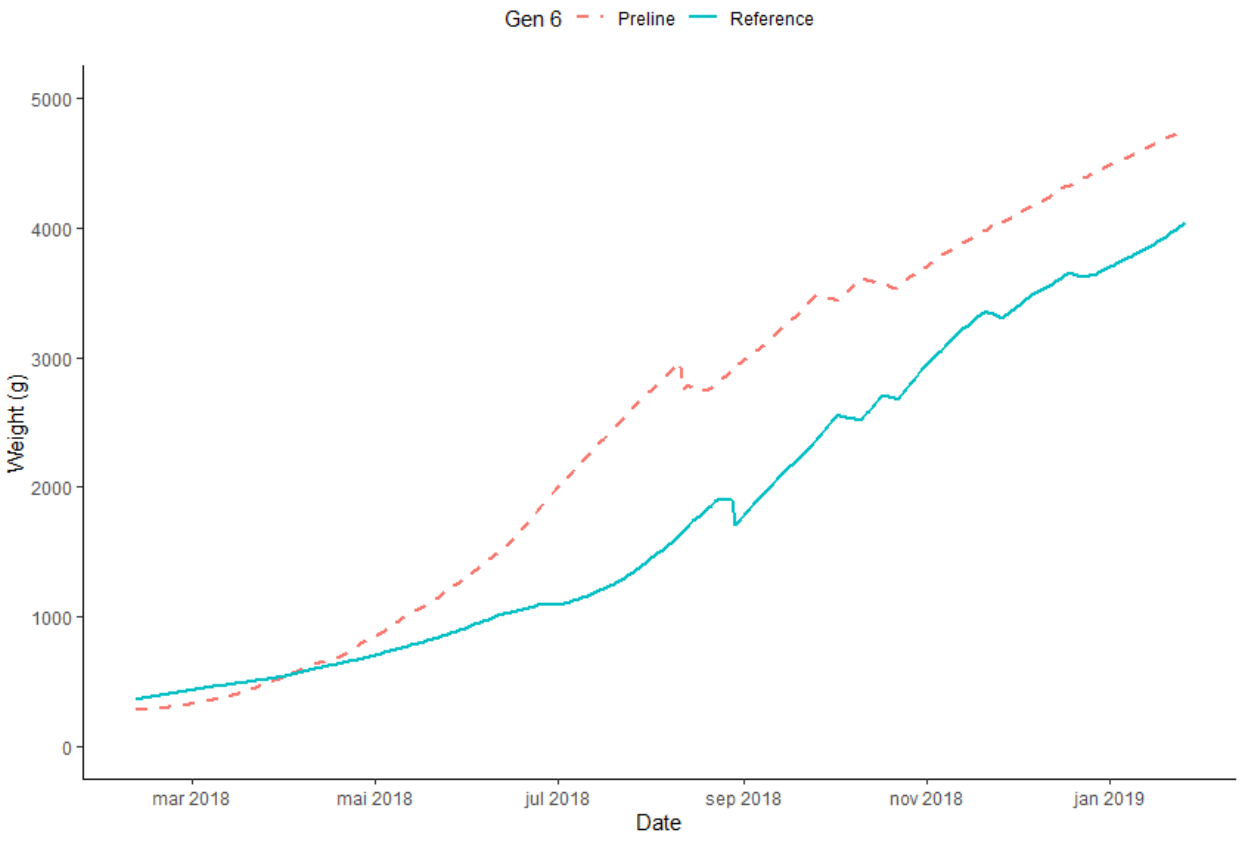

Figure 2.18. Growth during phase 2 in open net-pens for Preline group and reference group in generation 6 from 10 February to 26 January 2019.

Phase 2 - (Grow-Out): Feed Conversion Ratio, Mortality, Sea Lice Infestation and Growth performance

A significant effect on season was observed for feed conversion ratio ($p < 0.01$, two-way Anova). No difference was registered between the Preline and reference in FCR for the spring groups, while a lower FCR was observed in Preline during fall stockings (Figure 2.19 A). For mortality (Figure 2.19 B), a significant interaction effect between season and system was observed ($p < 0.05$, two-way Anova). In the spring stockings, higher mortality was registered in Preline compared to the reference group. However, in the fall stockings, a lower mortality was observed in the Preline group. The registered sea lice infestation (Figure 2.19 C) in the Preline group was lower during both spring and fall (no significant difference) compared to reference. Registered final weight in the spring and fall stockings was significantly higher ($p < 0.05$, two-way Anova) for the Preline fish in comparison to reference fish (Figure 2. 19 D). In addition, a significantly higher weight gain (w_2-w_1) was observed in fish from Preline compared to the reference fish ($p < 0.05$, two-way Anova). Further, interpretation of specific growth rate (SGR) and thermal growth coefficient (TGC), showed no significant difference between seasons (spring and fall) and systems (Preline and open net-pen).

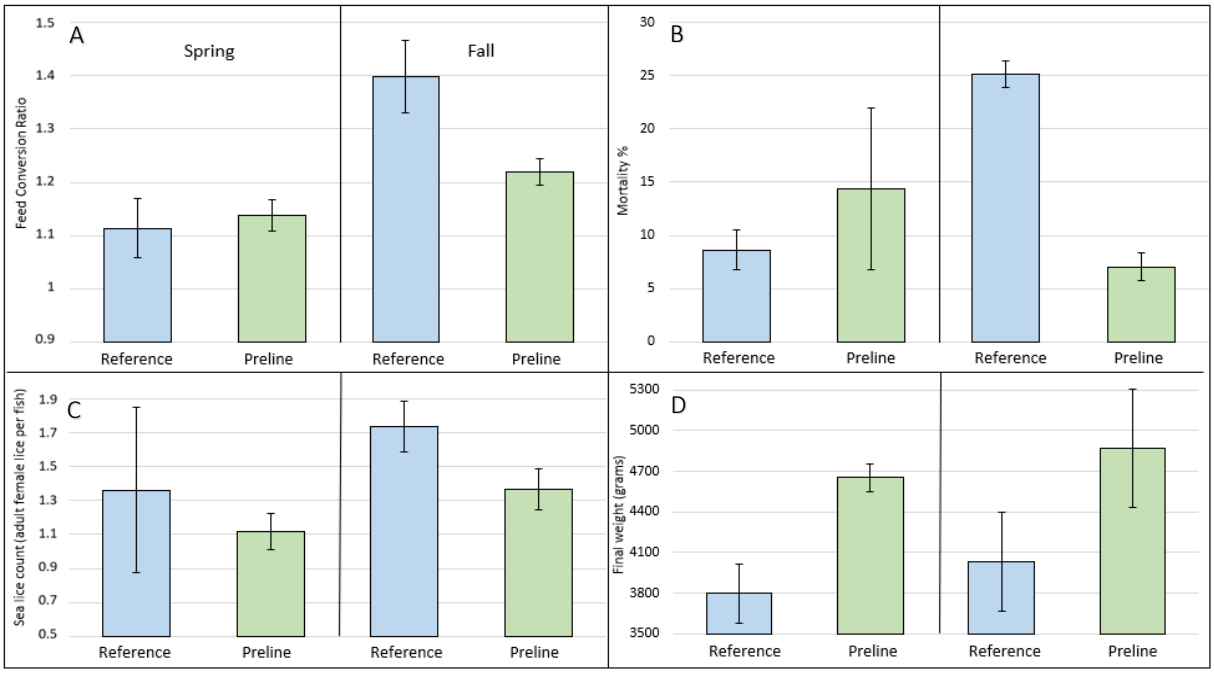

Figure 2.19. Registered feed conversion (A), mortality (B), sea lice infestations (C) and final weight (D) in phase 2 for spring (left) and fall (right) generations, mean ± SE (n =3).

Biomass Estimation

The biomass estimation in phase 1 and 2 was calculated by values (estimated final weight, observed mortality and fish n = 200,000) from empirical production data from both phases (post-smolt and grow-out) during spring and fall stockings (Table 2.6 and Table 2.7).

Biomass estimation in phase 1: Post-smolt

In a theoretical stocking scenario for phase 1 post-smolt, the estimated biological output varied during spring and fall stockings. The spring stockings shows a higher estimated gain in the reference group (125,374 kg) in comparison to the Preline groups (104,335 kg) due to a higher estimated final weight in the reference groups, despite a slightly higher mortality than the Preline group (Table 2.6).

Moreover, fall stockings in phase 1 shows a higher biomass gain favoring the Preline group (91,733 kg) compared to the estimated biological performance in the reference group (85,342 kg). This due to a higher estimated final weight in the reference groups, despite a higher mortality in Preline group.

Table 2.6: *Estimation of biomass for spring and fall stockings in Phase 1. Preline group and reference group for phase 1 (post-smolt). Average performance results (final weight and mortality) during the fall and spring generations (n=3).*

Phase 1	Season	Fish stock	Mortality %	Estimated Final weight per fish (kg)	Estimated Biomass in the system (kg)
Preline	Spring	200,000	1.01	0.527	104,335
Open net	Spring	200,000	1.59	0.637	125,374
Preline	Fall	200,000	5.43	0.485	91,733
Open net	Fall	200,000	2.13	0.436	85,342

Biomass estimation in phase 2: Grow-out

In theoretical output of the grow-out period, the spring stockings shows an estimated biomass favoring the Preline group (796,764 kg) compared to the Reference group (693,787 kg), explained by the higher final weight in this group (Preline), despite a higher mortality. The biomass gain was calculated to be approximately 100,000 kg higher in the Preline group.

In addition, in the fall stockings, the estimated biomass in Preline group (905,704 kg) is favored compared to the reference group (603,532 kg), as a combined effect of higher final weight and lower mortality in the Preline group in comparison to the reference group (Table 2.7). The biomass gain for the fall stockings was calculated to be approximately 300,000 kg higher in the Preline groups.

Table 2.7*: Estimation of biomass for spring and fall stockings in phase 2. Preline group and reference group for phase 2 – grow-out in open net-pens (final weight and mortality).*

Phase 2	**Season**	**Fish stock**	**Mortality %**	**Estimated Final weight per fish (kg)**	**Estimated Biomass in the system (kg)**
Preline	Spring	200,000	14.4	4.654	796,764
Open net	Spring	200,000	8.64	3.797	693,787
Preline	Fall	200,000	7.05	4.872	905,704
Open net	Fall	200,000	25.12	4.030	603,532

Discussion of Methods: Chapter 2 – Benchmark Analyses of Six Generations Reared in Preline and Reference Groups

All the generations in this study experienced similar rearing conditions during the freshwater stage and did originate from the same strain. This was of great importance when benchmarking biological performances in Atlantic salmon reared in two different systems (Preline and open sea cage). In a scientific context, full control of the experimental parameters is strived for, including control of environmental and physical parameters. Even though the environmental conditions and physical parameters cannot be controlled in this experiment, the results in this thesis represent field results as they would be in a real production situation and are constantly monitored.

When conducting large-scale production experiments, the experimental design should include replicates to strengthen the interpretation. In cooperation with the industry, large-scale experimental studies have some limitations. To cope with these limitations and logistical challenges, the arrangement of this experiment was designed to meet and accommodate most of the critical parameters for an acceptable benchmark between the two rearing systems (Preline and open net-pen). Consequently, it was decided to run and interpret six generations of stockings from May 2015 to February 2018. This allowed for an investigation in seasonal variations between the rearing systems, where three generations were stocked during spring and three generations were stocked during fall. The experimental design allowed for a realistic interpretation of salmon produced according to Norwegian aquaculture.

Variations in the measured parameters in the post-smolt phase were likely due to different rearing systems and conditions (Preline and open net-pen). As mentioned, the Preline system generates a relatively stable condition by pumping water from a fixed depth (~ 30 m). For the grow-out phase in open net-pens, the registered parameters varied as a result of different placement of the open net facilities. Further, the locations were exposed to surface layers with

more dynamic changes in oxygen and temperature. However, the water temperatures during the grow-out phase was similar for all the generations (see Table 2.4). Moreover, fish in the open net system distribute themselves over various depths, making it difficult to determine exactly which parameter the fish is exposed to.

In terms of growth in fish, temperature is regarded as one of the main factors that influence this process (Fry, 1971). In the post-smolt phase, a difference in final weight was expected due to dissimilarity in average temperature between the rearing systems during the season (Preline and open net). In this experiment, the weight estimations were generated by weight models using an FCR value of 1.1 as input in the Fishtalk calculation and TGC (Thermal Growth Coefficient) throughout the study. TGC summarizes fish growth by taking temperature into account, allowing for the comparison of fish influenced by different water temperatures (Iwama & Tautz, 2011; Thorarensen & Farrell, 2011). Recent studies of the 3rd generation in the Preline system showed that the estimations based on feed output (Fishtalk calculations, FCR = 1.1) correspond with the weight measurements that were conducted during the post-smolt phase (Moe et al., 2017). Thus, it should be expected that the weight estimations conducted during the grow-out phase are credible.

The thermal growth coefficient (TGC) in this experiment was not calculated from individually tagged fish. Ideally, additional measurements should be included in this study because it is essential to evaluate the growth potential in the fish and strengthen the overall credibility of the weight estimations. Moreover, according to Jobling (2003), the TGC estimations should be interpreted with caution when the temperature exceeds the optimum of 15–16°C. During the experimental period, the temperature exceeded 16°C in some of the generations during the grow-out phase. When temperatures are above the optimum, the growth rate starts to decrease; therefore, calculating TGC is most accurate between 7.5–12.5°C (Jobling, 2003). Except for generation 6 (> 40 days in reference and; >30 days in the Preline

group), the periods with a temperature above 16°C were relatively short within the generations in this study, suggesting that the TGC is most likely not affected. The SGR is affected by both temperature and body weight, where the specific growth rate increases with increasing sea temperature and decreases with increasing body weight (Talbot, 1993).

Since this is a large-scale industrial experiment, limitations were met to run several replicates of the experimental groups. A large amount of empirical data (more than 2.1 million fish) has been collected (daily and weekly) over several years in this study. FCR and TGC are observational values calculated from data generated from Lerøy Vest AS. In addition, mortality and sea lice count were registered weekly, for each generation. Since there were six replicates for each parameter, one for each generation, a statistical analysis could be conducted for these values. For a relevant comparison of the parameters (TGC, initial weight, final weight, gain, FCR, mortality and sea lice count), the values were analyzed within spring and fall generations (n=3). Ideally, periodic samplings of weight and length should be conducted during the experimental period. Moreover, the number of measurements (n = 6) in this study suggest differences between the groups but is insufficient to determine trends. Consequently, more repetitions of stockings would help determine differences among the measured parameters in this research.

Discussion of Results

Phase 1: Post-smolt

Growth

Growth in fish is influenced by environment and is commonly used as an indicator of animal performance (Thorarensen & Farrell, 2011), where the temperature is considered one of the main factors that influences growth (Fry, 1971). Moreover, the rate of metabolic function is controlled by temperature, and will influence the efficiency of biomass gain from feeding (Handeland et al., 2008). Hence, the initial weight in phase 1 was significantly different between the spring and fall generations, as could be expected, since the 1+ smolts were bigger than the 0+ smolts.

The growth performance varied through the different seasons, and in the fall stockings, the overall growth performance observed was better in fish from the Preline system, apart from generation 6. During spring, the observed overall growth performance was higher in the reference group, except for in generation 5, where the growth performance was better in the Preline fish. These variations in growth performances are probably related to variations in smolt quality from the freshwater facility. The observations are based on empirical production data (n =1), and for this dataset, a statistical analysis could not be performed. Therefore, this is a descriptive analysis of the data.

During phase 1, the average temperature varied between the Preline system and the reference group in open net-pens within the generations. This is likely affected by both the seasonal temperature conditions and bathymetric differences (Preline fish were exposed to water from 30 m of depth), whereas the fish in the reference group that were in open net-pens were exposed to surface water (Figure I.4). The temperature effect for growth is similar to the studies done for the 3rd Preline generation (also a part of this dataset), where fish from the

reference group (12.9°C on average) had a significantly higher final weight compared to the Preline fish (9.5°C on average) at the end of the post-smolt phase (Moe et al., 2017).

To mitigate the different temperature conditions and regional differences between the rearing systems, a model incorporating growth rate per day that is dependent on the daily temperature was employed (TGC). For the TGC in phase 1, no significant difference was shown between the systems, indicating that the fish in phase 1 had a similar growth rate when the difference in temperature was incorporated in the calculation.

FCR

During phase 1, the Preline fish were forced to swim against a moderate current, causing mild aerobic training, in contrast to the fish in the reference group that was reared in open net-pens. Raceway systems, like Preline, are designed to control water velocity, which is known to affect growth performance (Castro et al., 2011; Totland et al., 1987). Atlantic salmon exposed to long-term sustained swimming showed a 38% increase in growth with respect to the non-exercised fish (Castro et al., 2011; Totland et al., 1987) Moreover, the feed conversion is also affected by exercise, and several studies have shown that exercise decreases the Feed Conversion Ratio (FCR) in different salmonid species (Christiansen et al., 1992; East & Magnan, 1987; Leon, 1986), which might also affect the FCR in the Preline and the reference groups. Growth and FCR should be seen in the context of the possible reduction of stressors, aggressive behavior, fewer interactions and hierarchy development between the fish swimming in Preline system (Adams et al., 1995; Christiansen et al., 1991; Jobling et al., 1993; Solstorm et al., 2016). In addition, fish density impacts the growth performance of post-smolt, and findings in Calabrese et al (2017) demonstrated the density should not exceed 75kg/m^3. In the Preline system, the average stocking density was higher (M = initial density 10.3 kg/m^3; M = final density 42 kg/m^3) compared to the reference group (M = initial density 0.7 kg/m^3; M = final density 3.3 kg/m^3) in all generations. However, in this study, no significant difference was

registered for growth and FCR between the rearing systems in phase 1. The observed growth performance across the generations shows that temperature is the main factor influencing growth, independent of the rearing system.

Mortality

Mortality was measured daily based on individual inspections in each group by employees at Lerøy Vest AS. The registered mortality varied between the generations. Observations from the field have shown that mortality rate for post-smolt could be influenced by the transport from freshwater to seawater (Harald Sveier, Lerøy AS, 2020, pers. comm.). During the post-smolt phase, no overall difference in mortality was registered between the Preline group and the reference group. However, Totland et al. (1987) documented that fish exposed to an exercise regime tend to have an increased mortality rate during the first couple of days in the training regime. For phase 1, the rate of mortality in the Preline was especially affected by one particular generation (generation 6), were a higher mortality rate was observed during both post-smolt and grow-out phase. Whether this increased mortality was related to poor smolt quality, high density, the exercise achieved in the Preline system or other factors is difficult to determine.

Sea lice

In phase 1, infestations of sea lice were significantly lower in the Preline group compared to the reference group in open-net pens. This observation was expected since the water in the Preline system is controlled by a deep-water intake under the sea lice belt, in contrast to in open net-pens, where the fish are regularly exposed to the natural environment, in which sea lice is abundant at the open locations (Torrissen et al., 2013). The findings of reduced sea lice pressure on fish in the Preline are of great interest and suggest that new technology, such as S-CCS could contribute to reduce sea lice infestations on farmed post-smolt in sea.

Phase 2: Grow-out

Growth

At the end of phase 2, harvest, fish from the Preline group showed a significantly higher weight gain compared to the reference group. In addition, fish from the Preline group had a significantly higher final weight compared to the reference group. These results correspond to studies done for salmonids raised in closed containment systems (CCS) and exposed to moderate water velocity, where an increase in growth as an effect of exercise has been documented (Nilsen et al., 2019).

For the observed overall growth performance during fall, higher growth was observed in fish from the Preline system, except for generation 1. During spring, the observed overall growth performance was higher in fish originating from the Preline system. These growth performances are based on empirical production data, and for this dataset (n = 1), a statistical analysis could not be performed.

The observed growth performance of fish in this study indicates that rearing of salmonids in an S-CCS prior to the grow-out phase in open sea cages could have a positive effect. Interestingly, the temperature conditions between the (Preline and reference) did not differ during the grow-out phase. For the TGC, no significant difference was shown between the groups, indicating that the fish in phase 2 had a similar growth rate when incorporating the TGC, which takes into account the optimal season temperature for fish growth (Iwama & Tautz, 2011). For SGR in phase 2, no difference between the groups was found.

Moreover, several studies have indicated that water velocities (> 0.40 BL/s) can have positive effects on growth through muscle fiber hypertrophy (Ibarz et al., 2010; Totland et al., 1987). Moe et al. (2017) showed that fish in the 3rd Preline generation, at the end of the post-smolt phase, had 2.44 times higher frequency of muscle fibers in the smallest interval group (0–20μm), compared to the reference group. Taken together, these observations suggest that

the higher growth in Preline fish during the grow-out phase could be explained by hypertrophy of the newly recruited muscle fibers.

Mortality

At the end of the grow-out phase, a significant lower mortality for the fall stockings was observed in the Preline group in comparison to the reference group. In the spring stockings, the reference group showed a lower mortality rate in comparison to the Preline group. The mortality observations in phase 2 were also influenced by the one Preline generation that cumulated a higher mortality rate during this experiment (Generation 6).

The difference in mortality rate between Preline fish and reference might relate to the exercise achieved in the Preline system, where the exercised fish tend to be more capable of resisting environmental and physical challenges in the sea. These present findings are supported in the study by Moe et al. (2017).

FCR

In aquaculture, feed conversion ratio (FCR) is an important measurement that has an economic impact. In the grow-out phase, the FCR was significantly different during seasons between the Preline fish and reference fish. The FCR can be described as the amount of mass gained by the fish relative to the amount of feed consumed (Jackson, 2010). The FCR is affected by exercise, and several studies have shown that exercise decreases the FCR in different salmonid species (Christiansen et al., 1992; East & Magnan, 1987; Leon, 1986). Studies have also shown that weight gain is achieved with less feed when the appetite is stimulated as an effect of training (Davison, 1989). In open sea cages, freely swimming fish tend to form dominant hierarchies and show increased aggression; this can again lead to less food available for subordinate fish (Adams et al., 1995; Brännäs, 2009). In phase 2, during the fall, the FCR was lower in the Preline group in comparison to the reference group. The decrease in FCR could be related to a better appetite in the robust and exercised fish from Preline.

Sea lice

After a free-swimming larvae period, Sea lice (*L. salmonis*) settle, attach, and feed on its fish host. The sea lice cause stress and physical damage to the fish, adversely affecting growth and performance. Consequently, severe infestations of sea lice can lead to secondary infections and mass mortalities (Costello, 2006; Pike & Wadsworth, 1999; Torrissen et al., 2013). At the end of phase 2, no statistical differences in sea lice infestations was shown between fish from the Preline group and the reference group. However, the trend points to a lower infestation level in fish reared in Preline. In addition, according to observations from the field the need for sea lice treatments in Preline groups are reduced in comparison to groups in open net-pens (Harald Sveier, Lerøy AS, 2020, pers. comm.). This observation could be influenced by many factors, where one aspect could be the skin of the fish. The skin and associated mucus layer of Atlantic salmon constitutes its first line of defense against the environment. The skin of the fish protects both as a physical barrier and as an active and protective layer with immunological capacities that interacts with the surrounding environments (Sveen et al., 2016). In addition, the skin provides protection against external agents and has a high capacity for regeneration and healing (Richardson et al., 2016). Recent studies of the skin barrier, including epidermis and dermis, showed that thickness and mucus cell numbers increased in line with growth after seawater transfer in Atlantic salmon (Karlsen et al., 2018). Accounting for these results and observations, it could be suggested that the fish reared in the Preline system as post-smolt before the grow-out phase are more robust in terms of sea lice infestations. The reduced sea lice infestations could also help explain the lower mortality rate observed for Preline fish.

Estimated Biomass

Phase 1: Post-smolt

The estimated biomass in phase 1 was calculated based on the average performance in survivors at the end of the post-smolt phase (3–4 months) in fish reared in the Preline system and the reference group for the fall and spring stockings (n = 3). In the spring stockings, the estimated biomass output favored the open sea cage strategy with a higher final weight per fish giving a higher total biomass gain of 17 %, despite the higher mortality compared to the Preline group in phase 1.

The fall stockings showed an estimated biomass favoring the Preline strategy due to the higher estimated final weight per fish, with 7 % higher gain compared to reference, despite the higher mortality in Preline system during the fall stockings. This estimation should be seen in context to the Preline generation that showed very high mortality rate during the experimental period.

Moreover, these estimated differences in growth among the groups through spring and fall stockings in phase 1 are probably related to the "opposite season" temperature conditions generated by the water inlet (~30 m of depth) in the Preline system, since the growth is highly affected by temperature (Fry, 1971).

Phase 2: Grow-out

The estimated biomass output of the adult salmon at the end of phase 2 (grow-out) favors the Preline strategy in both spring and fall stockings. For the spring stockings, the mortality rate was higher in fish from the Preline system in comparison to reference groups. Still, the bigger final weight per fish reared in the Preline system (>600 g), gave 12% (100,000 kg) higher estimated biomass gain compared to the reference group.

For the fall estimations, fish reared in the Preline system prior to grow-out phase showed a higher final weight (>800 g) per fish compared to reference group. In combination with a

lower mortality rate, the fall performance in Preline fish suggests an estimated increase in biomass of 40% (approximately 300,000 kg), in comparison to the reference group.

In order to carry out these theoretical estimations, assumptions such as equal stocking density and initial weight were set for a realistic comparison. During phase 1, the estimated final weight per fish was higher in the reference group in the fall stockings and was quite similar for the spring stocking. Nevertheless, after undergoing the grow-out phase in fall and spring, the Preline fish showed a dramatically higher estimated final weight per fish in comparison to fish reared in open sea cages through both phases. As already discussed, this could be related to the hyperplasia fish achieved from exercise in the Preline system, documented in the study of Moe et al. (2017).

From an economical point of view, these estimations show that implementation of the S-CCS strategy could have a beneficial economic impact, by potentially achieving more produced biomass and increased welfare for the fish, due to lower mortality and stable growth performance in the grow-out phase. Based on these estimated biomasses, an economic analysis of implementing S-CCS in a conventional production regime for Atlantic salmon is depicted in appendix IV.

Conclusion

This benchmark study determined differences in phase 1- post smolt and phase 2 – grow-out of Atlantic salmon. The main finding for phase 1 was that fish reared in the Preline system had lower sea lice infestations. After having undergone the grow-out phase, fish from the Preline system had a higher final weight, higher weight gain and showed a lower mortality than the reference group in the fall stockings. Accounting for these findings, despite the low number of measurements in this experiment, assessment of S-CCS technology for rearing of post-smolt show a positive impact in production of Atlantic salmon.

Hypotheses for phase 1 (post-smolt):

- **$H0_{1a}$**: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on growth compared to the reference group, **is accepted.**
- **$H0_{2a}$**: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on feed conversion ratio compared to the reference group, **is accepted**.
- **$H0_{3a}$**: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on mortality compared to the reference group, **is accepted**.
- **$H0_{4a}$**: Rearing Atlantic salmon post-smolt in Preline system has no significant effect on sea lice infestations compared to the reference group, **is rejected**. Significantly lower infestations were observed in Preline system, so **$H1_{4a}$ is accepted:** Rearing Atlantic salmon post-smolt in Preline system has a significant effect on sea lice infestations compared to the reference group

Hypotheses for phase 2 (grow-out in open sea cages):

- **$H0_{1b}$**: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on growth during the grow-out phase compared to the reference group, **is rejected**. Due to the findings of significantly higher weight gain and final weight in Preline fish, **$H1_{1b}$ is accepted:** Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on growth during the grow-out phase compared to the reference group.
- **$H0_{2b}$**: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on feed conversion ratio **is rejected**. Results from feed conversion shows a significantly effect between the groups, where a better conversion is observed in Preline, thus, **$H1_{2b}$ is accepted:** Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on feed conversion ratio.
- **$H0_{3b}$**: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on mortality, **is rejected**. Since significant difference was found for interaction between season and rearing method during phase 2, therefore, **$H1_{3b}$ is accepted:** Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has a significant effect on mortality.
- **$H0_{4b}$**: Rearing Atlantic salmon post-smolt in Preline system before transfer to open sea cages for a grow-out phase has no significant effect on sea lice infestations, **is accepted**.

Synoptic Discussion: Acute challenge test and Benchmark analysis

As presented earlier, the salmon industry is facing several challenges, where sea lice infestations, escapees of farmed fish, diseases, pollution and environmental impact represents some of the major problems for a prospective growth (Oppedal et al., 2011; Torrissen et al., 2013; Glover et al., 2012; Rosten et al., 2011). Moreover, in the commercial production line, the post-smolt stage has shown to be a sensitive and critical phase for survival, and a high rate of the fish never reach marked size (Roberts & Pearson, 2005; Bleie & Skrudland, 2014; Holtby et al., 1990). The predominant production of Atlantic salmon occurs in open sea cages. Application of S-CCS as a strategy to reduce the period fish are exposed to open sea requires knowledge on biological requirements of post-smolt in these systems. The motivation of this study was to determine if growth performance and welfare of post-smolt are affected by rearing methods (S-CCS and open sea cage) prior to grow-out phase. The results from chapter (1); Acute challenge test and chapter (2); Benchmark analysis provide basic insight of the effect on rearing post-smolt in both S-CCS and open sea cages.

Acute challenge test: S-CCS *versus* open sea cages

In aquaculture, the fish are confined and are thus not able to escape from stressors; farmed fish could be exposed to several different stressors, such as suboptimal water quality, repeated handling, transport, and crowding. The effects of many of these challenges on fish have been investigated in various studies (Di Marco et al., 2008; Gorissen et al., 2012; Pottinger, 2010; Remen et al., 2012). In addition, the acute stress response of fish has been widely reviewed (Barton, 2002; Wendelaar Bonga, 1997). However, knowledge of long-term stress on salmonids remains limited. It is assumed that fish reared in open sea cages could be exposed to stressors that might potentially lead to a condition of chronic stress.

The ACT experiments in chapter 1 investigated the stress response in post-smolt after undergoing an acute stress challenge in two different environments (S-CCS and open sea cage).

Exposure to a low allostatic load (eustress) may have a positive impact on animal performance (Kupriyanov & Zhdanov, 2014), in contrast to exposure to a high allostatic load (long-term or repeated exposure for a stressor), which results in a chronic stress condition. In this state, the alarm signals and initial stress response could be minimal as a result of an allostatic overload (distress) on the animal (McEwen & Seeman, 1999).

Fish reared in the S-CCS (Preline and Neptune) showed a stronger response in cortisol release compared to their reference group after ACT. This observation is similar to the findings in Korte et al. (2007), where fish in a state of good welfare increase their cortisol levels when reacting to an acute challenge, according to the concept of allostasis. Further, the observations a lower cortisol response in the reference groups in open net pens correspond to studies done on Atlantic salmon exposed to chronic stress followed by an additional stressor which resulted in suppressed cortisol response (Grassie et al., 2013; Madaro et al., 2016). These observations also correlate to the general downregulation of the HPI axis, a regulation that is common in fish adapting to chronic stress (Barton, 2002; Barton et al., 1986; Vijayan & Leatherland, 1990).

As opposed to open net-pens, the S-CCS allow for control of the water current within the system. Active species, such as salmonids, can be made to swim facing a constant current. This swimming behavior tends to create schools where the interaction between the fish is reduced, making fewer dominant hierarchies. This contrasts with free-swimming fish in still water, which tend to form dominant hierarchies and show increased aggression (Adams et al., 1995; Winberg et al., 1991). The schooling behavior generated by the S-CCS might relate to the observed stress responses and less individual variability in the measurements from the S-CCS groups in chapter 1. The overall observation in measured parameters showed that the fish

reared in the S-CCS had a more homogeneous stress response (cortisol, plasmatic ions, lactic acid) in comparison to fish in the reference group.

The results from chapter 1 indicate that fish reared in open sea cages are more prone to reaching an allostatic overload as a result of repeated or long-term exposure to different stressors in the sea. This contrasts to fish reared in S-CCS, where a more controlled and stable environment is achieved, and therefore the fish show a stronger ability to handle an acute stressor. In addition, the low baseline response observed in the S-CCS groups imply that the fish experience a good state of welfare in these systems.

Benchmark analysis: six generations of stocking

The combined results from chapter 1 indicate that rearing post-smolt in S-CCS could influence growth performance during the grow-out phase, which lead to the benchmark analysis conducted in chapter 2. The aim of the analysis was to investigate and benchmark biological performance in fish reared in the Preline system (S-CCS), and fish reared in a traditional open cage system.

In phase 1 post-smolt, the continuous current forced the fish to swim and exercise in a sheltered and protected system. The findings from phase 1 show that growth is probably more influenced by temperature rather than the rearing system. The lower infestations of sea lice on Preline fish is an observation of great importance. For the wild salmon this finding shows that farmed fish in S-CCS imposes less impact on the surrounding environment in term of sea lice pressure. From phase 2 grow-out, the findings suggest that exercise and stable water conditions during post-smolt phase has a positive impact on the grow-out phase. The significantly lower mortality in fall stockings and less sea lice treatments observed on Preline fish indicate that the fish are more robust. Moreover, with the higher final weight and weight gain in phase 2, the findings suggest a trend favoring the Preline strategy. Especially accounting for the estimated

biomass giving a higher gain in Preline fish for spring (100,000 kg) and fall (300,000 kg) stockings.

Today, some of the S-CCS technologies in the industry are slowly emerging from prototypes to proper production facilities for rearing of post-smolts. The Preline system for instance was one of the first deployed and has been in operation for almost five years, during which the farmers have gained important experience. The implementation of S-CCS in conventional production cycle for salmon are affected of many factors, such as license fee for operating, political regulations, environmental impact, fish welfare and economically factors. To determine if S-CCS should be fully integrated in conventional salmon production, collaboration between researches, politicians and decision makers are important.

In summary, the findings in the acute stress test and benchmark study shows promising indications favoring S-CCS technology compared to full time exposure in open sea. It could be speculated that use of S-CCS is favorable during tough conditions and winter months, and that open sea cages might be preferred during calmer summer months. To determine an optimal use and strategy of floating semi closed containment systems, further research is needed in order to achieve a broader understanding and knowledge of producing post-smolt in S-CCS.

Future perspectives

Further studies are needed to understand the impact of implementing S-CCS in the post-smolt phase for Atlantic salmon, regarding seasonal and environmental differences in the rearing systems. Preferably, the samplings should follow in time series, allowing for a deeper understanding of the stress response development in the fish post ACT treatment. The samplings in this study were conducted during spring (March and April); ideally, seasonally samplings should also be included. All the fish in such an experiment should originate from the same strain and be of similar size and weight. Moreover, greater knowledge of normal biochemical parameters in fish reared in unstressed and chronic stressed conditions is essential to compare the effect of S-CCS compared to open net pens. At present, knowledge of the normal concentration range in the measured parameters (cortisol, chloride, sodium, calcium, glucose, magnesium, and lactic acid) is scarce for Atlantic salmon cultured in Norway. To mitigate the lack of this knowledge, fish (n = 30) was analyzed to account for individual variability and for a broader understanding in this study.

To strengthen the statistical power and general knowledge on how fish reared in S-CCS perform in open sea cages, additional measurements are needed to support the results in this study. Optimal swimming velocity in S-CCS and the effect of hyperplasia are areas that needs to be more investigated. In addition, the observations of less sea lice infestation on the Preline fish in open net pens rises intriguing questions that should be followed up. Moreover, environmental impact on the surroundings, sustainable use of the sludge from the S-CCS and optimization of stocking strategy are areas of interest for future research.

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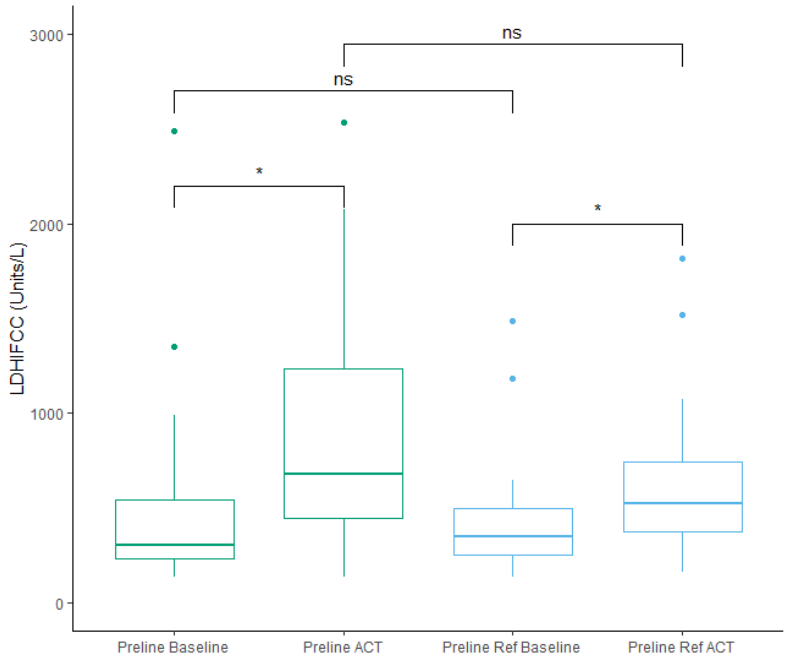
Appendix I – Additional results chapter 1

Appendix I include additional results that were not included in chapter 1. Significant differences are presented in the graphs. Since the additional results are not part of the main thesis, the statistical results are not included in Appendix II. The analysis of the additional plasma samples was conducted using the ABX Pentra as described in chapter 1.

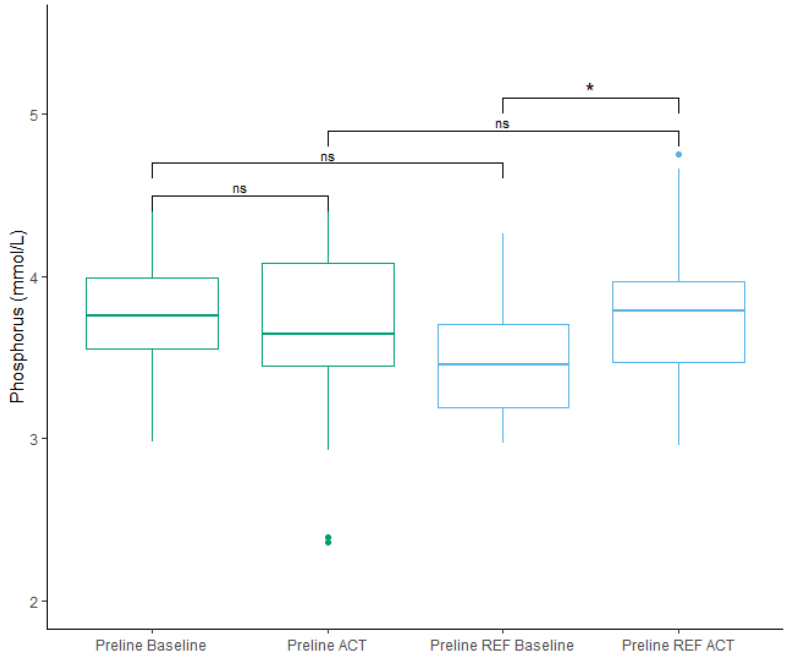
Experiment 1 – Preline system; additional results

Plasma Lactate Dehydrogenase concentration



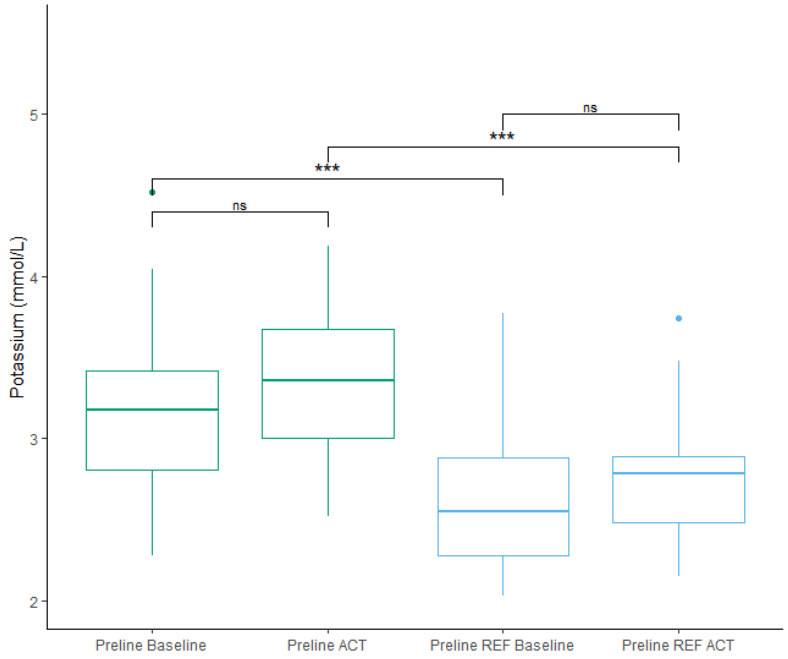
Appendix Figure 1: Plasma lactate dehydrogenase concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are $p<0.05$ (*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****), ns = no significance.

Plasma Phosphorus concentration



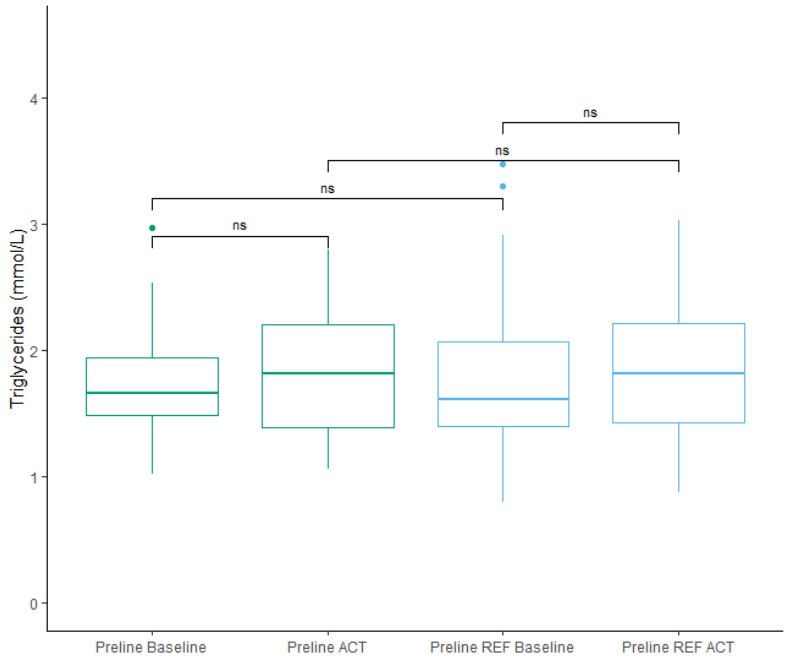
Appendix figure 2: Plasma phosphorus concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Potassium concentration



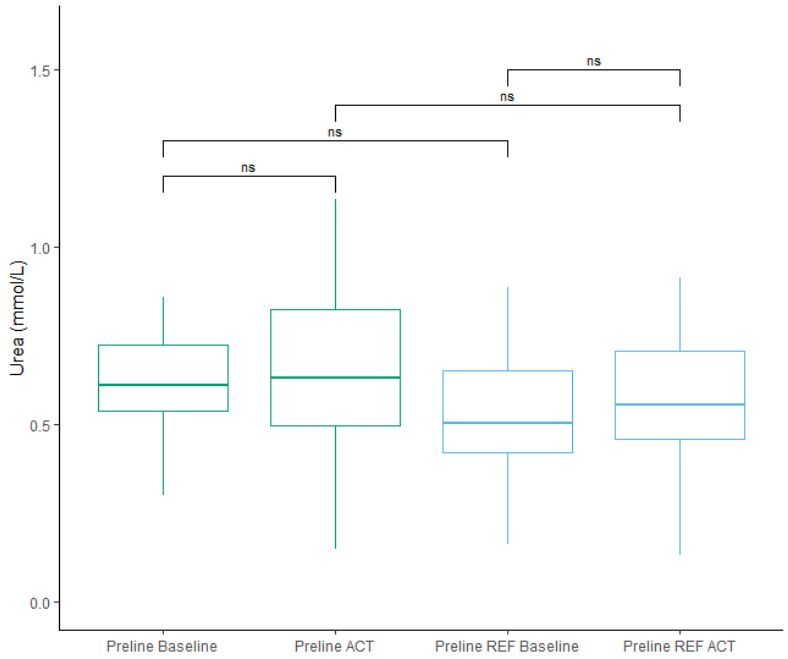
Appendix figure 3: Plasma potassium concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Triglycerides concentration



Appendix figure 4: Plasma triglycerides concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

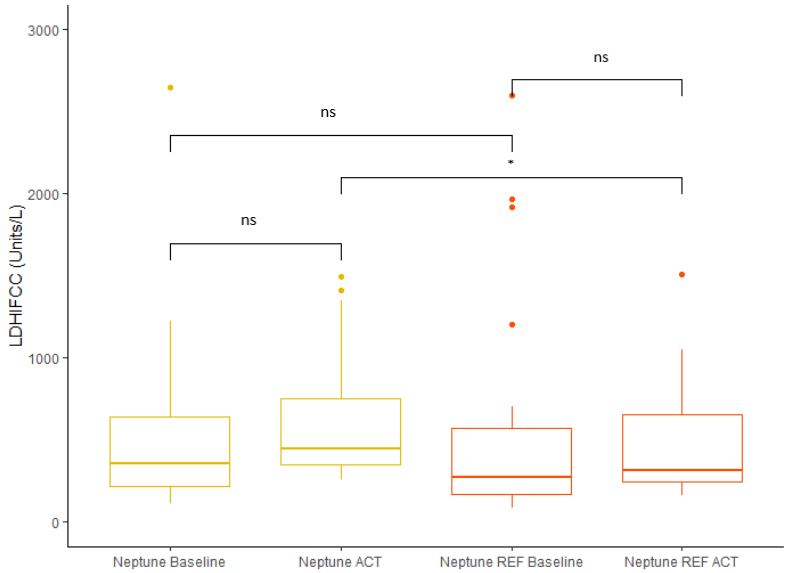
Plasma Urea concentration



Appendix figure 5: Plasma urea concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

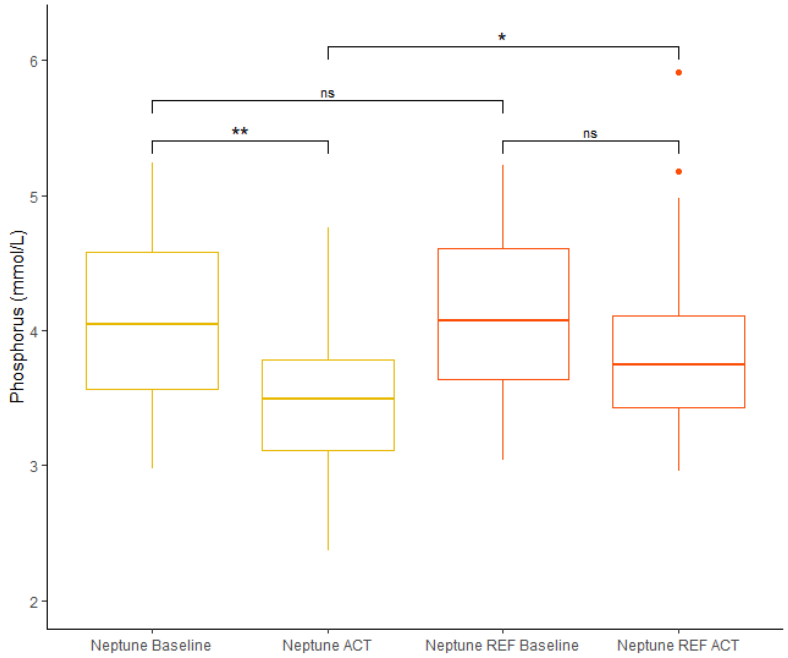
Experiment 2 – Neptune system; additional results

Plasma Lactate Dehydrogenase concentration



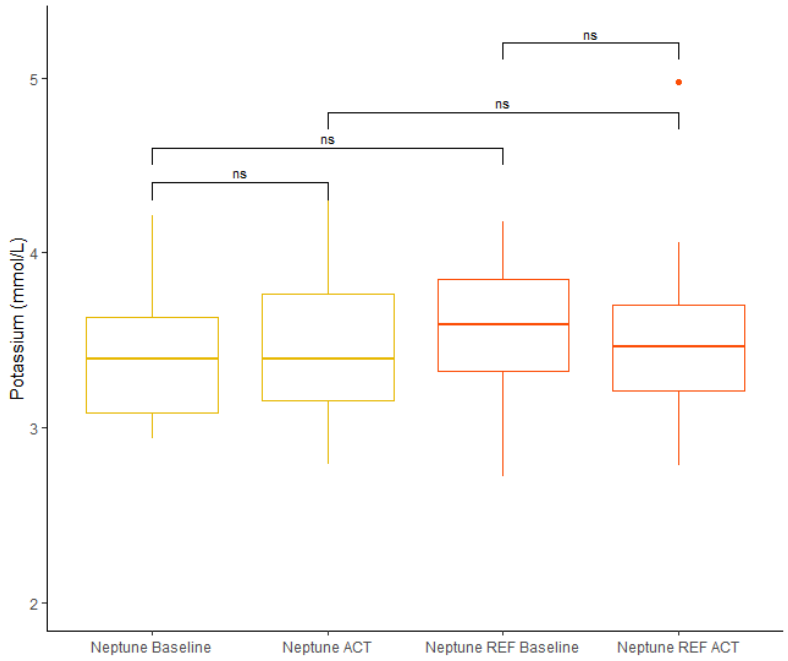
Appendix figure 6: Plasma lactate dehydrogenase concentration in Neptune S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Phosphorus concentration



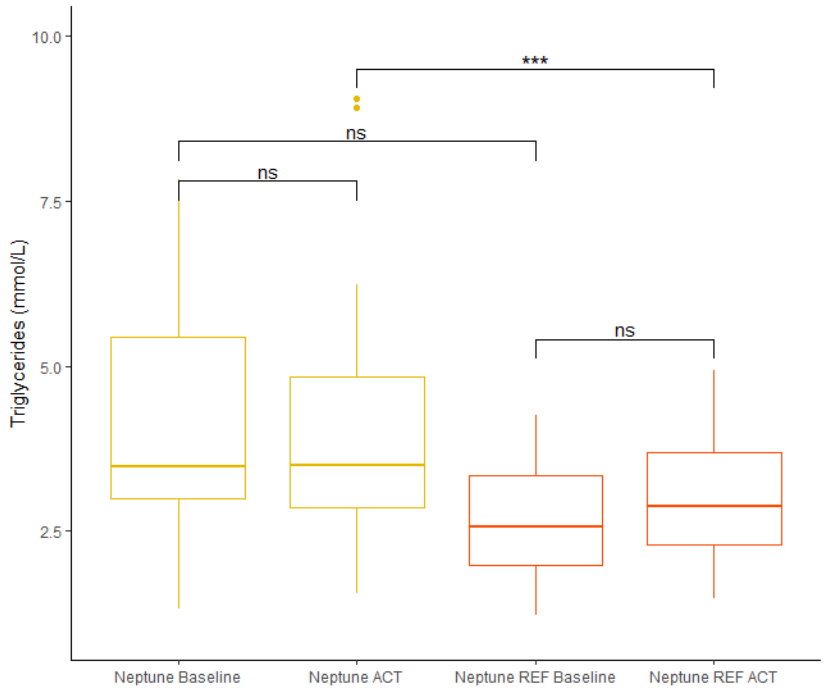
Appendix figure 7: Plasma phosphorus concentration in Neptune S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Potassium concentration



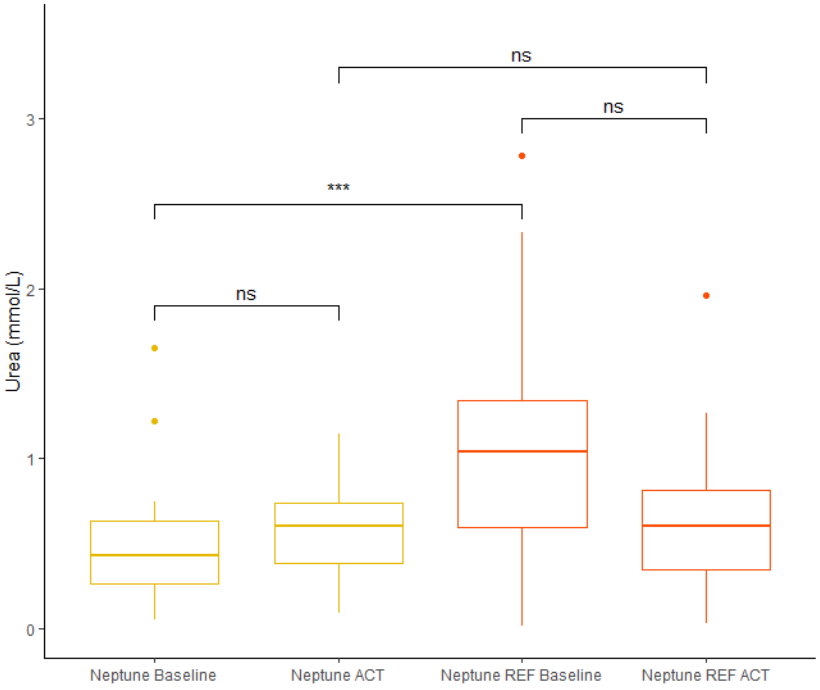
Appendix figure 8: Plasma potassium concentration in Neptune S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Triglycerides concentration



Appendix figure 9: Plasma triglycerides concentration in Neptune S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance.

Plasma Urea concentration



Appendix figure 10: Plasma urea concentration in Preline S-CCS. Baseline and the Acute Test Challenge (ACT) treatment. Comparative reference group are also included. The lines represent the medians, and the dots represent outliers. Significance levels are p<0.05 (*), p<0.01(**), p<0.001(***), p<0.0001(****), ns = no significance

Appendix II – Statistical Analyses Chapter 1

Experiment 1 – Preline system

Objective – ACT treatment in fish from Preline system and reference

Sampling - Cortisol

Appendix table 1: Test results from a nonparametric Kruskal-Wallis one-way ANOVA. Significant levels are indicated with asterisks, p < 0.05: (*); p < 0.01: (**); p < 0.001: (***); p < 0.0001 (****).

	K-W chi-squared	DF	p-value
Treatment	76	3	< 2.2e-16 ****

Appendix table 2: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	810	2.2e-16 ****
ACT – Ref ACT	787	8.214e-13 ****
Baseline – Ref Baseline	128	3.58e-05 ****
Ref ACT – Ref Baseline	417	0.2461

Sampling - Sodium

Appendix table 3: Test results from a nonparametric Kruskal-Wallis one-way ANOVA.

	K-W chi-squared	DF	p-value
Treatment	56.416	3	3.425e-12 ****

Appendix table 4: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	864	1.015e-15 ****
ACT – Ref ACT	405	0.657
Baseline – Ref Baseline	215	0.00037 ***
Ref ACT – Ref Baseline	678	0.00057 ***

Sampling - Calcium

Appendix table 5: Test results from a nonparametric Kruskal-Wallis one-way ANOVA. See Appendix table 1 for additional info.

	K-W chi-squared	DF	p-value
Treatment	57.324	3	2.191e-12 ****

Appendix table 6: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	900	2.984e-11 ****
ACT – Ref ACT	455.5	0.9411
Baseline – Ref Baseline	274	0.0094 **
Ref ACT – Ref Baseline	677.5	0.0007 ***

Sampling - Magnesium

Appendix table 7: Test results from a nonparametric Kruskal-Wallis one-way ANOVA.

	K-W chi-squared	DF	p-value
Treatment	50.743	3	5.548e-11 ****

Appendix table 8: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	840	6.552e-11 ****
ACT – Ref ACT	402	0.7853
Baseline – Ref Baseline	307.5	0.0538
Ref ACT – Ref Baseline	661.5	0.0006 ***

Sampling - Lactic Acid

Appendix table 9: Test results from a nonparametric Kruskal-Wallis one-way ANOVA. See Appendix table 1 for additional info.

	K-W chi-squared	DF	p-value
Treatment	40.831	3	7.101e-09 ****

Appendix table 10: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	557	$2.2e^{-16}$ ****
ACT – Ref ACT	557	0.1159
Baseline – Ref Baseline	345.5	0.1241
Ref ACT – Ref Baseline	585	0.0462 *

Sampling - Glucose

Appendix table 11: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	4.4	0.0054 **

Appendix table 12: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-0.048664	0.037984	-1.281	0.5767
ACT – Ref ACT	-0.067952	0.037983	-1.789	0.2839
Baseline – Ref Baseline	0.116477	0.038310	3.040	0.0153 *
Ref ACT – Ref Baseline	-0.000139	0.038310	-0.004	1.0000

Sampling - Chloride

Appendix table 13: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	29.172	3.968e-14 ***

Appendix table 14: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-20.973	2.623	-7.997	<0.001 ***
ACT – Ref ACT	-1.850	2.623	-0.706	0.8948
Baseline – Ref Baseline	6.907	2.623	2.634	0.0464 *
Ref ACT – Ref Baseline	-12.217	2.623	-4.658	<0.001 ***

Experiment 2 – Neptune system

Objective – ACT treatment in fish from Neptune and reference group.

Sampling – Cortisol

Appendix table 15: Test results from a nonparametric Kruskal-Wallis one-way ANOVA.

	K-W chi-squared	DF	p-value
Treatment	64.9	3	5.248e-14 ****

Appendix table 16: Test results from a Mann-Whitney-Wilcoxon post-hoc test. See Appendix table 1 for additional info.

Comparison	w-value	p-value
ACT – Baseline	780	3.009e-16 ***
ACT – Ref ACT	662	0.001443 **
Baseline – Ref Baseline	203	0.0552
Ref ACT – Ref Baseline	522	0.001192 **

Sampling – Chloride

Appendix table 17: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	14.933	2.837e-08 ***

Appendix table 18: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-19.387	3.908	-4.961	< 0.001 ***
ACT – Ref ACT	4.666	3.942	1.184	0.63830
Baseline – Ref Baseline	9.223	3.908	2.360	0.09076
Ref ACT – Ref Baseline	-14.829	3.942	-3.762	0.00155 **

Sampling - Sodium

Appendix table 19: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	46	2.2e-16 ***

Appendix table 20: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-30.900	3.147	-9.818	< 1e-05 ***
ACT – Ref ACT	1.558	3.264	0.477	0.964
Baseline – Ref Baseline	14.880	3.120	4.769	2.7e-05 ***
Ref ACT – Ref Baseline	-17.578	3.238	5.428	< 1e-05 ***

Sampling - Calcium

Appendix table 21: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	82.606	< 2.2e-16 ***

Appendix table 22: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-0.96467	0.07614	-12.669	<0.001 ***
ACT – Ref ACT	-0.04033	0.07614	-0.530	0.9517
Baseline – Ref Baseline	0.22500	0.07614	2.955	0.0191 *
Ref ACT – Ref Baseline	-0.69933	0.07614	-9.185	<0.001 ***

Sampling - Glucose

Appendix table 23: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	17.064	3.02e-09 ***

Appendix table 24: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-0.4757	0.1667	-2.854	0.0262 *
ACT – Ref ACT	0.6970	0.1667	4.182	<0.001 ***
Baseline – Ref Baseline	0.6923	0.1667	4.154	<0.001 ***
Ref ACT – Ref Baseline	-0.4803	0.1667	-2.882	0.0237 *

Sampling - Magnesium

Appendix table 25: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	142.14	< 2.2e-16 ***

Appendix table 26: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-1.01278	0.07114	-14.236	< 0.001 ***
ACT – Ref ACT	0.23518	0.07114	3.306	0.00678 **
Baseline – Ref Baseline	0.25513	0.07054	3.617	0.00237 **
Ref ACT – Ref Baseline	-0.99283	0.07054	-14.075	< 0.001 ***

Sampling – Lactic Acid

Appendix table 27: Test results from a one-way ANOVA. See Appendix table 1 for additional info.

	DF	F-value	p-value
Sampling	3	238.81	< 2.2e-16 ***

Appendix table 28: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	estimate	Std. error	t-value	p-value
ACT – Baseline	-1.34002	0.05621	-23.841	<1e-04 ***
ACT – Ref ACT	-0.11449	0.05621	-2.037	0.181
Baseline – Ref Baseline	0.66170	0.05621	11.773	<1e-04 ***
Ref ACT – Ref Baseline	-0.56383	0.05621	-10.032	<1e-04 ***

Appendix III – Statistical Analyses from chapter 2

Appendix table 29: Summary of Statistical Analysis of Empirical Production Data in Phase 1 and Phase 2 for The Initial Weight(W_1), Final Weight(W_2), Gain (W_2 -W_1), Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC), Mortality, Feed Conversion, and Sea Lice Infestations (n = 6, PL = Preline System, Ref = reference).

Phase 1	System		Significance	Season		Significance	Significanse interaction
	Preline	Reference	PL vs. Ref	Spring	Fall	Season	System x Season
Initial weight (g)	115.3	117.4	NS	124.6	108.1	*0.05*	NS
Final weight (g)	505.9	536.9	NS	582.2	460.5	NS	NS
Gain weight (g)	390.6	419.5	NS	457.6	352.4	NS	NS
SGR	1.32	1.33	NS	1.49	1.17	NS	NS
TGC	2.859	2.835	NS	2.95	2.74	NS	NS
Mortality	3.22	1.86	NS	1.3	3.78	NS	NS
FC	1.18	1.19	NS	1.17	1.2	NS	NS
Sea lice	0.019	0.39	*0.05*	0.093	0.32	NS	NS
Phase 2	System		Significance	Season		Significance	Significanse interaction
	Preline	Reference	PL vs. Ref	Spring	Fall	Season	System x Season
Initial weight (g)	508.7	543.2	NS	461	590.7	NS	NS
Final weight (g)	4,763	3,944	*0.05*	4,256	4,451	NS	NS
Gain weight (g)	4,254	3,401	*0.05*	3,795	3,860	NS	NS
SGR	0.764	0.684	NS	0.778	0.67	NS	NS
TGC	2.938	2.562	NS	2.671	2.829	NS	NS
Mortality	10.72	16.88	NS	11.52	16.08	NS	*0.05*
FC	1.17	1.25	NS	1.125	1.308	*0.01*	NS
Sea lice	1.24	1.54	NS	1.24	1.55	NS	NS

Phase 1 – Post-smolt phase

Appendix table 30: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.0817	0.7822
Season	1	1.0806	0.3290
System: Season	1	0.6280	0.4506

Appendix table 31: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	51.26	-407.06	509.59	0.983
Cage Spring – Cage fall	185.4	-272.92	643.72	0.590
PL Spring – Cage fall	76.26	-382.06	534.59	0.948
Cage Spring – PL Fall	134.13	-324.19	592.46	0.786
PL Spring – PL fall	25.00	-433.32	483.32	0.997
PL Spring – Cage Spring	-109.13	-567.56	349.19	0.868

Initial weight (w_1)

Appendix table 32: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.0838	0.779
Season	1	5.4199	0.048 *
System: Season	1	0.0047	0.947

Appendix table 33: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-2.533333	-34.59846	29.53179	0.9938
Cage Spring – Cage fall	16.000000	-16.06512	48.06512	0.4309
PL Spring – Cage fall	14.433333	-17.63179	46.49846	0.5106
Cage Spring – PL Fall	18.533333	-13.53179	50.59846	0.3190
PL Spring – PL fall	16.966667	-15.09846	49.03179	0.3855
PL Spring – Cage Spring	-1.566667	-33.63179	30.49846	0.9985

SGR (Specific Growth rate)

Appendix table 34: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.0009	0.9767
Season	1	4.9431	0.0568
System: Season	1	0.2152	0.6550

Appendix table 35: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	0.06282596	-0.5930671	0.7187190	0.9892
Cage Spring – Cage fall	0.38918028	-0.2667127	1.0450733	0.2999
PL Spring – Cage fall	0.31763436	-0.3382587	0.9735274	0.4542
Cage Spring – PL Fall	0.32635432	-0.3295387	0.9822473	0.4331
PL Spring – PL fall	0.25480840	-0.4010846	0.9107014	0.6189
PL Spring – Cage Spring	-0.0715459	-0.7274389	0.5843471	0.9843

TGC (Thermal Growth Coefficient)

Appendix table 36: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.0062	0.9391
Season	1	0.4938	0.5022
System: Season	1	1.5560	0.2475

Appendix table 37: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-0.3457917	-1.6859585	0.994375	0.8407
Cage Spring – Cage fall	-0.1611908	-1.5013576	1.178976	0.9792
PL Spring – Cage fall	0.2312744	-1.1088923	1.571441	0.9432
Cage Spring – PL Fall	0.1846009	-1.1555658	1.524768	0.9695
PL Spring – PL fall	0.5770661	-0.7631006	1.917233	0.5443
PL Spring – Cage Spring	0.3924652	-0.9477015	1.732632	0.7863

Lice infestations

Appendix table 38: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	8.4755	0.01955 *
Season	1	3.1516	0.11377
System: Season	1	2.3649	0.16265

Appendix table 39: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-0.5814569	-1.1733312	0.01041731	0.0513
Cage Spring – Cage fall	-0.4329937	-1.0248680	0.15888054	0.1670
PL Spring – Cage fall	-0.6124912	-1.2043655	-0.0206169	0.0427 *
Cage Spring – PL Fall	0.14846323	-0.4434110	0.74033749	0.8511
PL Spring – PL fall	-0.0310342	-0.6229085	0.56083997	0.9981
PL Spring – Cage Spring	-0.1794975	-0.7713718	0.41237675	0.7690

FCR (Feed conversion ratio)

Appendix table 40: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.0718	0.7955
Season	1	0.7750	0.4043
System: Season	1	0.1927	0.6723

Appendix table 41: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	0.00684659	-0.1745369	0.1882301	0.9993
Cage Spring – Cage fall	-0.0176781	-0.1990617	0.1637054	0.9886
PL Spring – Cage fall	-0.0459938	-0.2273773	0.1353897	0.8472
Cage Spring – PL Fall	-0.0245247	-0.2059083	0.1568588	0.9710
PL Spring – PL fall	-0.0528404	-0.2342239	0.1285431	0.7888
PL Spring – Cage Spring	-0.0283156	-0.2096992	0.1530679	0.9568

Mortality

Appendix table 42: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.5102	0.4982
Season	1	0.4032	0.5457
System: Season	1	0.0004	0.9849

Appendix table 43: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	3.3000000	-6.095182	12.695182	0.6856
Cage Spring – Cage fall	-0.5400000	-9.935182	8.855182	0.9975
PL Spring – Cage fall	-1.1233333	-10.518515	8.271849	0.9795
Cage Spring – PL Fall	-3.8400000	-13.235182	5.555182	0.5828
PL Spring – PL fall	-4.4233333	-13.818515	4.971849	0.4760
PL Spring – Cage Spring	-0.5833333	-9.978515	8.811849	0.9969

Final weight (w_2)

Appendix table 44: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System	1	0.1048	0.7545
Season	1	1.6163	0.2393
System: Season	1	0.6937	0.4291

Appendix table 45: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	48.73333	-384.7293	482.1960	0.9828
Cage Spring – Cage fall	201.4000	-232.0626	634.8626	0.4863
PL Spring – Cage fall	90.70000	-342.7626	524.1626	0.9054
Cage Spring – PL Fall	152.6666	-280.7960	586.1293	0.6839
PL Spring – PL fall	41.96667	-391.4960	475.4293	0.9888
PL Spring – Cage Spring	-110.700	-544.1626	322.7626	0.8445

Statistical Results Phase 2 – Grow-out

Gain (w_2-w_1)

Appendix table 46: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	7.0667	0.0288 *
Season (Spring and fall)	1	0.0414	0.8437
System: Season	1	0.1068	0.7522

Appendix table 47: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	957.63333	-495.2221	2410.4888	0.2284
Cage Spring – Cage fall	39.53333	-1413.3221	1492.3888	0.9997
PL Spring – Cage fall	787.50000	-665.3555	2240.3555	0.3671
Cage Spring – PL Fall	-918.10000	-2370.9555	534.7555	0.2560
PL Spring – PL fall	-170.13333	-1622.9888	1282.7221	0.9807
PL Spring – Cage Spring	747.96667	-704.8888	2200.8221	0.4066

SGR (Specific growth rate)

Appendix table 48: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	1.6391	0.2363
Season (Spring and fall)	1	2.9509	0.1242
System: Season	1	0.2181	0.6530

Appendix table 49: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	0.10985929	-0.1748899	0.3946085	0.6237
Cage Spring – Cage fall	0.13736922	-0.1473799	0.4221184	0.4571
PL Spring – Cage fall	0.18850568	-0.0962435	0.4732549	0.2256
Cage Spring – PL Fall	0.02750993	-0.2572392	0.3122591	0.9889
PL Spring – PL fall	0.07864639	-0.2061028	0.3633956	0.8130
PL Spring – Cage Spring	0.05113646	-0.2336127	0.3358857	0.9368

TGC (Thermal growth coefficient)

Appendix table 50: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	2.7826	0.1338
Season (Spring and fall)	1	0.5005	0.4994
System: Season	1	0.7109	0.4236

Appendix table 51: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	0.5660641	-0.4547707	1.5868988	0.3498
Cage Spring – Cage fall	0.0305819	-0.9902529	1.0514167	0.9996
PL Spring – Cage fall	0.2165376	-0.8042972	1.2373723	0.9021
Cage Spring – PL Fall	-0.5354822	-1.5563169	0.4853526	0.3922
PL Spring – PL fall	-0.3495265	-1.3703613	0.6713083	0.7014
PL Spring – Cage Spring	0.1859557	-0.8348791	1.2067904	0.9343

Sea Lice infestations

Appendix table 52: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	1.3240	0.2831
Season (Spring and fall)	1	1.3537	0.2782
System: Season	1	0.0556	0.8195

Appendix table 53: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-0.3696496	-1.577106	0.8378062	0.7642
Cage Spring – Cage fall	-0.3730688	-1.580525	0.8343871	0.7594
PL Spring – Cage fall	-0.6169814	-1.824437	0.5904745	0.4125
Cage Spring – PL Fall	-0.0034191	-1.210875	1.2040367	0.9999
PL Spring – PL fall	-0.2473317	-1.454788	0.9601242	0.9105
PL Spring – Cage Spring	-0.2439125	-1.451368	0.9635433	0.9137

Feed conversion ratio (FCR)

Appendix table 54: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	2.5792	0.1469
Season (Spring and fall)	1	14.4636	0.0052 **
System: Season	1	4.4741	0.0673

Appendix table 55: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-0.1793056	-0.3975257	0.038914	0.1118
Cage Spring – Cage fall	-0.2851734	-0.5033935	-0.066953	0.0130 *
PL Spring – Cage fall	-0.2606368	-0.4788569	-0.042416	0.0210 *
Cage Spring – PL Fall	-0.1058678	-0.3240879	0.1123523	0.4528
PL Spring – PL fall	-0.0813312	-0.2995513	0.1368889	0.6471
PL Spring – Cage Spring	0.0245366	-0.1936835	0.2427567	0.9828

Mortality

Appendix table 56: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL and Sea cage)	1	2.3425	0.1642
Season (Spring and fall)	1	1.2886	0.2891
System: Season	1	8.7834	0.0180 *

Appendix table 57: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	-18.073333	-36.28581	0.1391442	0.0517
Cage Spring – Cage fall	-16.483333	-34.69581	1.7291442	0.0767
PL Spring – Cage fall	-16.483333	-28.93248	7.4924775	0.3057
Cage Spring – PL Fall	1.590000	-16.62248	19.8024775	0.9917
PL Spring – PL fall	7.353333	-10.85914	25.5658108	0.5916
PL Spring – Cage Spring	5.763333	-12.44914	23.9758108	0.7466

Final weight (w_2)

Appendix table 58: Test results from a two-way ANOVA lm-model. See Appendix table 1 for additional info.

	DF	F-value	p-value
System (PL - Sea cage)	1	6.6626	0.02530 *
Season (Spring - fall)	1	0.3774	0.55608
System: Season	1	0.0053	0.94382

Appendix table 59: Test results from a Tukey HSD post-hoc test. See Appendix table 1 for additional info.

Comparison	Diff	Lwr	Upr	p-value
PL fall – Cage fall	841.3667	-594.3984	2277.1317	0.3089
Cage Spring – Cage fall	-171.7000	-1607.4650	1264.0650	0.9795
PL Spring – Cage fall	623.5667	-812.1984	2059.3317	0.5378
Cage Spring – PL Fall	-1013.0667	-2448.8317	422.6984	0.1871
PL Spring – PL fall	-217.8000	-1653.5650	1217.9650	0.9601
PL Spring – Cage Spring	795.2667	-640.4984	2231.0317	0.3507

Stocking density in Preline and Sea cages (chapter 2)

Appendix table 60: Stocking density at the start and end of experimental period in Preline and reference group during phase 1 (100 to 284-844 g)-. Rearing volume: Preline 2000 m^3 and sea cage (spissnot) 27,000 m^3.

Phase 1				
Generation	Preline initial	Preline final	Reference initial	Reference final
		Kg/m^3		
1	9.8	42.0	0.8	4.5
2	7.8	55.6	0.7	3.9
3	8.8	37.7	0.6	5.0
4	5.1	20.7	0.7	2.1
5	14.5	59.8	0.8	2.2
6	15.9	35.8	0.7	2.4

Appendix table 61: Stocking density at the start and end of experimental period in Preline and reference group during phase 2 (285 to 3,360-5,700 g). Rearing volume: Preline 2000 m^3 and sea cage 27,000 m^3.

Phase 2				
Generation	Preline initial	Preline final	Reference initial	Reference final
		Kg/m^3		
1	3.12	21.8	4.6	24.3
2	4	23.4	3.9	22.1
3	2.8	23.8	5.1	18.8
4	1.5	13.6	2.1	22.4
5	4.4	43.9	2.2	13.5
6	1.5	20.6	1.9	19.8

Appendix IV– Economic Analysis: Implementation of semi-closed containment system (S-CCS) in a conventional production regime for Atlantic salmon in Norway.

Background

To reduce the challenges highlighted in the introduction, the industry demands new technology. Implementation of Preline closed containment system (S-CCS) is suggested as a new strategy in abating the challenges and farmers are now considering these capital-intensive technology alternatives to realize increased production.

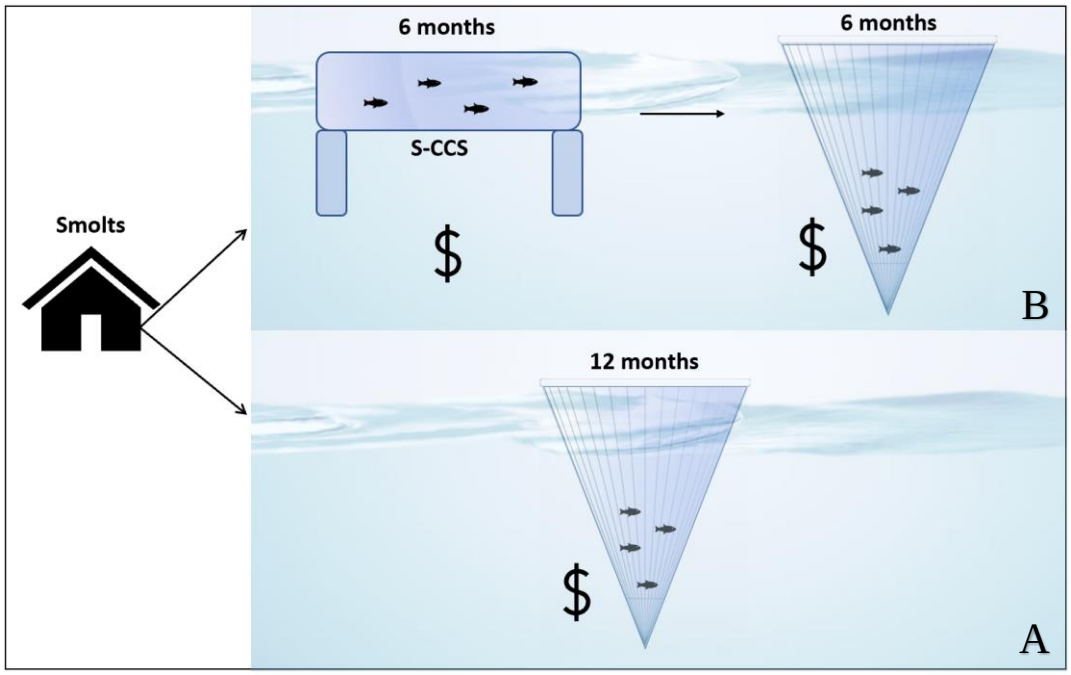
This appendix will analyse implementation of S-CCS system for post-smolt production in a conventional production regime for Atlantic salmon in Norway.

Objective:

The aim of this analysis is to benchmark the investment cost of a new technology (S-CCS) regime implemented into a conventional production regime for Atlantic salmon and compare it to a traditional sea cage strategy.

The economic model includes two strategies. First; Conventional rearing of small smolt in traditional conventional sea-cage strategy until harvest (Appendix Figure 11A).

Second; Rearing post-smolt in S-CCS prior to grow-out phase in sea cage until harvest (Appendix Figure 11B).



Appendix figure 11. Overview over the two production strategies. These are equivalent to the strategy performed in chapter 2 *(Note. The stocking period in the S-CCS was (>6 months) for the post-smolt in chapter 2*).

Investments scenarios

Scenario 1: Spring estimations

A: Sea cage strategy: 12-month production, based on spring estimations

B: S-CCS + Sea cage strategy: 6 months (post-smolt) in S-CCS and 6 months in Sea cage, based on spring estimations

Scenario 2: Fall estimations

A: Sea cage strategy: 12-month production, based on fall estimations

B: S-CCS + Sea cage strategy: 6 months (post-smolt) in S-CCS and 6 months in Sea cage, based on fall estimations

Assumptions

The empirical data is part of a full-scale industrial production cycle. Stocking density, placement of locations, number of fish, initial weight and more factors varied among the generations. In consequence, some corrections and assumptions were implemented in the economic model.

Assumptions for the economic model:

- Initial number of fish in all scenarios are 200,000 individuals due to MTB (780 tons) restrictions, and for a comparable estimation between the groups.
- Initial weight of the post-smolt is set equal (115 g) in both phases for all comparisons.
- The mortality and final weight per fish is estimated from the performances in six generations of stocking, mean values (n = 3) from spring and fall stockings (Appendix table 61).
- To determine the feed cost of fish that dies during the stocking period, the fish size is fixed at 2 kg.
- Investment in the S-CCS also included a 100% investment in a conventional sea-based construction to fulfil a full production regime for Atlantic salmon production.
- Initial investment of conventional Sea-cage: 50. million NOK (1 MTB)
- Initial investment of S-CCS system: 35.8 million NOK
- In S-CCS phase the cost of lice-treatment is 0 NOK/kg.
- Operating period for the model is 12 months. (in field, the average period for the stockings was 13.2 months for the experiment).

- Feed cost (NOK/kg), smolt price (NOK/kg), salary (per hour), consultant fee (per hour) are equal in both systems.
- In the Sea cage phase the fish is stocked at the same location the whole period from stocking to harvest size.
- The fallowing time ("*brakkleggingstid"* in Norwegian) for the S-CCS: two weeks.
- The license fee for operating with Atlantic salmon in sea ("*konsesjonskostnad"* in Norwegian) are not included in this analysis.
- Discount rate is 10% for both production systems.
- For NPV and IRR calculations depreciation period is 10 year and corporation tax are not included.
- The depreciation cost and interest cost for all the systems are fixed at 1 year in both scenarios (12 months).
- Maintenance cost, 10% of depreciation applies for 1 year in both scenarios (12 months)

Note: In scenario 1 and 2, the depreciation calculations and maintenance cost presented for the S-CCS strategy (1B and 2B) are for 6 months production in each system, but in the analysis the systems are depreciated for 12 months. This applies to the depreciation cost and maintenance cost in appendix table 63,64,68 and 69, and explains the difference in Cost, NOK in the appendix table 65 and 70.

Estimated input data in the economic model

The economic model is based on empirical biological data from the fall and spring generations studied in chapter 2, as depicted in Appendix table 61.

Appendix table 61. *Estimation of biomass for spring and fall stockings. Preline group and reference group open net-pens (final weight and mortality).*

Estimations	Season	Fish stock N	Mortality %	Initial weight	Estimated Final weight per fish (kg)	Estimated Biomass in the system (kg)
Preline	Spring	200,000	14.4	0.115	4.654	796,764
Open net	Spring	200,000	8.64	0.115	3.797	693,787
Preline	Fall	200,000	7.05	0.115	4.872	905,704
Open net	Fall	200,000	25.12	0.115	4.030	603,532

The formula of the Net Present Value (NPV) is used to calculate the present value of an investment by the discounted sum of all cash flows from the project. To determine the NPV the investment cost, cash flow, lifetime, and discount rate is required.

The Net Present Value (NPV):

$$NPV = -C_0 + \sum_{i=0}^{T} \frac{C_i}{(1+r)^i}$$

$-C_0$ = Initial Investment
C = Cash Flow
r = Discount Rate
T = Time period (10 years)

The internal rate of return (IRR) is used in capital budgeting in order to estimate the profitability of potential investment. The internal rate of return is a discount rate that makes the net present value (NPV) of all cash flow from a project equal to zero.

Internal Rate of Return (IRR) – discount rate r which makes NPV = 0;

$$IRR = \frac{(Cash\ flows)}{(1+r)^T} - Initial\ investment = 0$$

Cash flows = Cash flows in the time period

r = Discount rate

T = Time period (10 years)

Scenario 1A – Sea cage strategy 12 months (spring)

Appendix table 62. *Overview of production related cost in the 12-month sea cage strategy (Spring).*

Expense item	**Unit**	**Cost**
Time of production, months	12	
Smolt cost, NOK/per item	NOK 20.00	NOK 4 000 000.00
Smolt weight, kg	21 211	
Feed cost, NOK/kg	NOK 12.00	NOK 10 241 650.64
Lice cost, NOK/kg	NOK 5.00	NOK 3 363 875.20
Personnel cost, number of employees	4	
Salary per employee, incl. fees NOK	NOK 900 000.00	
Cost salary per production, NOK		NOK 3 600 000.00
Investment, complete facility	NOK 50 000 000.00	
Depreciation, % per year	10	
Depreciation cost		NOK 5 000 000.00
Interests cost, NOK	3	NOK 1 500 000.00
Maintenance cost, % depreciation	10	NOK 500 000.00
Hiring of equipment/personnel, hours	400	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 1 000 000.00
Energy cost per month	NOK 10 000.00	
Energy cost total		NOK 120 000.00
Total		**NOK 29 325 525.84**

Appendix table 63. *Estimated production cost NOK/kg Sea cage 12 months (spring).*

Estimated production cost	
Number of fish in	200 000
Number of fish out	182 720
Weight in, kg	0.115
Weight out, kg	3.797
Produced biomass, round kg	693 788
Processed weight, kg (gutted fish)	575 844
Cost, NOK	**NOK 29 325 525.84**
Selling price, NOK/kg	NOK 60.00
Sales value total, NOK	**NOK 34 550 634.43**
Prod cost, NOK/kg	**NOK 42.27**
Profit, NOK	**NOK 5 225 108.60**

Scenario 1B – Preline S-CCS strategy and open sea cage (spring)

Appendix table 63. *Overview of production related cost 6-month in Preline S-CCS (Spring).*

Expense item	**Unit**	***6 months***
Time of production, months	6	
Smolt cost, NOK/per item	NOK 20.00	NOK 4 000 000.00
Smolt weight (spring and fall), kg	22 885	
Fees cost, NOK/kg	NOK 12.00	NOK 1 040 986.56
Lice cost, NOK/kg	NOK 0.00	NOK 0.00
Personnel cost, number of employees	2	
Salary per employee, incl fees NOK	NOK 900 000.00	
Cost salary per production, NOK		NOK 900 000.00
Investment, S-CCS	NOK 35 800 000.00	
Depreciation, % per year-month	10	
Depreciation cost		NOK 1 790 000.00
Interest cost, NOK	3	NOK 1 074 000.00
Maintenance cost, % depreciation	10	NOK 179 000.00
Hiring of equipment/personnel, hours	180	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 450 000.00
Energy cost per month	NOK 30 000.00	
Energy cost total		NOK 180 000.00
Total		**NOK 9 613 986.56**

Appendix table 64. *Overview of production related cost 6-month in Sea cage (Spring).*

Expense item	**Unit**	***6 months***
Time of production, months	6	
Feed cost, NOK/kg	NOK 12.00	NOK 10 434 413.75
Lice cost, NOK/kg	NOK 5.00	NOK 3 569 340.71
Personnel cost, number of employees	4	
Salary per employee, incl. fees NOK	NOK 900 000.00	
Cost salary per production, NOK		NOK 1 800 000.00
Investment, complete facility	NOK 50 000 000.00	
Depreciation, % per year-month	10	
Depreciation cost		NOK 2 500 000.00
Interest cost, NOK	3	NOK 1 500 000.00
Maintenance cost, % depreciation	10	NOK 250 000.00
Hiring of equipment/personnel, hours	200	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 500 000.00
Energy cost per month	NOK 10 000.00	
Energy cost total		NOK 60 000.00
Total		**NOK 20 613 754.46**

Appendix table 65. *Summary of scenario 1 output – profit in both strategies marked in green.*

Total	**Sea cage per 12 months**	**S-CCS – Sea cage per 12 months**
Number of fish produced per year	182 720	171 191
Produced biomass, round kg	693 788	796 725
Processed weight, kg (gutted fish)	575 844	661 282
Cost, NOK	**NOK 29 325 525.84**	**NOK 37 520 982.30***
*Prod cost, NOK/kg	NOK 42.27	NOK 47.09
Selling price, gutted, NOK/kg	60	60
Sales value total, NOK	**NOK 34 550 634.43**	**39 676 894.04**
Profit, NOK	**5 225 108.60**	**2 155 911.74**

**Includes total depreciation and maintenance cost for 12 months.*

Scenario 2A – Sea cage strategy (fall)

Appendix table 66*: Overview of production related cost in the 12-month Sea Cage strategy (Fall).*

Expense item	**Unit**	**Cost**
Time of production, months	12	
Smolt cost, NOK/per unit	NOK 20.00	NOK 4 000 000.00
Smolt weight, kg	17 222	
Feed cost, NOK/kg	NOK 12.00	NOK 9 158 821.06
Lice cost, NOK/kg	NOK 5.00	NOK 2 931 552.00
Personnel cost, number of employees	4	
Salary per employee, incl. fees	NOK 900 000.00	
Cost salary per production, NOK		NOK 3 600 000.00
Investment, complete facility (Sea cage)	NOK 50 000 000.00	
Depreciation, % per year	10	
Depreciation cost		NOK 5 000 000.00
Interest cost, NOK	3	NOK 1 500 000.00
Maintenance cost, % depreciation	10	NOK 500 000.00
Hiring of equipment/personnel, hours	400	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 1 000 000.00
Energy cost per month	NOK 10 000.00	
Energy cost total		NOK 120 000.00
Total		**NOK 27 810 373.06**

Appendix table 67: *Estimated production cost NOK/kg in sea cage.*

Estimated production cost	
Number of fish in	200 000
Number of fish out	149 760
Weight in, kg	0.115
Weight out, kg	4.03
Produced biomass, round/whole kg	603 533
Processed weight, kg (gutted fish)	500 932
Cost, NOK	**NOK 27 810 373.06**
Selling price, NOK/kg	NOK 60.00
Sales value total, NOK	**NOK 30 055 933.44**
Prod cost, NOK/kg	**NOK 46.08**
Profit, NOK	**NOK 2 245 560.38**

Scenario 2B - Preline S-CCS and open sea cage strategy (fall)

Appendix table 68: *Overview of production related cost 6-month in Preline S-CCS.*

Expense item	**Unit**	***6 months***
Time of production, months	6	
Smolt cost, NOK/per item	NOK 20.00	NOK 4 000 000.00
Smolt weight (spring and fall), kg	22 770	
Feed cost, NOK/kg	NOK 12.00	NOK 1 171 541.76
Lice cost, NOK/kg	NOK 0.00	NOK 0.00
Personnel cost, number of employees	2	
Salary per employee, incl. fees NOK	NOK 900 000.00	
Cost salary per production NOK		NOK 900 000.00
Investment, S-CCS	NOK 35 800 000.00	
Depreciation, % per year-month	10	
Depreciation cost		NOK 1 790 000.00
Interest cost, NOK	3	NOK 1 074 000.00
Maintenance cost, % depreciation	10	NOK 179 000.00
Hiring of equipment/personnel, hours	180	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 450 000.00
Energy cost per month	NOK 30 000.00	
Energy cost total		NOK 180 000.00
Total		**NOK 9 744 541.76**

Appendix table 69: *Overview of production related cost 6-month in Sea cage (Fall).*

Expense item	**Unit**	***6 months***
Time of production, months	6	
Smolt cost, NOK/per item		NOK 0.00
Smolt weight (spring and fall), kg		
Feed cost, NOK/kg	NOK 12.00	NOK 11 737 909.69
Lice cost, NOK/kg	NOK 5.00	NOK 4 014 985.59
Personnel cost, number of employees	4	
Salary per employee, incl. fees NOK	NOK 900 000.00	
Cost salary per production, NOK		NOK 1 800 000.00
Investment, complete facility	NOK 50 000 000.00	
Depreciation, % per year-month	10	
Depreciation cost		NOK 2 500 000.00
Interests cost, NOK	3	NOK 1 500 000.00
Maintenance cost, % depreciation	10	NOK 250 000.00
Hiring of equipment/personnel, hours	200	
Hourly cost, hiring	NOK 2 500.00	
Hiring cost		NOK 500 000.00
Energy cost per month	NOK 10 000.00	
Energy cost total		NOK 60 000.00
Total		**NOK 22 362 895.28**

Appendix table 70: *Summary of scenario2 output – profit in both strategies marked in green.*

Total	**Sea cage per 12 months**	**S-CCS – Sea cage per 12 months**
Number of fish produced per year	149 760	185 922
Produced biomass, round kg	603 533	905 812
Processed weight, kg (gutted fish)	500 932	751 824
Cost, NOK	**NOK 27 810 373.06**	**NOK 39 400 437.04***
Prod cost, NOK/kg	NOK 46.08	NOK 43.50
Selling price, gutted, NOK/kg	60	60
Sales value total, NOK	**NOK 30 055 933.44**	**NOK 45 109 436.80**
Profit, NOK	**NOK 2 245 560.38**	**NOK 5 708 999.77**

**Includes total depreciation and maintenance cost for 12 months.*

Summary of the two scenarios

Appendix table 71. *Production cost/kg, profit, NPV and IRR.*

Scenarios	Production cost/kg	Profit	NPV	IRR
1 A: Sea cage (Spring)	42.27 kr	5.225,108	22.189,422	19.56 %
1 B: S-CCS + Sea cage (Spring)	47.09 kr	2.155,911	12.253,797	13.230 %
2 A: Sea cage (Fall)	46.08 kr	2.245,560	3.737,680	11.713 %
2 B: S-CCS + Sea cage (Fall)	43.50 kr	5.708,999	30.930,402	17.634 %

Discussion

According to the positive NPV values, all the scenarios in this case are profitable. The NPV measurement treats the projects equally, and The NPV results are independent of the investor`s risk preferences. The method gives an acceptable level of precision and is a widely used tool to predict the profit in investment projects. An assumption in the method is that there is an overall goal and vision to maximize the financial values of the interest groups (owners, investors, stakeholders, partners).

The internal rate of return method (IRR) is another tool to analyse if a project is profitable. The IRR is rate of return that gives NPV = 0. The decision rule for the method is to accept those investment projects that have a capital cost less than the projects' internal rate of return. In other words, if the cost of capital is less than the IRR, the net present value will be positive. The IRR gives a good indication of the profitability of projects in the same way as the NPV, subject to its proper use.

Scenario 1 – Spring estimations

For the spring estimations, both strategies prove to be profitable. Here, the 12-month sea cage strategy is preferred by taking the NPV, IRR, Production cost/kg and profit into account. This is probably related to the 50% lower estimated mortality in the sea-cage strategy giving a 11% lower production cost/kg for sea-cage strategy in comparison to the S-CCS

strategy, despite the higher total gain (~12 % higher) achieved in the S-CCS strategy. Besides, in this scenario the S-CCS system is not used while the fish is transferred to open sea cages, giving a low NPV with this strategy and the optimal utilization is not achieved. Consequently, for the spring estimations, the Sea cage strategy is favoured, giving a profit over twice as high compared to the S-CCS strategy.

Scenario 2 – Fall estimations

Both strategies suggest a profitable investment. In comparison between the systems, the NPV, IRR, Production cost/kg and profit values favour the S-CCS strategy based on the fall estimations. Considering the lower mortality and higher estimated biomass gain in the S-CCS strategy (~ 40% higher gain than sea cage strategy), this result was expected. The NPV values is almost ten times larger than the sea cage strategy and the estimated production cost per kg is 6 % lower in the S-CCS strategy, giving a profit twice as high for the S-CCS strategy.

In summary, all the scenarios in this analysis are profitable. In scenario 1 and 2, the S-CCS system is not fully utilized as it only operates once per year for an equal comparison with the sea cage strategy. A strategy where the capital investment is only utilized for 6 months, is not a realistic scenario in an industry context, and from a farmer's point of view a strategy maximizing the MTB ("*Maksimalt tillatt biomasse*" in Norwegian) is strived for.

By implementing S-CCS in an optimal production line, it allows for an additional stocking every 6 months (within 1 production year), giving a total of two stockings per year in the S-CCS strategy to maximize the MTB. The optimization of this strategy was not evaluated in this analysis but are of interest for future research. Considering the findings summarized in chapter 1 and 2, implementation of S-CCS systems in conventional Atlantic salmon show a positive economic impact by achieving more biomass.

Moreover, this analysis was based on estimated biological performance and various assumptions. Further analyses should investigate comparisons with land-based CCS, additional

cost regarding sea lice pressure, reduced mortality, feed cost, electricity cost, environmental impact, and other relevant production factors. The license fee for operating with salmon was not included in this analysis and is of great importance in future decisions for assessing new S-CCS technology in Norwegian salmon aquaculture.