Master thesis

University of Bergen, Norway

Field study on Atlantic salmon (*Salmo salar*) acoustic delicing: Fish welfare and salmon lice (*Lepeophtheirus salmonis*) dynamics

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

By

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Abstract

High density of salmon lice is one of the biggest challenges in Norwegian salmon farming. For the aquaculture industry it is important to develop methods that include high efficiency in the removal of salmon lice, low costs and minimal negative effects on fish and the environment, while avoiding wastage and labour-intensive operations. AcuLice treatment has been developed to fulfil these requirements and uses a composite acoustic sound image with low-frequency sound to remove salmon lice from Atlantic salmon. This master thesis examines and documents the stress effects on Atlantic salmon as well as the effect on salmon lice dynamics in large-scale of the AcuLice system. The stress effects of salmon were characterized by measuring the concentration of cortisol (primary stress response) and glucose, lactic acid, chloride, calcium and magnesium (secondary stress response) in the blood, as well as calculating specific growth rate (tertiary stress response). These measurements were compared with a control sample prior to AcuLice treatment (primary – and secondary response) or the reference group (tertiary response). The effect of AcuLice treatment on salmon lice dynamics was measured by weekly salmon lice counting's at the facilities from week 30, 2019 to week 20, 2020. The number of salmon lice treatments in the same period, was also recorded and compared between the AcuLice - and reference group. In addition, numbers of weeks until the first salmon lice treatment (mechanical treatment) was compared between the two groups. The findings from the stress analyses showed no significant difference in the primary stress response measurements between the AcuLice and control group. In addition, apart from slight increase in plasma glucose, no significant difference was observed in in the secondary or tertiary stress response measured. Furthermore, a significant higher number of small salmon lice was found in the AcuLice facilities compared to the reference. For the mature female salmon lice, a significant lower number was shown for the AcuLice group. In addition, a lower number of salmon lice treatments and a longer production period before the first salmon lice treatment occurred was observed at the AcuLice facilities. The experiments suggest that AcuLice treatment does not have a negative effect on Atlantic salmon when it comes to acute stress and that the treatment has a positive effect on reduction of salmon lice pressure at the production site.

1 Introduction

1.1 Aquaculture in Norway: Challenges and potential

The ever-increasing world population brings with it an increasing demand for food resources (Tsatsakis et al., 2017). A key problem is sufficient access to animal protein sources and there is an urgent need to find new sources of protein. The earth's surface consists of 70% ocean and only 17% of the current production of edible meat is from the sea (Costello et al., 2020). Aquaculture will thus have the potential to contribute with the supply of good protein sources to an increasing population.

In Norway, the Atlantic salmon (*Salmo salar)* farming industry started in the early 1970s (Taranger et al., 2015). Salmon farming began when the Grøntvedt brothers put 200 000 smolts into the sea in self-developed octagonal floating cages (Berge, 2014). This was both the start of the Atlantic salmon production and the commercial farming equipment. Present farming of Atlantic salmon is based on production in freshwater (FW) on land, combined with further growth in the ocean in seawater (SW). Although, there has been a major change in the technical standard for salmon farming, the open cages are still the most widely used and are both cost-effective and an efficient utilization of coastal sea areas. The open sea cages have a size of up to 160 m in circumference and can contain up to 200 000 individuals per cage (Directorate of Fisheries, 2010).

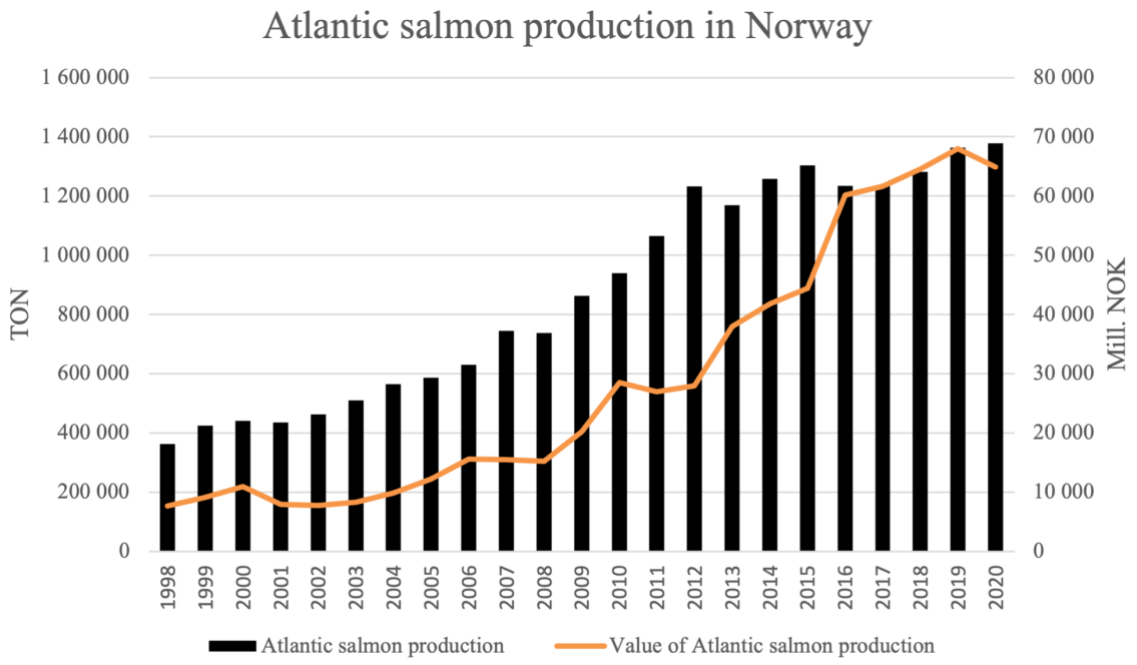

Figure 1. Atlantic salmon (Salmo salar) aquaculture production and first-hand value in Norway in the period from 1998 to 2020. Source: Directorate of Fisheries, 2021.

The production of Atlantic salmon has increased rapidly from 531 tons in 1971 to 1.38 million tons in 2020 (Directorate of Fisheries, 2021) (Figure 1), making Norway the largest producer of Atlantic salmon worldwide (Ytrestøyl et al., 2015). The production volume reached 1.2 million tons in 2012 and has stagnated around this volume since. The intensive cultivation of Atlantic salmon accounts for approximately 95% of the total volume of seafood produced in Norway (Statistics Norway, 2020). There is also a further political desire for the industry to have a fivefold increase in aquaculture production by 2050, which will mean a production of 5 million tons (Olafsen et al., 2012). The aquaculture industry has not had an increase in production since 2012 and this can be a consequence of the challenges the industry has faced. Problems due to escaped farmed salmon and pathogens are some of the biggest challenges the industry is facing.

Escaped farmed salmon can pose a threat to the wild salmon stock due to the potential danger associated with crossing farmed with wild salmon (McGinnity et al., 1997). In some years, cases of escaped farmed salmon have been reported where the number has been higher than the number of adult wild salmon returning to the rivers to spawn (Directorate of Fisheries, 2021b; Forseth et al., 2017). Most of the escaped farmed salmon disappear into the sea, but some survive after escaping and several thousand migrate up the rivers (Skilbrei, 2013; Diserud et al., 2019). This can lead to a genetic influence on the wild salmon which in turn will be able to change the characteristics of the wild salmon populations, weaken the populations' adaptability and reduce the number of wild salmon produced.

Pathogens are another serious problem for the aquaculture industry in Norway. In connection with viral diseases, there are between 400 and 500 disease outbreaks along the Norwegian coast annually (Grefsrud et al., 2021). This entails financial losses for the industry as well as reduced welfare for the fish. In addition, outbreaks in farmed fish can affect the environment by spreading disease to wild fish (Murray, 2013). A pathogenic challenge for the industry is related to amoebic gill disease (AGD) which is caused by the amoeba *Paramoeba peruranshar* (Rodger, 2014). The amoeba migrates and parasitizes the gills of salmon, which has caused high mortality in the southern parts of Norway. It can be treated with FW or hydrogen peroxide bath treatment. The biggest pathogenic threat to the aquaculture industry is nevertheless considered to be related to salmon lice (*Lepeophtheirus salmonis*) (Mackinnon, 1998; Mustafa et al., 2000). Salmon lice feed on blood, skin and mucus from the salmonid, where the extent damage of the host varies from mild skin damage to more serious wounds on the individual

salmon which can cause death (Bowers et al., 2000; Dawson et al., 1999). Furthermore the infestation can lead to several negative factors as decreased growth rate, appetite and pre-convection efficiency (Dawson et al., 1999; Pike et al., 1999). Salmon lice will also have a negative impact on wild salmonids (Torrissen et al., 2013). In addition, the parasite constitutes a huge economic cost for the aquaculture industry due to treatment and preventive efforts, and did cost the industry approximately NOK 5 billion in 2016 (Iversen et al., 2017), which corresponds to about 9% of the income of the individual production facility (Abolofia et al., 2017). The increasing salmon lice pressure has also led to the parasite becoming a decisive factor when it comes to establishment of new aquaculture concessions and have led to a reputation challenge for the aquaculture industry.

1.2 Salmon lice

Salmon lice (*Lepeophtheirus salmonis*) is a marine ectoparasitic copepod of the order *Harpacticoida*. The parasite occurs naturally in all sea areas in the northern hemisphere and has a high degree of host specificy where it attacks salmonids (including wild and farmed salmon and trout (*Salmo trutta*), as well as char (*Salvelinus alpinus*). Salmon lice have been shown to detect a number of host-related and environmental stimuli. This includes stimuli in the form of movements in water masses, salinity, temperature, light and chemicals. In particular, chemical and hydrodynamic signals have been shown to be used by salmon lice to identify and infect the right host (Heuch & Karlsen, 1997; Komisarczuk et al., 2017).

The salmon lice parasite develops through eight developmental stages that are categorized by exoskeleton replacement, where the stages can be divided into three different phases: free-living, sedentary and mobile (Hamre et al., 2013) (Figure 2). In the first phase, the stages are called *nauplius* 1, - 2 and copepodite. In this phase, the salmon lice are free-living and float with the water masses so that it can spread over relatively large areas. The free-living salmon lice can move vertically in the water column and do search for the light in the surface. The copepodite does not eat at this stage and can survive from a week to a month, depending on environmental conditions like temperature (Dalvin, 2020). If the copepodite finds a salmonid, it hooks itself on to the fish and then finds a place on the skin to settle. Afterwards, the salmon lice starts to feed on the salmon skin and exoskeleton replacement leads it into a new immobile phase. During this phase, the salmon lice goes through the following stages: *Chalimus* 1 and -

2 where it grows further while it is attached to the host fish (The Sea Lice Research Centre, 2020). As the salmon lice approaches the adult stage, it will enter the third phase, which is the mobile phase and this includes the following stages: preadult 1, - 2 and adult. The salmon lice can in these stages move around on the skin of the salmon. Further on the salmon lice will reproduce and the adult females will produce a pair of egg strings, containing 150-400 eggs per string, carry these eggs until they hatch and are released to the water masses as *nauplius* (Dalvin, 2020).

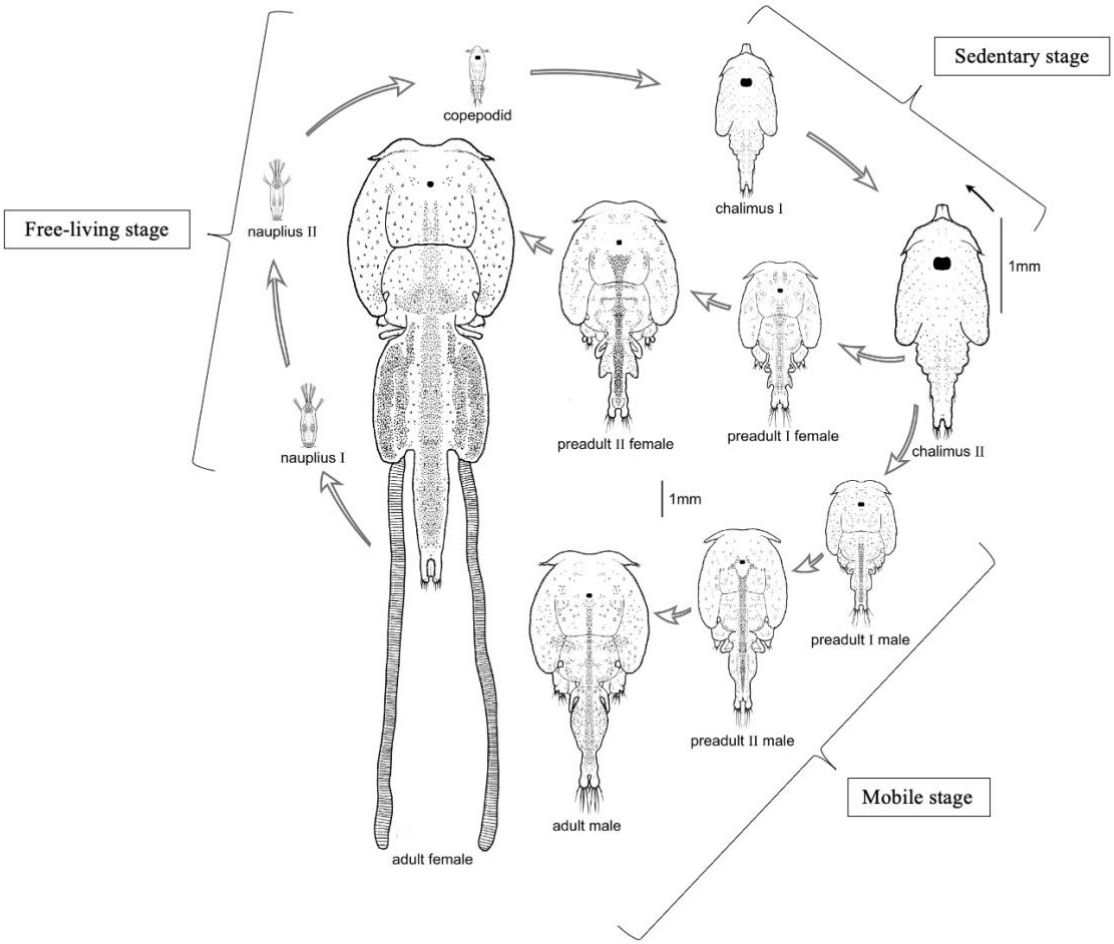

Figure 2. Salmon louse (Lepeophtheirus salmonis salmonis) life cycle. The three main phases: free-living (including: nauplius 1, 2 and copepodite), sedentary (chalimus 1 and 2) and mobile (preadult 1, 2 and adult). Illustration by: (The Sea Lice Research Centre, 2020).

Salmon lice development time and survival is strongly affected by temperature and salinity in the water masses. At 10°C, the generation time for salmon lice is estimated at 7.5-8 weeks (Johnson & Albright, 1991), where a few degree changes will have a major impact on the generation time (Johnson & Albright, 1991; Tully, 1989, 1992). The number of generations of salmon lice will vary between seasons and years as the sea temperature varies (Tully, 1989,

1992). Johannessen (1975) found that a reduction in temperature of 2.5°C from 11.5°C to 9°C resulted in the females carrying the egg strings 3 times longer. Salmon lice are also sensitive to salinity and it has been shown that the survival of free-living copepodites is compromised at salinity levels below 29‰ (Bricknell et al., 2006), while nauplii will not develop into copepodites at salinities below 25‰ (Johnson & Albright, 1991). Salmon lice fall off the host quickly if they are exposed to FW, but studies have nevertheless shown that salmon lice that were attached to a host could survive from 48 hours (McLean et al., 1990) to 3 weeks (Finstad et al., 1995) in FW.

The salmon lice feed on the skin, blood and mucus of the host salmonid. For the smolts salmon lice infestation can lead to death and is especially a welfare problem and case for wild post-smolts. The infestation cause sores and damage which can lead to a weakening of the salmonids' immunological capacity. The fish can then become more susceptible to other infections such as viruses, bacteria and fungi (Dalvin et al., 2018). In addition, infestation of salmon lice could cause problems related to the fish's salt balance and increased levels of the stress (Gallardo et al., 2019; Torrissen et al., 2013).

1.3 Stress response

High density of salmon lice, including salmon lice treatments, diseases and noise are some factors that can cause increased levels of stress in teleost (Handeland et al., 1996; Smith et al., 2004; Torrissen et al., 2013; Gallardo et al., 2019). Stress can be defined as a condition in which the physiological balance (homeostasis) is disturbed or threatened as a result of an external or internal stimulus, defined as stressors (Selye, 1950; Wedemeyer, 1996; Wendelaar Bonga, 1997). A stressor for fish can be divided into two main types: acute - and chronic stress (Brattelid et al., 2009). Acute stress occurs due to a sudden or surprising event that needs a quick response (Tort, 2011). Responses that take effect as a result of a single case of stress can be considered adaptable to deal with a given challenge and will be temporary. Nevertheless, strong or lasting stressors will lead to maladaptive responses where the fish's acute stress responses will not be sufficient to maintain its vital equilibria (Schreck & Tort, 2016). Chronic stress occurs if the stress response is activated repeatedly or is persistent. Due to the magnitude and duration of exposure to a stressor, the stress response can be divided into primary, secondary and tertiary stress responses (Figure 3).

The primary stress response activates the hypothalamic-pituitary-interrenal (HPI) axis. Exposure of a stressor initiates a rapid neural stimulated excretion of the stress hormones catecholamines (adrenaline and noradrenaline) and corticosteroids (cortisol) into the circulatory system (Wendelaar Bonga, 2011) (Figure 3). In teleost adrenaline and noradrenaline are released from the chromaffin tissue located in the head kidney and from the adrenergic nerves' endings (Randall & Ferry, 1992). Cortisol is secreted from the interrenal tissue located in the head kidney in response to pituitary hormones, such as adrenocorticotropic hormone (ACTH) (Iwama, 2006; Wendelaar Bonga, 2011).

The secondary stress response has an activating effect on the fish (Figure 3). After the stressor has mobilized an increase in production of stress hormones (catecholamines and cortisol) from the head kidney, the fish metabolism is affected. The increase in stress hormones is coupled with the release of glucose for energy production (Wendelaar Bonga, 1997). This will in SW results in higher heart rate, perfusion of the gills, and increment in metabolism, lactate levels and haematocrit. In addition, the increase of cortisol will cause a leakage of chlorine trough the tight junctions in the epithelium (McDonald & Milligan, 1997).

The tertiary stress response is triggered as a result of long-time or repeatedly changes in the physiology (Brattelid et al., 2009). In this chronic stress state, the fish will not be able to maintain homeostasis, and it can lead to major changes in the health performance, with altered behavior in addition to reduced growth rate, disease resistance and survival (Schreck, 2010; Wendelaar Bonga, 1997) (Figure 3). In Aquaculture chronic stressors could be poor water quality, transport, disease, salmon lice infestation, malnutrition, vaccination and noise (Chrousos, 1998; Smith et al., 2004; Iversen & Eliassen, 2014; Madaro et al., 2015).

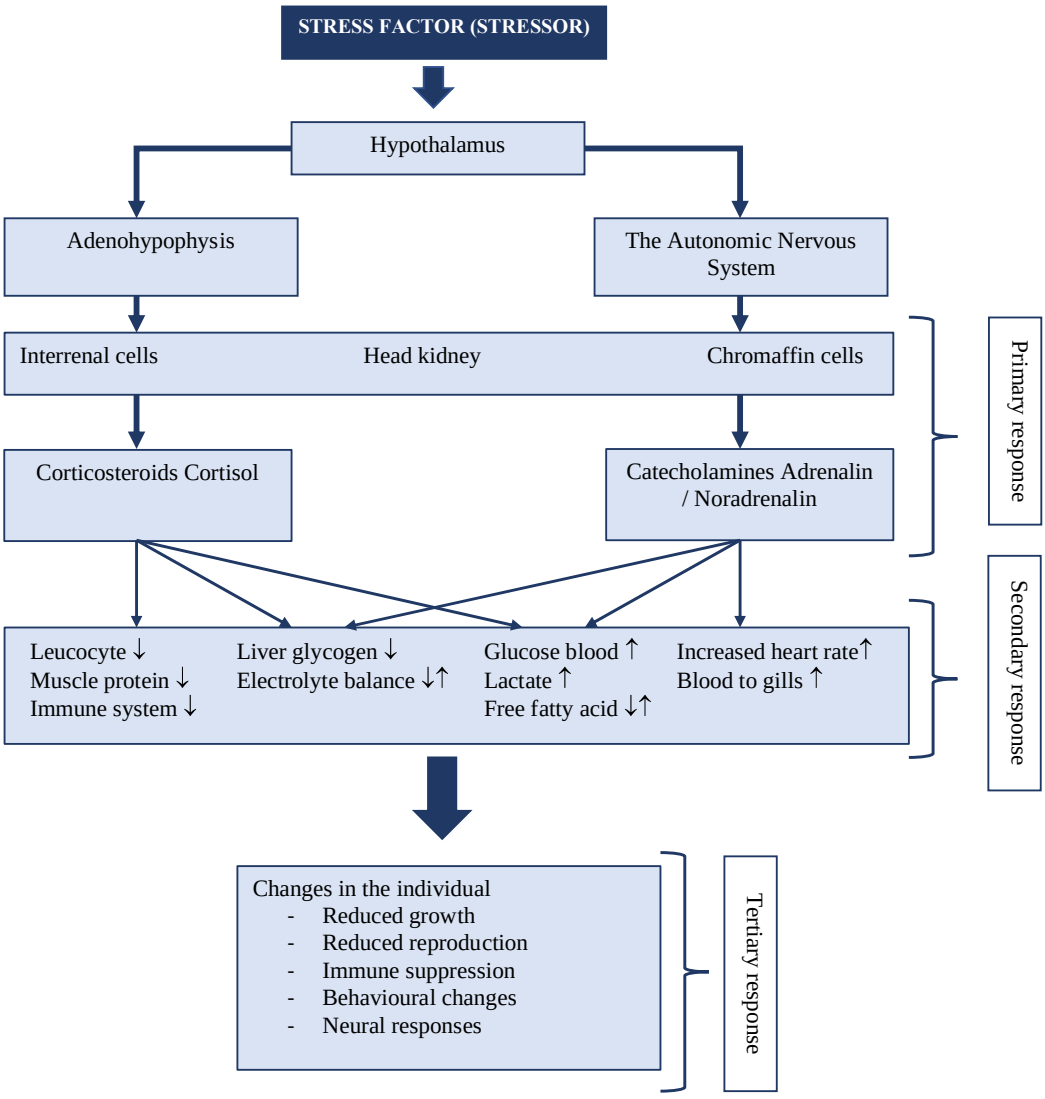

Figure 3. Schematic representation of stress response in fish, including endocrine metabolic and osmotic response. An adaptive response will try to maintain homeostatic and increase individual survival (Figure based on: Tort, 2011; Wendelaar Bonga, 2011).

To determine if a fish is exposed to a stressor the changes that occur during a possible impact can be measured. The primary response can be measured by analyzing the concentration of cortisol in blood. Since secretion of catecholamines occurs faster than for cortisol (Saligaut et al., 1999), and the biological half-life of adrenaline and noradrenaline being as short as 10 minutes in fish blood (Wendelaar Bonga, 2011), the catecholamines are not an useful indicator of primary stress response. To determine the secondary stress response, measurements of plasma concentration of calcium, chloride, glucose, lactic acid and magnesium are performed.

The stress-induced homeostasis caused by primary and secondary stress response will usually fall back to almost normal values in a chronic state of stress (Brattelid et al., 2009; Schreck, 2010; Sterling, 2012). This makes measurements of these parameters challenging in the context of detecting a tertiary stress response. However, the tertiary stress response can be analyzed by measuring, for example, survival and the specific growth rate.

Stressors in the aquaculture industry is related to conditions that negatively affect the immune system, growth and reproduction for the fish (Wendelaar Bonga, 1997). This may include noise and disturbance to which the fish are exposed. High density of salmon lice can lead to an acute stressor for the salmon due to handling and delicing methods with a major impact on the fish. In addition, salmon lice themselves could be a chronic stressor for the fish as it will be able to weaken the fish's health and immune system.

1.4 Regulatory managements

Salmon lice have become an increasing problem for the aquaculture industry due to its impact on wild salmon stocks as well as for the welfare of farmed salmon. The growing problem is due to factors such as increased host availability, in the form of increased production of salmonids in SW, and increased sea temperatures, which reduce the generation time of salmon lice (Grimnes et al., 1996). The parasite has thus become a challenge for further sustainable growth in the aquaculture industry, which has led to the establishment of monitoring systems and restrictions on how many salmon lice the fish farm can have.

To manage the salmon lice challenge, regulatory authorities have implemented a traffic lights system to control the salmon production growth. The Norwegian coast is divided into 13 production areas (Figure 4) where each area is given a colour: green, yellow or red, in relation to the mortality risk from salmon lice infestations for wild salmon (Produksjonsområdeforskriften, 2017). Green is low -, yellow is moderate-, while red is high for mortality due to salmon lice. The traffic light will further reflect whether each area can increase, decrease or maintain the production volume. If an area has high salmon lice pressure the system will thus force a reduction in production volume (Vollset et al., 2017; Myksvoll et al., 2018). The goal of the traffic light system is to protect the wild salmon stocks and increase salmon welfare.

Figure 4. The Norwegian coast divided into 13 salmon production areas to control the production with a traffic light system. With the production areas (PO – short for production area in Norwegian): PO 1: Swedish border to Jæren, PO 2: Ryfylke, PO 3: Karmøy to Sotra, PO 4: Nordhordland to Stadt, PO 5: Stadt to Hustadvika, PO 6: Nordmøre and Sørlige Trøndelag, PO 7: Nordlige Trøndelag with Bindal , PO 8: Helgeland to Bodø, PO 9: Vestfjorden and Vesterålen, PO 10: Andøya to Senja, PO 11: Kvaløya to Loppa, PO 12: Vest-Finnmark, PO 13: Øst-Finnmark.

The aquaculture industry also has a requirement to report weekly salmon lice numbers, any salmon lice treatments and disease on the fish to an official and open database called BarentsWatch (barentswatch.no). Barentswatch is a Norwegian monitoring and information system for the northern sea and coastal areas and part of the Government High North strategy. On this page, information of all facilities along the Norwegian coast is publicly available, contributing to openness and transparency for the industry based on the salmon lice problem.

To keep the salmon lice density down in Norwegian salmon farming, there is a requirement for a maximum of 0.5 mature female salmon lice per farmed salmon at the facility (0.2 in summer). This means that the industry wants to avoid the infestation of salmon lice or have to remove salmon lice when the mature female salmon lice limit has been reached. Until now, several different delousing solutions have emerged, both in form of preventing salmon lice infestations and effective ways to remove salmon lice.

1.5 Prevention of salmon lice in aquaculture

One way to avoid multiple handling and delousing events has been to have the fish longer on land so that the time the fish is at sea (and exposed to salmon lice) is minimized. In addition, new systems have been created for fish farming in the sea in the form of closed or semi-closed systems. Here, SW is collected from a depth of 25 meters to avoid the upper water masses where the salmon lice are present (down to 3 meters). Moreover, such systems have a better overview of the environmental challenges such as escapes, disease outbreaks, organic waste and contamination of any delousing agent. The principle of avoiding the upper water masses has also been done by lowering the cage or having a lice skirt around each cage so that the salmon lice are kept out. All these methods have been shown to work, to varying degrees, to limit salmon lice (Overton et al., 2019), but they involve higher costs and greater form of maintenance compared to the traditional open cages.

In addition, solutions have been developed to remove or eliminate salmon lice when they have attached a salmonid. These solutions are in the form of mechanical (including thermal -, FW -, rising and brushing treatments), chemical, biological or laser delice methods. In the case of thermal delice, the farmed salmon are introduced into a bath with temperate SW over a short period of time (Iversen et al., 2017), while the FW treatment exposes them to FW over a period of 4-8 hours. Another mechanical delice method uses rinsing or brushing of the fish to remove the salmon lice. Chemical delice in the form of drug treatment and which is done by either bath treatment with the salmon lice agent (cypermethrin, deltamethrin, azamethiphos or hydrogen peroxide) or by adding the solvent directly to the feed (diflubenzuron, teflubenzarone or emamectin benzoate). Removal of salmon lice by laser treatment takes place continuously by the laser purposefully and automatically removing salmon lice from the fish. Finally, biological delice are when using cleaner fish (lumpfish, *Cyclopterus lumpus* (Imsland et al., 2014, 2018) or ballan wrasse, *Labrus bergylta* (Skiftesvik et al., 2013). Here, a certain proportion of cleaner fish (typically a density between 3% and 15%) is added, which is intended to eat the salmon lice off the salmon.

However, the delice methods bring with them some unfortunate side effects. The most effective methods of removing salmon lice often have a significant impact on the fish due to stress caused by handling as well as the possible treatment that often provides unfavorable conditions for the fish (hot water, FW, brushing, etc.) (Overton et al., 2019). In addition, the chemical treatments

will cause environmental problems due to the release of chemicals into the sea and pollution of the environment. Many of the chemical treatments are aimed at the physiological process in arthropods, which means that a high effect can also be expected on other arthropods present in the environment (Kragesteen et al., 2019). Biological delice, on the other hand, also have challenges, especially related to the animal welfare of the cleaner fish where high mortality is observed.

Nevertheless, salmon lice have been shown to be good at developing resistance to the treatment methods that have been used to remove salmon lice in the aquaculture industry. As early as 1990, cases were reported in which salmon lice had become resistant to chemicals in the organophosphate groups (Jones et al., 1992). Since then, cases of salmon lice resistance to other chemicals have been reported (Aaen et al., 2015). The salmon lice short generation time in connection with the high number of individuals are two reasons that form the basis for the salmon lice's good habituation to the treatment methods. The consequence of the resistance of salmon lice is that it will be more difficult to limit the density of salmon lice in fish farms and some of the effectiveness of the various methods could decrease (Torrissen et al., 2013).

Given the problems associated with the salmon lice treatment methods that are in use in the aquaculture industry in addition to be able to increase the production volume, there is a great desire to develop new delice methods that reduce the impact on the welfare of farmed salmon and are effective in removing salmon lice.

1.6 AcuLice

Based on the desire to develop methods that include high efficiency, low costs and minimal negative effects on fish, while avoiding wastage, labor-intensive operations and negative effects on the environment, a treatment called AcuLice has been developed. AcuLice is a new method to prevent the spread of salmon lice with the use of a complex acoustic sound image (Figure 5) which produces and sends out constant low frequency sound to the water masses.

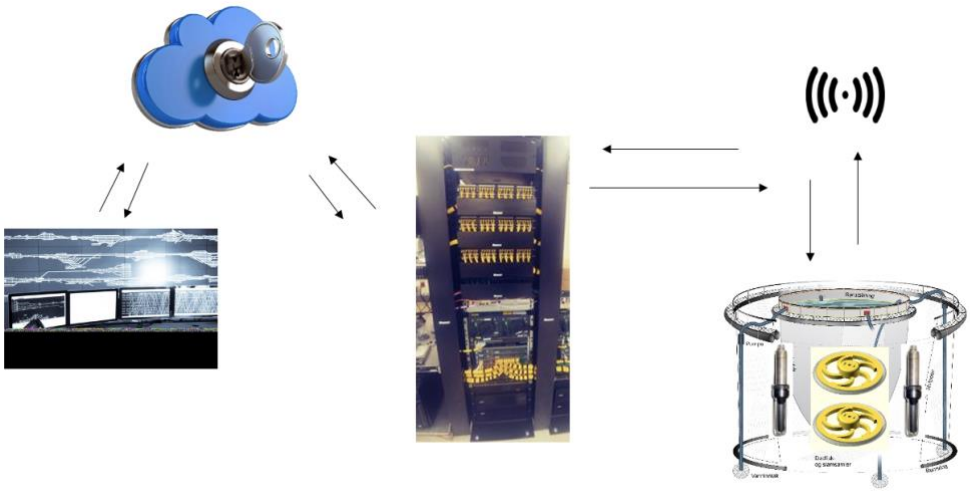

Figure 5. The AcuLice system consists of a central control room with monitoring, an electronic processor located on the facility and a component in the sea (that sends out low frequency sound waves). The whole system is connected to internet.

The system emits sound waves and sound occurs when water molecules are set in motion and pressed closer together so that the pressure increases (Kvadsheim et al., 2017). The sound levels generated by an acoustic source will propagate in the water mass and be attenuated with distance. In water, the velocity is five times higher than in air, but varies through the water column depending on the water temperature, salinity and hydrostatic pressure. Sound will also be deflected towards depths where the sound speed is lowest and thus lead to the formation of sound channels. In water, high-frequency energy will be absorbed quickly, while low-frequency energy is almost not absorbed. This means that 99% of the energy in a sound pulse of 100 kHz will be absorbed after approximately 10m, while the corresponding distance for a sound pulse of 100 Hz is 10 km.

A previous study has shown that salmon lice react with ‘aggressive behavior’ if they are exposed to low-frequency sound in the frequency range 1-5 Hz (Heuch and Karlsen 1997). The study showed that the copepodites increased the swimming activity in this frequency range and

at a frequency of 3 Hz the highest activity was observed. The frequency area of 1-5 Hz is the same that salmon produce when swimming. Hydrodynamic signals have been observed to be one of the factors salmon lice use to select the right host fish to infect. The AcuLice system is thought to disturb the salmon lice so that it enters a state of dormancy where it does not absorb food and dies or leave the host salmon to find a more suitable host. In a previous pilot study, there was observed effects regarding removal of salmon lice over time and no welfare challenges were shown in measurements of stress, fin condition and growth when exposed to AcuLice treatment. Based on the positive results in the previous pilot study, it was decided to conduct a large-scale study to follow the effects in an ordinary production situation with focus on fish welfare and health, as well as the effect on salmon lice.

Aims and objectives

This study aims to map the effect of AcuLice on the welfare, stress and health situation of salmon in intensive farming and document the effect of AcuLice on the risk of infestation for salmon lice in farmed salmon in a commercial perspective. The experiment was divided into two main parts: Experiment 1 – Acute stress effect of AcuLice treatment, Experiment 2 – Effect of AcuLice treatment in field. In Experiment 1, the acute stress effects (primary and secondary stress response) when Atlantic salmon was exposed for AcuLice treatment for one hour were studied. In Experiment 2, the effect of AcuLice treatment on tertiary stress response for Atlantic salmon and the effect on salmon lice dynamics was investigated. The thesis includes a large amount of data (n ≈ 400 000) and did start up before I was included in the project. Therefore, some of the materials was conducted by others.

The experiment was based on the following hypotheses:

Experiment 1

$H0_1$: AcuLice treatment for one hour has no significant effect on plasma cortisol concentrations in Atlantic salmon (Primary stress response).

> **$H1_1$:** AcuLice treatment for one hour has a significant effect on plasma cortisol concentrations in Atlantic salmon (Primary stress response).

$H0_2$: AcuLice treatment for one hour has no significant effect on plasma glucose concentration in Atlantic salmon (Secondary stress response).

> **$H1_2$:** AcuLice treatment for one hour has a significant effect on plasma glucose concentration in Atlantic salmon (Secondary stress response).

$H0_3$: AcuLice treatment for one hour has no significant effect on plasma lactic acid concentration in Atlantic salmon (Secondary stress response).

> **$H1_3$:** AcuLice treatment for one hour has a significant effect on plasma lactic acid concentration in Atlantic salmon (Secondary stress response).

$H0_4$: AcuLice treatment for one hour has no significant effect on plasma chloride concentration in Atlantic salmon (Secondary stress response).

> **$H1_4$:** AcuLice treatment for one hour has a significant effect on plasma chloride concentration in Atlantic salmon (Secondary stress response).

$H0_5$: AcuLice treatment for one hour has no significant effect on plasma calcium concentration in Atlantic salmon (Secondary stress response).

$H1_5$: AcuLice treatment for one hour has a significant effect on plasma calcium concentration in Atlantic salmon (Secondary stress response).

$H0_6$: AcuLice treatment for one hour has no significant effect on plasma magnesium concentration in Atlantic salmon (Secondary stress response).

$H1_6$: AcuLice treatment for one hour has a significant effect on plasma magnesium concentration in Atlantic salmon (Secondary stress response).

Experiment 2

$H0_7$: AcuLice treatment has no significant effect on specific growth rate in Atlantic salmon (Tertiary stress response).

$H1_7$: AcuLice treatment has a significant effect on specific growth rate in Atlantic salmon (Tertiary stress response).

$H0_8$: AcuLice treatment has no significant effect on small salmon lice counts on Atlantic salmon.

$H1_8$: AcuLice treatment has a significant effect on small salmon lice counts on Atlantic salmon.

$H0_9$: AcuLice treatment has no significant effect on mature female salmon lice counts on Atlantic salmon.

$H1_9$: AcuLice treatment has a significant effect on mature female salmon lice counts on Atlantic salmon.

$H0_{10}$: AcuLice treatment has no significant effect on number of salmon lice treatments during the treatment period.

$H1_{10}$: AcuLice treatment has a significant effect on number of salmon lice treatments during the treatment period.

$H0_{11}$: AcuLice treatment has no significant effect on the production time of Atlantic salmon in open sea cages before the first salmon lice treatment.

$H1_{11}$: AcuLice treatment has a significant effect on the production time of Atlantic salmon in open sea cages before the first salmon lice treatment.

2 Material & Methods

This master's thesis contains three different activities:

1) Experiment 1 – Acute stress effects of AcuLice treatment
2) Experiment 2 – Effects of AcuLice treatment in field
3) Field observation (described in Appendix I)

2.1 Experiment 1 – Acute stress effects of AcuLice treatment

2.1.1 Fish Material and Rearing Conditions

The Atlantic salmon, used in Experiment 1 – Acute stress effects of AcuLice treatment, originated from the Salmobreed strain (n = 60) and was reared from hatching to smolt at a recycling facility drifted by Hardingsmolt AS in Tørvikbygd, Kvam, as described below.

The hatchery production starts with the incubation of eggs in cold freshwater (approximately 6 – 8°C) until hatching. The alevins are then fed approximately 380 – 420-degree days (dC) post-hatching, in circular fiberglass tanks (rearing volume 5-50 m^3) at constant light and in heated water (approximately 12 – 14°C). The light is provided by fluorescent tubes mounted above the water surface. When the fish reaches a weight between 6–8 g, they are transferred from start feeding tanks to grow out RAS tanks (8 – 12 m, circular, fiberglass, volume 90 – 150 m^3). Following transfer, the fish are reared at constant light and further fed a standard dry diet (Ewos, Skretting, Norway), according to temperature and fish size (Austreng et al., 1987). All groups are vaccinated at a size of 40 – 60 g and then transferred to new (grow larger out tanks) 12 – 15 m tanks (circular, fiberglass, volume 150 – 350 m^3) where they are supplied with environment temperature freshwater and reared as described above. Oxygen content in outlet water was measured regularly and kept above 80%. During the experimental period, the fish experienced a freshwater temperature ranging from 12 to 14°C. A traditional photoperiod regime was conducted to stimulate parr smolt transformation (Handeland & Stefansson, 2001).

After the Hardingsmolt fish has completed the parr-smolt transformation, the fish that were used in Experiment 1 were farmed 7 weeks in a semi-closed system at Koløy, Fitjar (GreenBag). According to Kobbevik and Furuholmen AS the farming process at Koløy was normal and information about water temperature and other parameters is available from them (Ingebrigt Gunnar Landa, CEO). When the fish did reach approximately 500 g the group was transferred to open sea cages at Brattavika by a well boat on the 21st October 2019. The transfer was

completed according to ordinary procedures and regulations (Ivar Bergstø, Operations Manager).

2.1.2 Experimental Facility

Experiment 1 took place in Langenuen, Austevoll (Figure 6), at the full-scale facility Brattavika (60.044°N, 5.303°E, location number 11488) drifted by Kobbevik & Furuholmen Oppdrett AS. The facility consists of ten traditional open sea cages with a circumference of 160 m and volume of 37.000 m^3.

Figure 6. Location of the experimental facility Brattavika (60.044° N, 5.303° E, location number 11488) in Langenuen in Austevoll marked in green.

The facility produces salmon for commercial consumption following a standard protocol for salmon farming on sea. This does include weekly salmon lice counting, daily feeding from automatic feeders (commercial dry diets) and daily registration of SW temperature (approximately 6-9 m depth), oxygen levels (at -3 m depth) and dead fish. The daily and weekly husbandry was conducted by the facility employees.

2.1.3 General AcuLice Installation Process

The installation process for AcuLice treatment was performed in collaboration with the equipment supplier. The process is the same for all the facilities included in this thesis (Experiment 1 and 2).

Prior to the installation itself, extensive local measurements were made at the respective fish farms to be able to model and map the local fixed factors. After preliminary investigations and modeling of the site, the sound image was adapted to each individual site. The system was then installed at sea by the supplier (Figure 7). This involves connecting the speaker, usually in the centre of the site (depth of 10 – 20 m), placing the processor and connecting the component to internet. During this phase, a complete requirements specification for maintenance and operation was also prepared. The system is continuously monitored electronically. Once the AcuLice system was installed, it could be turned on by the equipment supplier whenever desired.

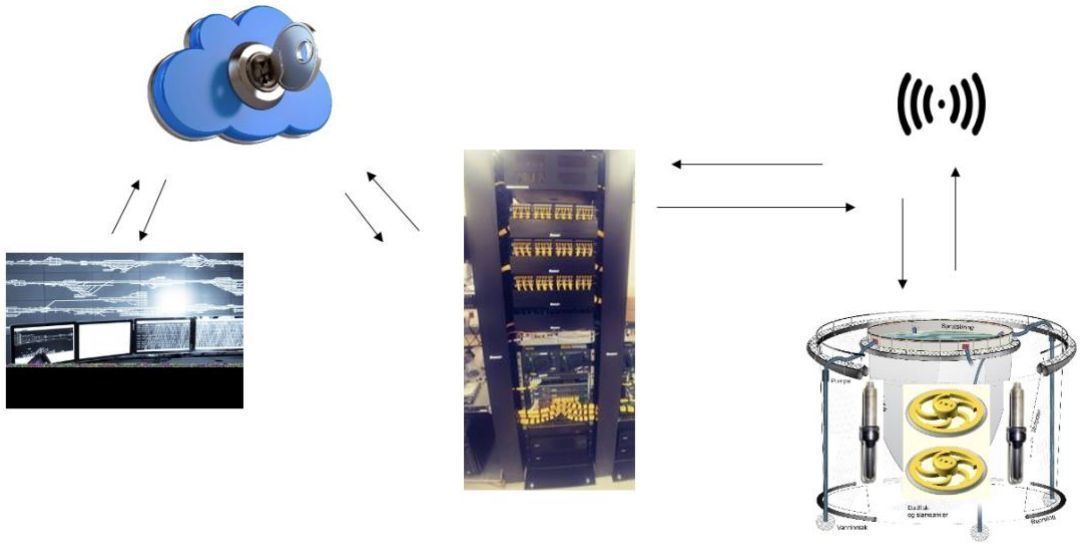

Figure 7. The AcuLice system consists of a central control room with monitoring, an electronic processor located on the facility and a component in the sea (that sends out low frequency sound waves). The whole system is connected to internet.

2.1.4 Experimental Design

Experiment 1 was carried out on the 24th of October 2019 and did include a control sampling and a treatment sampling (Experimental design is shown in Figure 8). The control sampling (Sampling 1) took place prior to start of the AcuLice treatment (not exposed to low frequency sound) and the treated group (Sampling 2) were done one hour after start of the AcuLice (exposed to low frequent sound). During the start of AcuLice treatment, the farmed salmon were monitored using an underwater camera, and no changes in the fish's behavior were observed. All measurements and plasma collections were performed at the feed barge at the facility. To get to and from the edge of the sea cage a boat was used. Daily feeding started at the same time as the AcuLice treatment.

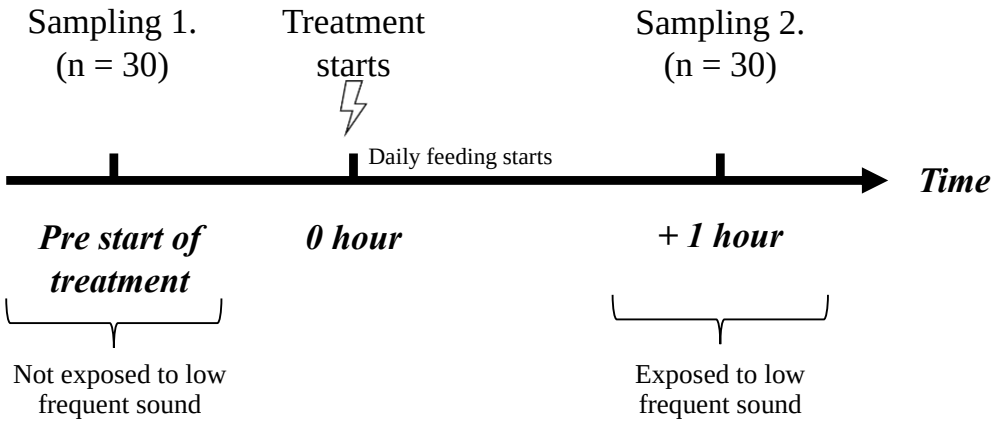

Figure 8. Experimental design for Exp1 at Brattavika. Sampling 1 took place before treatment (Control) and when the sampling was conducted the AcuLice treatment (and daily feeding) were started. One hour after treatment start, Sampling 2 took place.

2.1.5 Sampling Protocol

A total of 60 fish were part of Experiment 1. Fish were retrieved as semi-replicates from the same cage at the respective site. In each sampling there were 3 subsets (n = 10) and all fish were selected randomly from pen number 7 (Table 1).

Table 1. Overview of the samplings and subsets for the short time experiment at Brattavika on 24 October 2019

Sampling	Date	Subset	Cage number	Treatment	Fish N
1	24.10.2019	1	7	Control	10
		2	7	Control	10
		3	7	Control	10
2	24.10.2019	4	7	AcuLice	10
		5	7	AcuLice	10
		6	7	AcuLice	10

Pellets were used to attract the fish, before the fish were captured using hand net. Then the fish was humanly euthanized with an anaesthetic overdose of Benzocaine (Benzoak vet.® 20%, ACD Pharma AS, Norway). The blood was collected within 1 – 3 min to limit the effects of stress. The blood (2 mL) was taken from the caudal vein using heparinized syringes with 21G needles. The plasma was separated from blood cells by centrifugation (4 min at 5000 rpm). The fish was then measured in size (weight (g) and length (cm)) to the nearest 0.1 g and 0.1 cm. When the first subset with n = 10, was done, the same procedure was followed for the two next subsets, subset number 2 and 3. When all the three subsets in sampling 1 were done, the AcuLice was turned on, and sampling 2 was performed one hour after. See Figure 9 for a schematic setup of the sampling protocol.

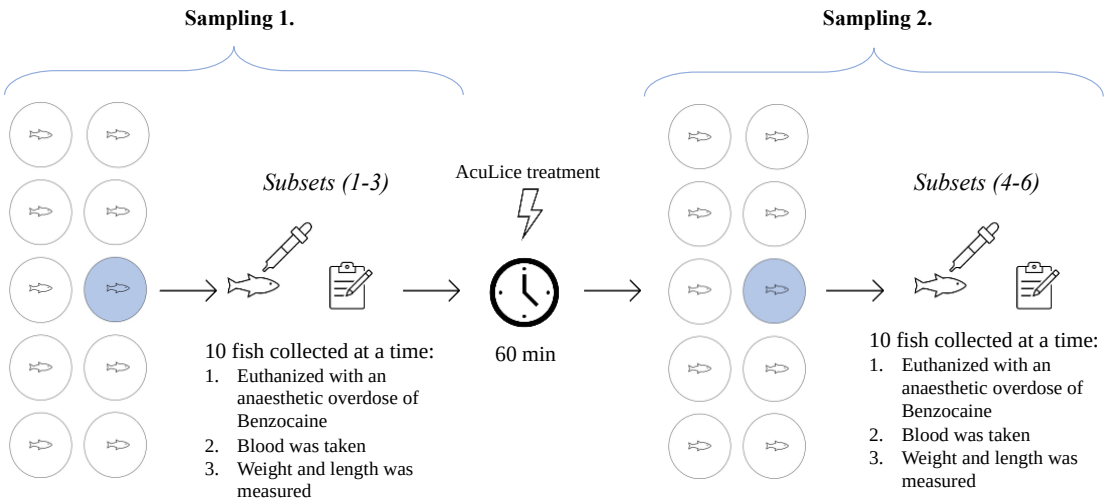

Figure 9. Schematic setup of the sampling protocol for the Experiment 1 - Stress effects with use of AcuLice at the facility Brattavika. 10 fish were taken in each subsampling. They were euthanized with an anaesthetic overdose of Benzocaine, thereafter blood was taken, and the fish's weight and length were measured.

2.1.6 Analysis

Primary stress response (plasma cortisol concentration)

Cortisol quantification from plasma (n = 60) was carried out using competitive ELISA (DEMEDITEC Cortisol ELISA Kit) following the manufacturer protocol (Demeditec Diagnostics GmbH, DEH3388, Kiel, Germany). The plasma samples were analysed in triplicates (10 µL) in a 96-well microplate. Every plate included two internal control samples and standards of a known concentration. Sample (10 µL), control or calibrator were dispensed into each well. Enzyme conjugate (200 µL) was dispensed using a multi-channel pipette. Enzyme conjugate consist of cortisol linked to horseradish peroxidase. The plate was then incubated for 60 minutes on an orbital shaker at room temperature.

After incubation the unbound conjugate is washed off. Substrate Solution, which included TBM (3,3',5,5'-Tetramethylbenzidine), was added. The plate was incubated for 30 minutes at room temperature and in dark to let the reaction happen. After the incubation, a stop solution (containing hydrochloric acid to decrease the pH) was added to each well. The intensity of colour developed is measured at 450 nm in a multimode microplate reader (Tecan Spark®, Tecan, Männedorf, Switzerland). With the help of known concentration standards, cortisol concentrations were calculated using a 4 Parameters Marquardt logistic regression with an extrapolation factor of 1 in the SparkControl Magellan v2.2.10 software.

Analysis of secondary stress responses

The concentration of chloride in the plasma samples was measured by potentiometry using the Pentra c400 clinical chemistry analyser with Ion-Selective Electrode (ISE) module (HORIBA, Kyoto Prefecture, Japan). Calibration of the ISE module was done using the ABX Pentra Standard 1, ABX Pentra Standard 2, and ABX Pentra Reference. The samples (>180 µL) were measured using a specific electrode. Chloride in the sample induce a change in the potential difference across the electrode membrane which is then compared with the reference electrode (Buck, 1981).

The measurements of glucose, lactic acid, calcium and magnesium were analysed using the Pentra c400 by colorimetric spectrophotometry determination. Each required reagent was calibrated using the ABX Pentra Multical and a quality control was performed using ABX Pentra P and N controls, as stated in the manufacturer's protocol.

Glucose was measured using a quantitative in vitro diagnostic assay using ABX Pentra Glucose HK CP reagent (HORIBA). The method used is the hexokinase method where the production of the phosphorylated glucose-6-phosphate is coupled with the subsequent production of D-gluconate-6-phosphate and reduction of NAD $^{+}$. The concentration of glucose is proportional to the increase in the NADH concentration. This is measured by spectrophotometry at 340/380 nm (Burrin & Price, 1985).

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

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

(4 AAP = 4–aminoantipyrine)

Lactic acid is analysed by a quantitative in vitro diagnostic determination using ABX Pentra lactic acid reagent (HORIBA). Lactate oxidase triggers the release of hydrogen peroxide which further reacts with 4-aminoantipyrine and ESPAS (N-ethyl-N-sulfopropyl-m-anisidine) to form a coloured complex. The reaction is dependent on peroxidase access and is measured bichromatically at 550/700 nm. The concentration of lactic acid is proportional to the intensity of the colour (Trinder, 1969).

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

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

(ESPAS = N-ethyl-N-sulfopropyl-m-anisidine)

To measure the calcium concentrations in the plasma samples, a method based on the metallochromogen Arsenazo III was used using ABX Pentra Calcium AS CP reagent (HORIBA). In the reaction, calcium ions (Ca^{2+}) will react with Arsenazo III (2.2 '- [1,8-Dihydroxy-3,6-disulfonapthyylene-2,7-bisarzo] -bisbenzenearsonic acid). The result of the reaction is a purple-colored chromophore with a pH of 6.75. The sample (5 µL) was mixed with reagent (300 µL) and water (10 µL). The absorbance of the Ca-arsenazo III complex was then measured bichromatically at 660/700 nm (Michaylova & Ilkova, 1971). The increase in absorbance of the reaction mixture is directly proportional to the calcium concentration.

$$Ca^{2+} + Arsenazo\ III \xrightarrow{pH\ 6.75} Ca - Arsenazo\ III\ complex\ (purple)$$

Magnesium was determined from a quantitative in vitro diagnostic assay using ABX Pentra Magnesium RTU Reagent (HORIBA). For each assay, sample (2.5 µL), distilled water (10 µL) and reagent (250 µL) are mixed. The magnesium ions in an alkaline solution will form a purple-coloured complex. GEDTA (glycolethylenediamine tetraacetic acid) is included in the reagent and forms complexes with calcium ions, which ultimately renders the reaction specific. The magnesium concentration is proportional to the intensity of the coloured magnesium complex measured.

2.2 Experiment 2 – Effect of AcuLice treatment in field

2.2.1 Fish Material and Rearing Conditions

As a result of different companies being involved in Experiment 2A, the fish at the various facilities came from Salmobreed stain but were farmed at different hatcheries, as described in Table 1. All the fish did follow a general procedure for hatchery-production as the previously described (Material and method for Experiment 1, section 2.1.1 Fish material and rearing conditions). The descriptive procedure is a general description and there may be minor differences between the hatcheries. Differences due to use of RAS systems, small differences in water temperature at the various stages and differences in vessel size can be present between the hatcheries.

The smolt was transferred to open sea cages, with use of a well boat, in the period of week 11 to 27 in 2019 (Table 2). This was done according to standard protocols and regulations given by the Norwegian Food Safety Authority (2017). When transfer to sea cages, the fish weighted from 140 to 450 g. At Seglberget we do not have access to this weight. The facilities have four to eight open sea cages each.

Table 2. Information about the fish involved in this experiment and its origin. Includes production site in the sea, the origin of roe, where the fish hatched and was reared from hatching to smolt stage, time and weight when transferred to sea. Weight of the fish at Seglberget we did not have access to.

	Production facility in SW (Site name)	Hatching location (from hatching to smolt stage)	Transfer to sea cages (week in 2019)	Weight when transferred to SW (g)
AcuLice	Breivik S	Sævareid Fiskeanlegg, BS Gjeravåg, BS Skålevik	15	192
	Grimsholmen	Sjøtroll	14	157
	Hattasteinen	BS Trovåg	27	281
	Hillersvik	Erko Seafood	18	295
	Loddetå	Sævareid Fiskeanlegg	14	166
	Svollandsneset	BS Trovåg	12	451
Reference	Maradalen	Alsaker Fjordbruk AS	11	317
	Seglberget	Alsaker Fjordbruk AS	14	
	Mælen	Alsaker Fjordbruk AS	11	140

2.2.2 Experimental Facilities and Locations

Experiment 2 – Salmon lice treatment took place in Sunnhordaland at 9 full scale facilities (Table 3) within the fjords: Bømlafjorden, Klosterfjorden, Ålfjorden og Skåneviksfjorden, collectively, they are further referred to as Hardangerfjorden. Assignment of facilities into reference or AcuLice treatment groups were based on current regime of the area. This has been done in order to avoid that salmon lice released from an AcuLice treated facility infest a reference facility placed downstream.

Table 3. Experiment 2 facilities divided into the two treatment groups (AcuLice or reference) with site number, company that operates the facility and coordinates of the location of the sites.

	Site name	Site number	Company that operates the facility	Coordinates (ºN/ºE)	
AcuLice	Breivik S	11574	Bremnes Seashore AS	59.671	5.312
	Grimsholmen	11559	Sjøtroll Havbruk AS	59.657	5.404
	Hattasteinen	11511	Bremnes Seashore AS	59.628	5.252
	Hillersvik	10300	Erko Seafood AS	59.608	5.312
	Loddetå	28996	Bremnes Seashore AS	59.692	5.543
	Svollandsneset	22955	Bremnes Seashore AS	59.685	5.589
Reference	Maradalen	12134	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.762	5.687
	Seglberget	17015	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.730	5.788
	Mælen	12127	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.699	5.724

An overview of the experimental area is given in Figure 10. Facilities with the AcuLice treatment installed is marked with green dots in the figure, while the reference facilities without the AcuLice instrument are marked with orange dots.

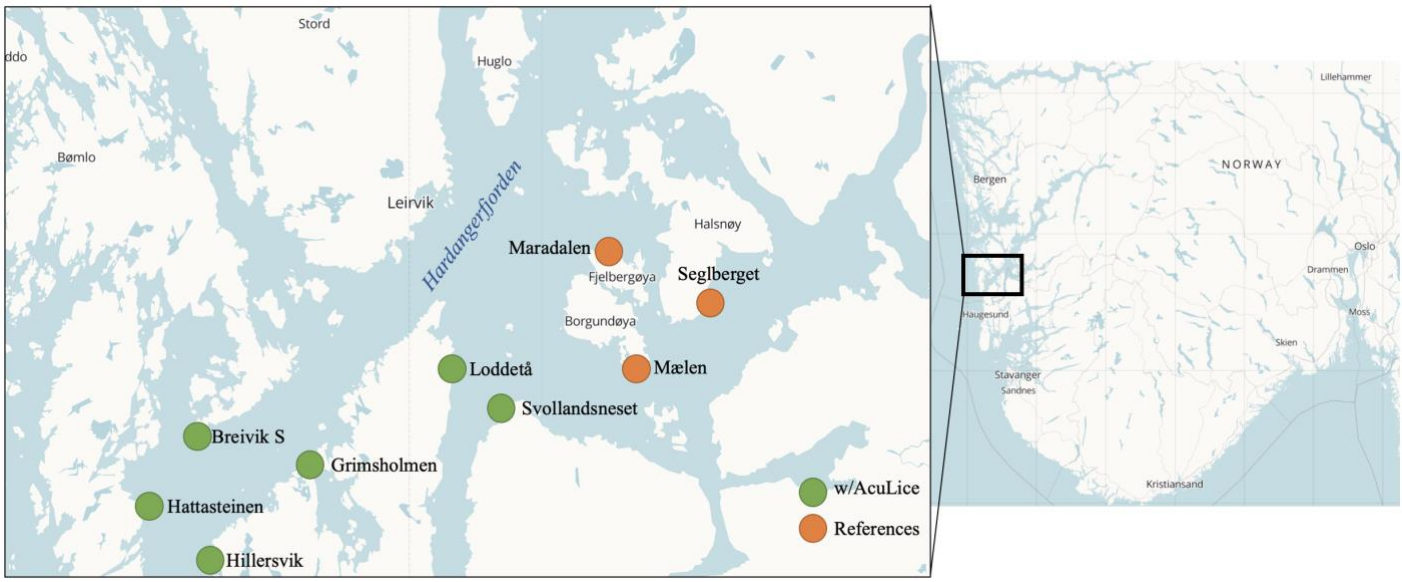

Figure 10. The facilities in Experiment 2 with green dots (AcuLice treatment) and orange dots (references without AcuLice treatment) located in Hardangerfjorden.

Daily and weekly measurements

The facilities did follow an ordinary production protocol for salmon farming for commercial consumption. This included weekly salmon lice counting, daily feeding (commercial dry diet fed from automatic feeders) and daily registration of SW temperature (at approximately 6-9 m depths, Figure 11), oxygen levels (at -3 m depth) and dead fish registration. All the facilities are fjord facilities with a salinity of 30 – 32‰. In addition, all the facilities had a density of 3-5% of cleaner fish present in the cages. The daily husbandry was conducted by the facility employees.

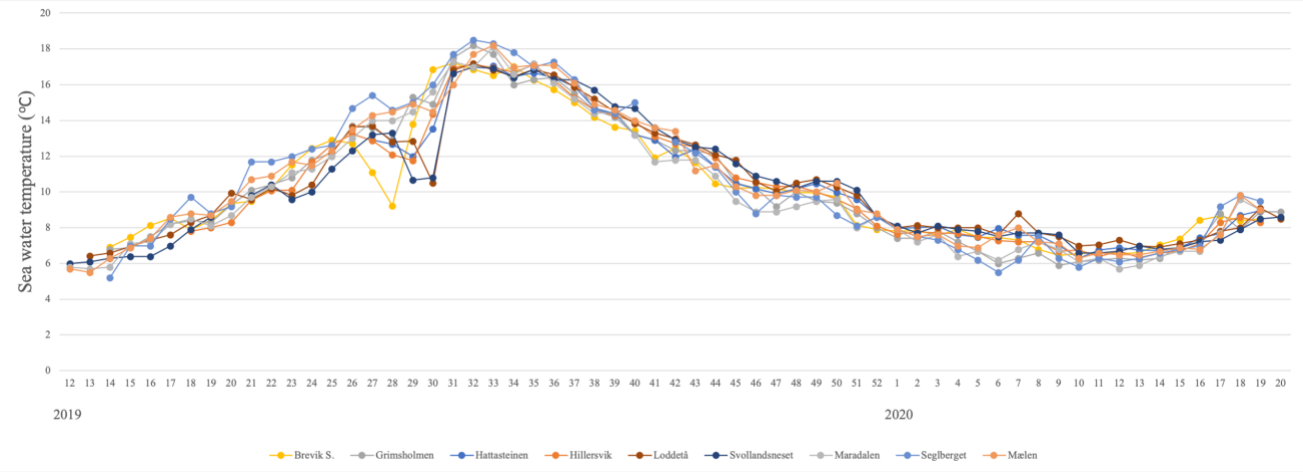

Figure 11. The SW temperature at all the facilities in Exp2A from week 12 in 2019 until week 20 in 2020, located in Hardangerfjorden measured at approximately 6-9 meters depth.

2.2.3 Experimental Design

Each experimental group was followed from onset into sea during the spring of 2019, until week 20 in 2020. The start-up at each locality varied due to different times for when production fish were transferred to sea cages and other company-internal conditions. The installation of the AcuLice equipment at the facilities in Experiment 2 follows the general description as previously described (Exp1 2.1.3 AcuLice installation). All the experimental facilities had started the AcuLice treatment no later than week 30, 2019. The experimental period for salmon lice counting was set from week 30 in 2019 to week 20 in 2020 (a period of 43 weeks). Number of salmon lice treatments was also counted for this period. In addition, numbers of weeks between fish were transferred to SW cages to first salmon lice treatment (defined as mechanical delice in the present study) was required, was measured and calculated (Described in 2.2.6 Data Analyzing). All equipment maintenance during the period was performed by the supplier. Daily follow-up was carried out by employees at the facility. Due to ordinary operation of the facilities

included in Experiment 2, the facilities had to follow the regulations on delice if mature female salmon lice exceed the limit. Throughout the production period delice treatments did occur when required for all the facilities.

In addition, a field observation was carried out at two facilities with AcuLice treatment (Breivik S and Ihlholmen) where the long-term stress effect of Atlantic salmon was investigated (tertiary stress response). The salmon welfare was registered using the Salmon Welfare Index Model (SWIM) and measurements of stress parameters (cortisol, glucose, lactic acid, etc.), was performed. More info in Appendix I.

2.2.4 Sampling Protocol

As an integral part of the experiment, production data was collected from each locality throughout the trial period (AcuLice and reference) with a focus on weight, weekly salmon lice infestation and numbers of salmon lice treatments. The facilities did follow a salmon lice counting protocol in accordance with regulations (The Veterinary Institute, 2009). Fish were randomly collected from three to six different, randomly chosen, cages (n = 20 from each pen) using hand nets. The fish was then anesthetized as directed on the given agent used.

The salmon lice counting was done by qualified salmon lice counters by carefully examining each individual fish. Salmon lice were classified into the stages: sessile salmon lice, mobile salmon lice and adult salmon lice (adult male and female salmon lice) (Figure 2). As a result of anaesthetic treatment salmon lice that fall off in the tub were counted and categorized. Subsequently, the average for each category and cage was calculated and registered in the Barentswatch database (BarentsWatch).

2.2.5 Data Processing and Calculations

Data collected during the 43-week period (week 30, 2019 – week 20, 2020) were processed. First the classifications of the salmon lice life stages that were registered during salmon lice counting were split up and changed into the new categories: small salmon lice (including copepodite, *chalimus* 1 and 2 life stages) and mature female salmon lice. Total average values for each different category were calculated for the data collected in the 43-week period at each facility in Experiment 2.

Specific growth rate on weight (SGR) was calculated for the period in the reference and AcuLice groups, with the exception of Seglberget due to missing weight measurements. The formula for SGR is:

$$\text{SGR} = \frac{lnW_2 - lnW_1}{\Delta T},$$

where W_1 is weight at the first measuring point, T_1, and W_2 is weight at second measuring point T_2. ΔT is numbers of days between T_2 and T_1.

2.2.6 Data Collection

For analyzing the numbers of weeks until the first salmon lice treatment was required, data for all the production groups in Experiment 2 was collected from the database BarentsWatch (https://www.barentswatch.no/fiskehelse/, "Tiltak mot lakselus"), in the period from SW transfer of Atlantic salmon (given in Table 2) until the first salmon lice treatment did occur. Number of mature female salmon lice the week before first salmon lice treatment occurred was also retrieved from the database (Table 4). A salmon lice treatment in this study is defined as a mechanical salmon lice treatment solely conducted due to an excessive number of female mature salmon lice (regulated limit of 0.5 mature female salmon lice per salmon (0.2 in summer)). The use of biological treatment (cleaner fish) and chemical treatment (bath or added to feed) is omitted from the definition in this thesis. Requirements for salmon lice treatment were decided by the company veterinarian based on weekly salmon lice counts.

Table 4. Number of mature female salmon lice at each production facility in Experiment 2 the week before the first mechanical salmon lice treatment and number of salmon lice treatments in the period from week 30, 2019 to week 20, 2020. Measured as mature female salmon lice per fish.

	Facilities	Number of mature female salmon lice the week before first salmon lice treatment (per fish)
AcuLice	Breivik S	0.35
	Grimsholmen	0.41
	Hattasteinen	0.49
	Hillersvik	0.33
	Loddetå	0.37
	Svollandsneset	0.35
References	Maradalen	1.21
	Seglberget	0.44
	Mælen	0.46
	Mean number AcuLice	**0.38**
	Mean number References	**0.70**

2.3 Statistical analysis

All statistical analysis and figures were performed using the Statistica™, v.13 (TIBCO Software Inc, Palo Alto, CA, US) software. Data in all graphical illustrations are presented by the means of each group and standard error of means (SEM). Statistical outliers with values greater than 1.5 times the interquartile range were excluded from the datasets using the Tukey fence method in Microsoft® Excel v. 16.41 (Microsoft, Redmond, Washington, US). The distributions of all response variables were checked for normality and homogeneity of variance using the Shapiro-Wilk test and the Levene test. A General Linear Models (two-way random effects nested ANOVA) analysis was fitted between each of the response variables and the predictor variables, "AcuLice sites" and "control regime site"/"reference regime sites", and with replicate subsamplings (random effect) as a nested factor within the predictor variables. A student t-test was used to analyse the specific growth rate, numbers of salmon lice treatments and numbers of weeks from Atlantic salmon was transferred to SW cages until first salmon lice treatment did occur between the AcuLice and reference group. A significance level of α=0.05 was used for all statistical models while asterisks was used to indicate significant differences between groups, NS = no significant difference, ($p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)).

3 Results

3.1 Experiment 1 – Acute stress effects of AcuLice treatment

Primary stress response (Plasma Cortisol Concentration)

The mean value of plasmatic cortisol concentration was 29.72 mmol/L at the first sampling (Control), and 35.50 mmol/L at the second sampling (1 hour with AcuLice treatment) (Figure 12) and did not vary (two-way nested ANOVA, $p > 0.05$, Table 10 in Appendix II) between the two sampling points.

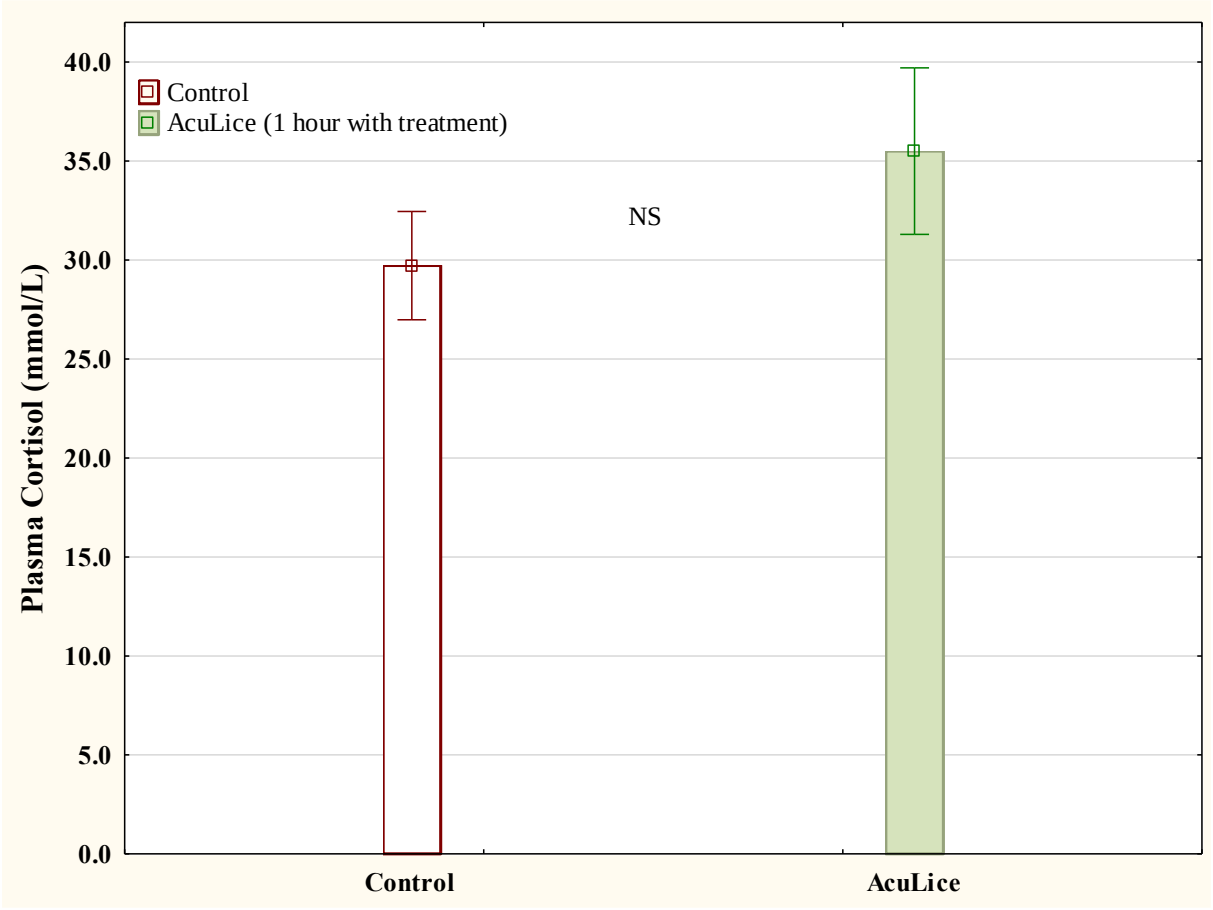

Figure 12. Plasma cortisol concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, n=30.

Secondary stress response (Glucose, lactic acid and ions)

Plasma Glucose Concentration

For the plasmatic glucose concentration, it was observed an increase in the mean value from 5.75 mmol/L at the first sampling (Control), to 6.13 mmol/L at the second sampling (1 hour with AcuLice treatment) (Figure 13). The test revealed a significant increase (two-way nested ANOVA, $p < 0.05$, Table 12 in Appendix II).

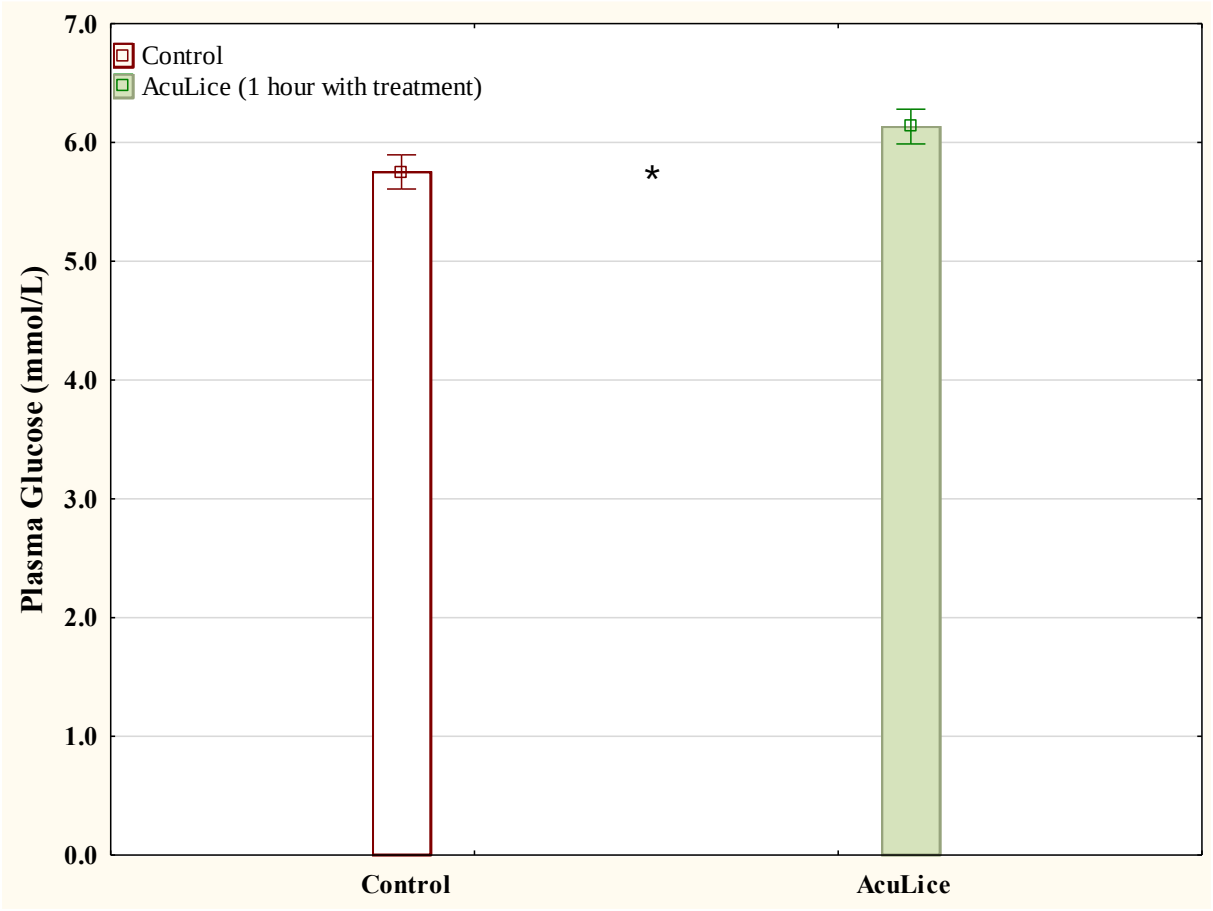

*Figure 13. Plasma glucose concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). Asterisks indicates the level of significance between the two groups: * $p < 0.05$. Each data sampling is present as a mean ± SEM, n=30.*

Plasma Lactic Acid Concentration

The mean value of plasma lactic acid concentration was 2.70 mmol/L at the first sampling (Control), and 2.68 mmol/L at the second sampling (1 hour with AcuLice treatment) (Figure 14). The concentration did not vary (two-way nested ANOVA, $p > 0.05$, Table 14 in Appendix II) between the two sampling points.

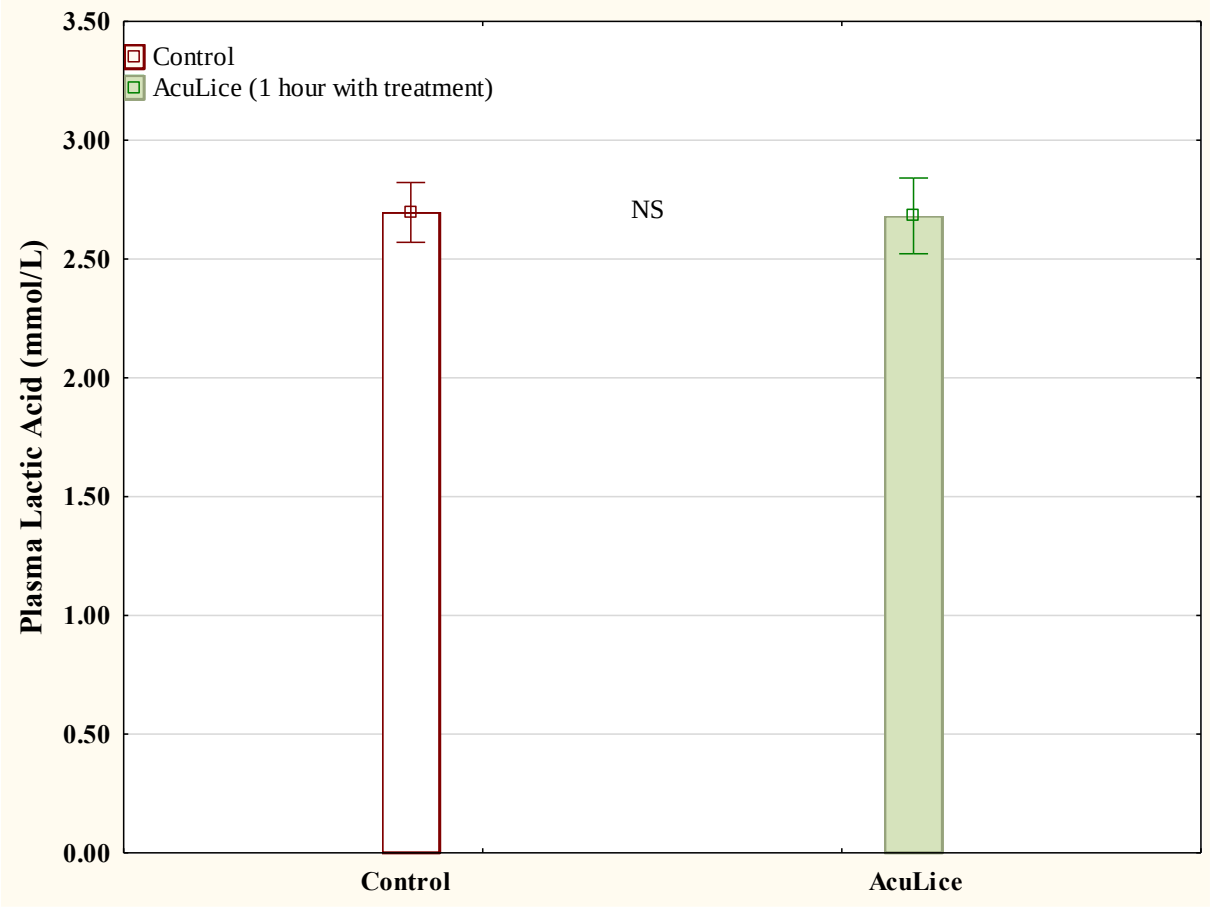

Figure 14. Plasma lactic acid concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, n=30.

Plasma Chloride Concentration

The two-ways nested ANOVA test revealed that the decrease of chloride concentration was not significant (NS) between the control and AcuLice groups ($p > 0.05$, Table 16 in Appendix II). The mean value of plasmatic chloride concentration was 127.40 mmol/L at the control sampling, and 126.28 mmol/L at the second sampling (1 hour with AcuLice treatment, Figure 15).

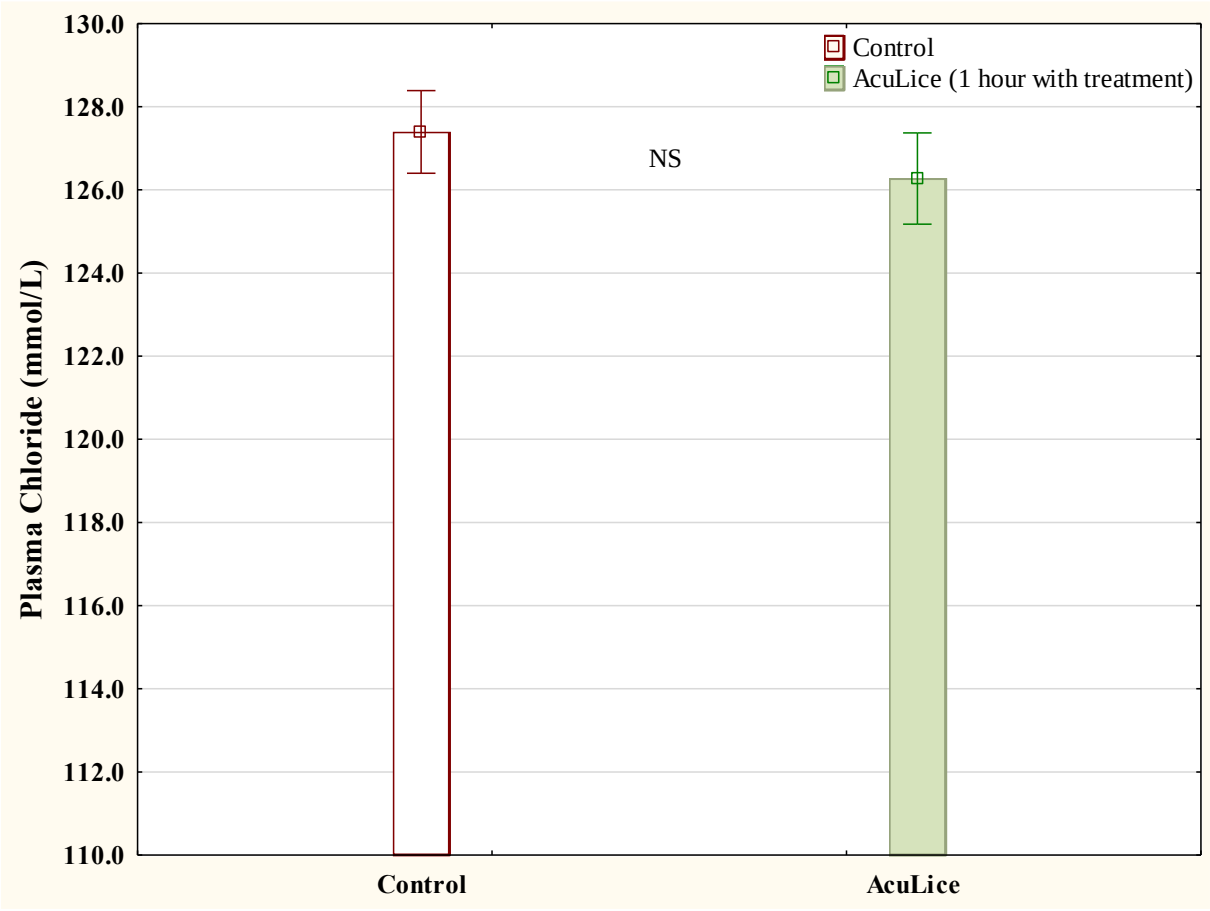

Figure 15. Plasma chloride concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, n=30.

Plasma Calcium Concentration

The mean value of plasma calcium concentration was 2.67 mmol/L at the first sampling (Control), and 2.68 mmol/L at the second sampling (1 hour with AcuLice treatment) (Figure 16). The two-ways nested ANOVA test showed no significant (NS) difference between the control and AcuLice group ($p > 0.05$, Table 18 in Appendix II).

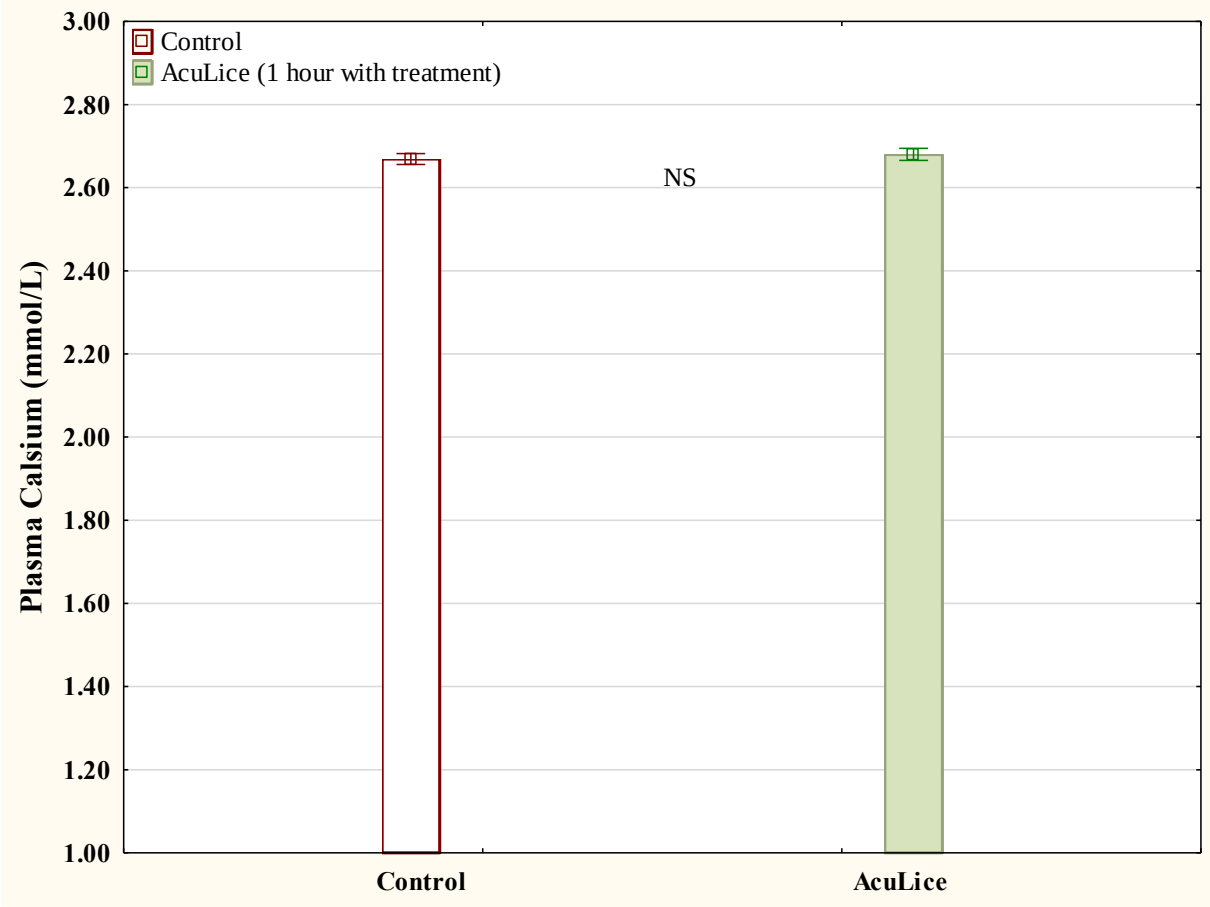

Figure 16. Plasma calcium concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, n=30.

Plasma Magnesium Concentration

No significant difference in mean magnesium concentration between the first sampling (Control) and second sampling (1 hour with AcuLice treatment) was revealed in the two-ways nested ANOVA test ($p > 0.05$, Table 20 in Appendix II). The mean value was 0.89 mmol/L in the control and 0.85 mmol/L in the AcuLice treated group (Figure 17).

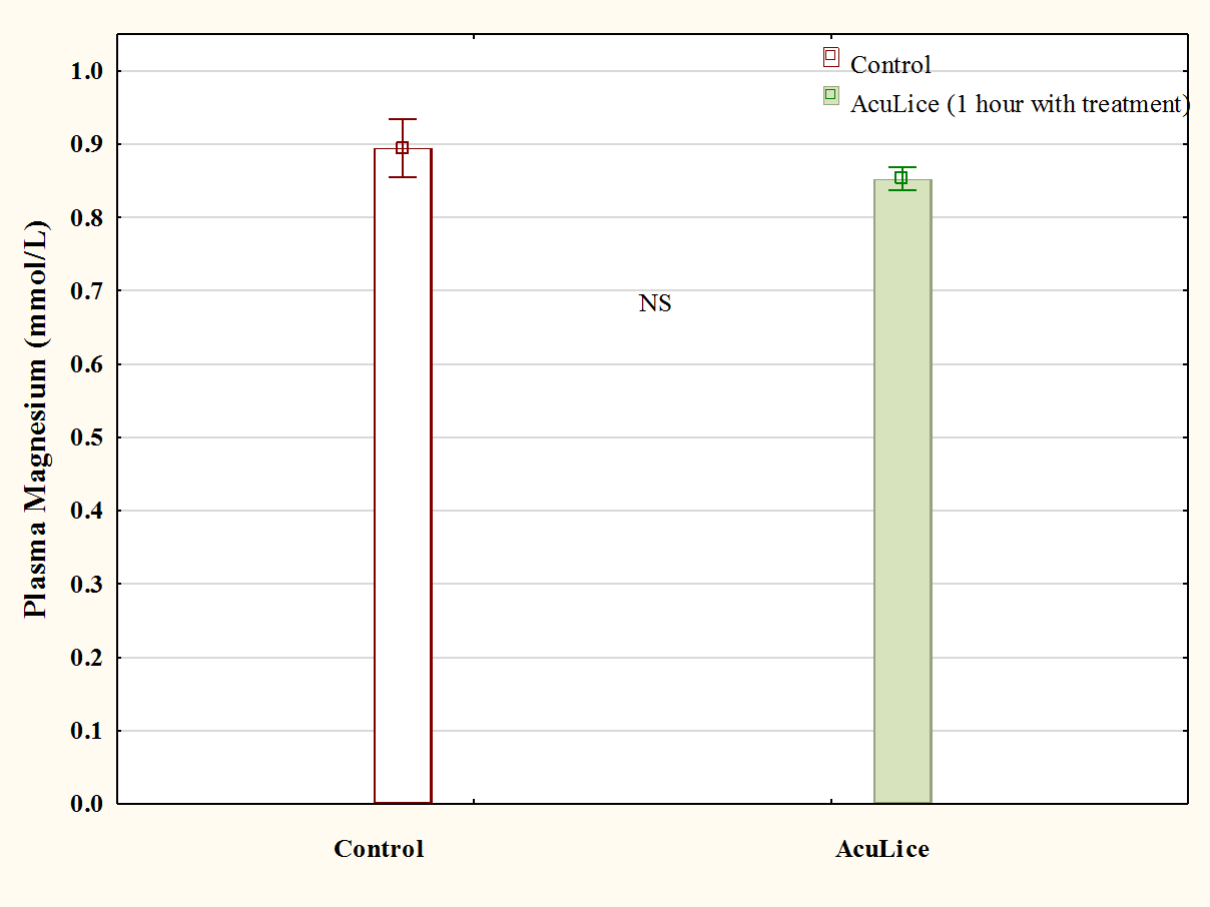

Figure 17. Plasma Magnesium concentration in mmol/L. Average value for salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, n=30.

3.2 Experiment 2 – Effect of AcuLice treatment in field

Tertiary stress response (Specific growth rate)

SGR in groups of Atlantic salmon exposed to low frequent sound compared to references

The AcuLice treated groups had a minimum value of SGR in weight at 0.32 % * day^{-1} (Svollandsneset) and a maximum growth rate at 0.52 % * day^{-1} (Grimsholmen, Hattasteinen) in the period from week 30, 2019 to week 20, 2020. For the reference group the minimum growth rate was 0.37 % * day^{-1} (Maradalen) and maximum were 0.48 % * day^{-1} (Mælen) in the same period. Due to lack of values for Seglberget, this locality is not included in the SGR survey. See Figure 18.

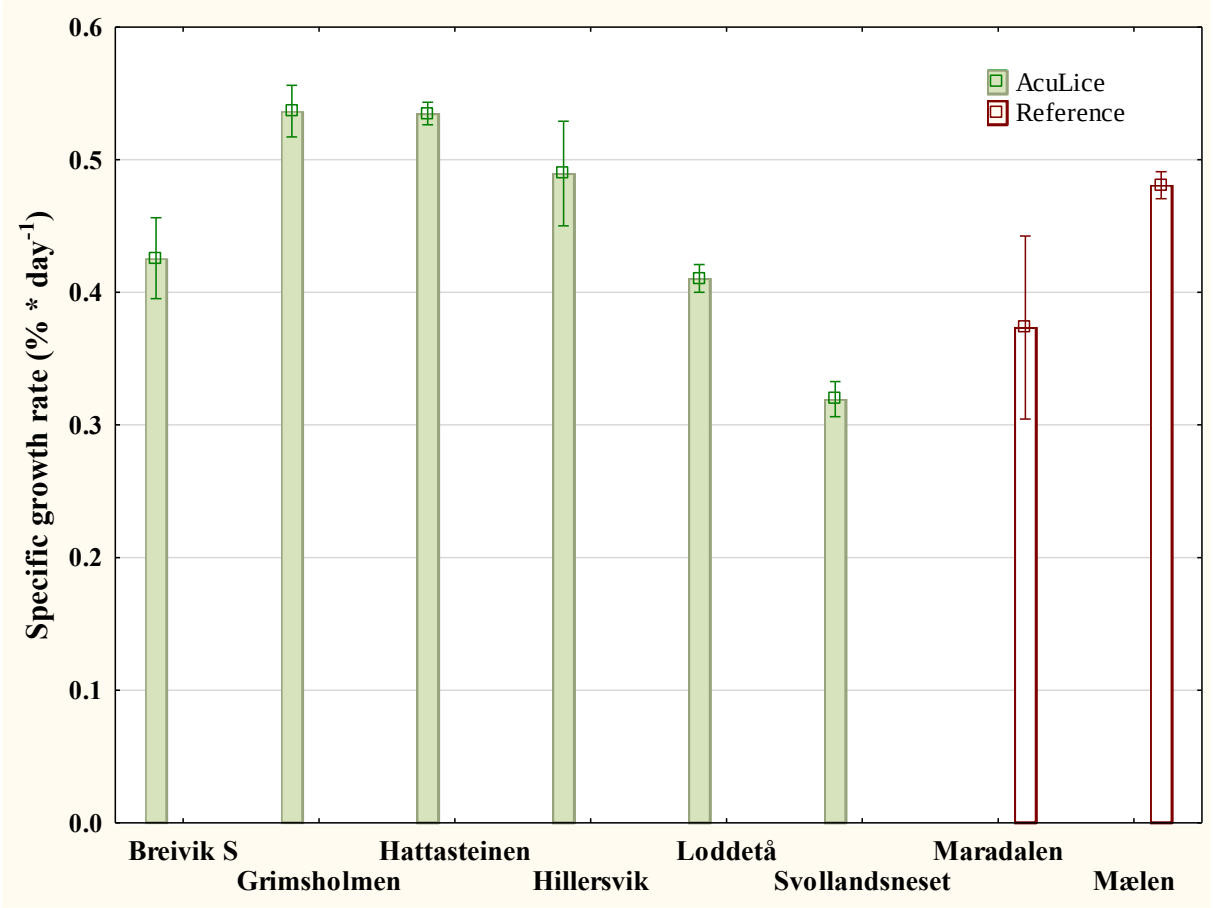

*Figure 18. Mean growth rate (SGR (% * day^{-1})) calculated for the Atlantic salmon in each experimental facility in Experiment 2A in the period from week 30, 2019 to week 20, 2020. The AcuLice treated facilities are marked in green, and the reference group are marked in red. Due to lack of values for Seglberget, this locality is not included in the survey. Data from each facility is presented as mean ± SEM.*

Mean SGR in AcuLice treated group compared to reference group

There were not revealed any significant difference in the mean SGR (% * day^{-1}) for each facility in Experiment 2, between the reference group and the AcuLice treated group (Student's t-test, $p > 0.05$, Table 22 in Appendix III), in the period (week 30, 2019 to week 20, 2020). The mean SGR for AcuLice treated group was 0.45 % * day^{-1} and for the reference group 0.43 % * day^{-1} (Figure 19).

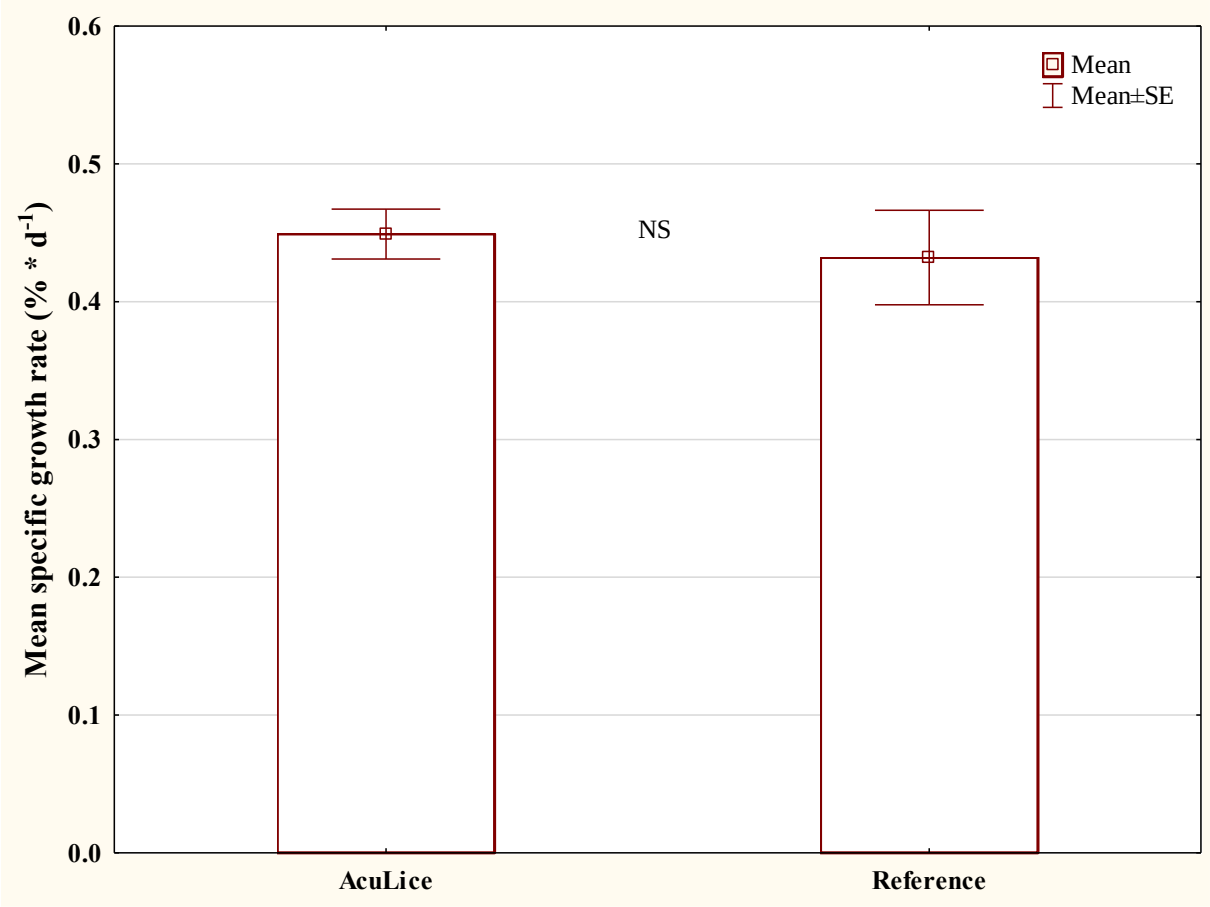

*Figure 19. Mean growth rate (SGR (% * day^{-1})) calculated for Atlantic salmon in the two experimental groups: AcuLice treated group and the reference group. Data from each facility is presented as mean ± SEM. NS indicates that there is no significant difference between the two groups.*

Effect on salmon lice dynamics

Numbers of small salmon lice in AcuLice and reference groups

The AcuLice treated groups showed a mean number of small salmon lice from 0.39 (Loddetå) to 1.22 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Figure 20). The reference group had, in the same period, a mean number of small salmon lice from 0.07 (Mælen) to 0.24 (Maradalen).

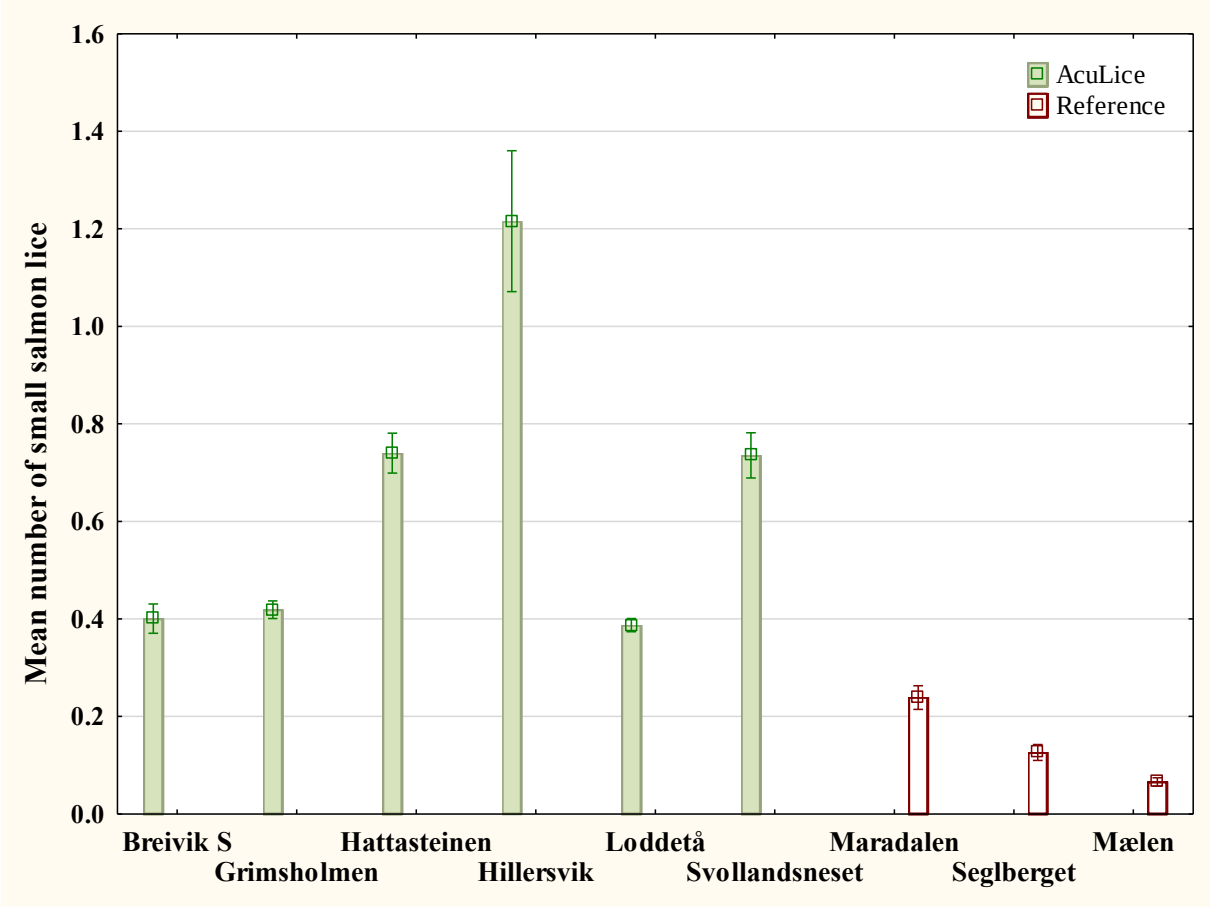

Figure 20. Mean number of small salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to AcuLice treatment (AcuLice) compared to the reference group (Reference). Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

Number of mature female salmon lice in AcuLice - and reference groups

The AcuLice treated groups had a mean number of mature female salmon lice from 0.12 (Breivik S) to 0.31 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Figure 21). The reference group had in the same period a mean number of mature female salmon lice from 0.39 (Maradalen) to 0.49 (Mælen).

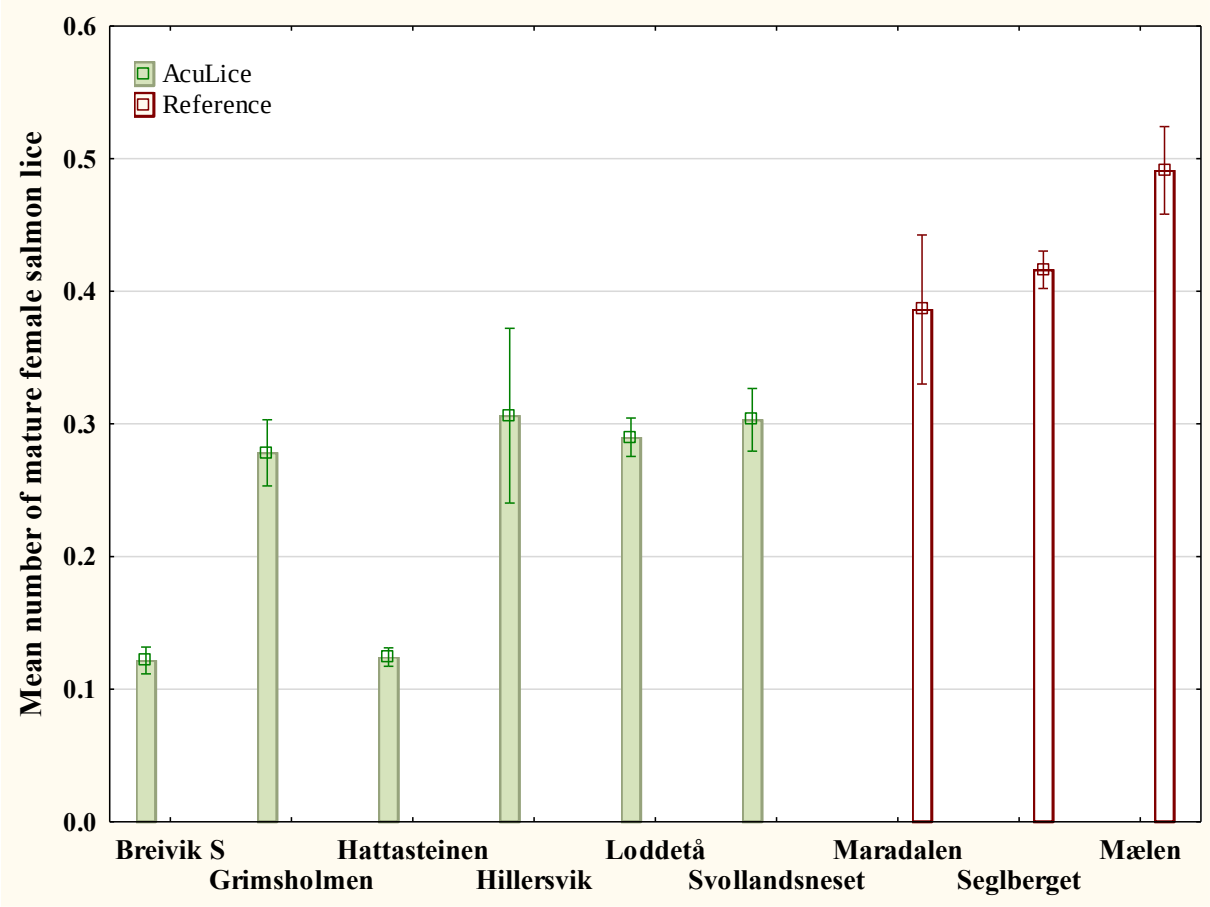

Figure 21. Mean number of mature female salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to AcuLice treatment (AcuLice) compared to the reference group (Reference). Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

Effect of AcuLice treatment in salmon lice population composition

Higher number of small salmon lice was observed for the AcuLice treated groups compared to the reference groups during the experimental period ($p < 0.001$, Table 24 in Appendix III). In contrast, a lower number of mature female salmon lice was observed for the AcuLice treated groups compared to the reference in the same period ($p < 0.001$, Table 26 in Appendix III). See Figure 22.

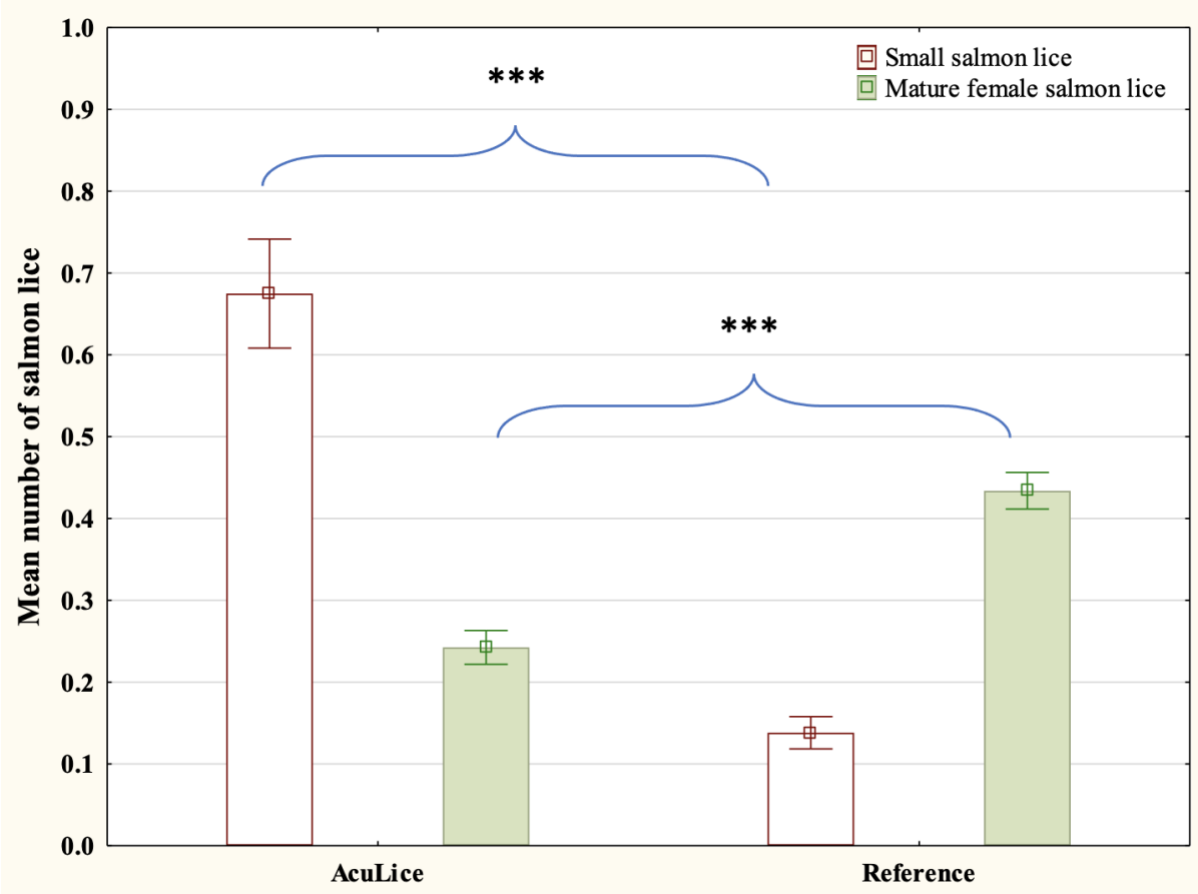

*Figure 22. Mean number of small salmon lice (red columns) and mature female lice (green columns) measured as per Atlantic Salmon between group exposed to AcuLice treatment compared to the reference group, during the period (week 30, 2019 to week 20, 2020). Asterisks indicates the level of significance between the two groups: *** $p < 0.001$. Data from each treatment group is present as a mean ± SEM.*

Numbers of salmon lice treatments during the 43 weeks period (week 30, 2019 to week 20, 2020)

The AcuLice treated group had significant lower number of salmon lice treatments (Student's t-test, $p < 0.05$, Table 32 in Appendix III) during the 43 weeks period (week 30, 2019 to week 20, 2020) compared to the reference group. The mean number of salmon lice treatments in the experimental period was 3.1 treatments per cage in the AcuLice group and 6.3 in the reference group (Figure 23).

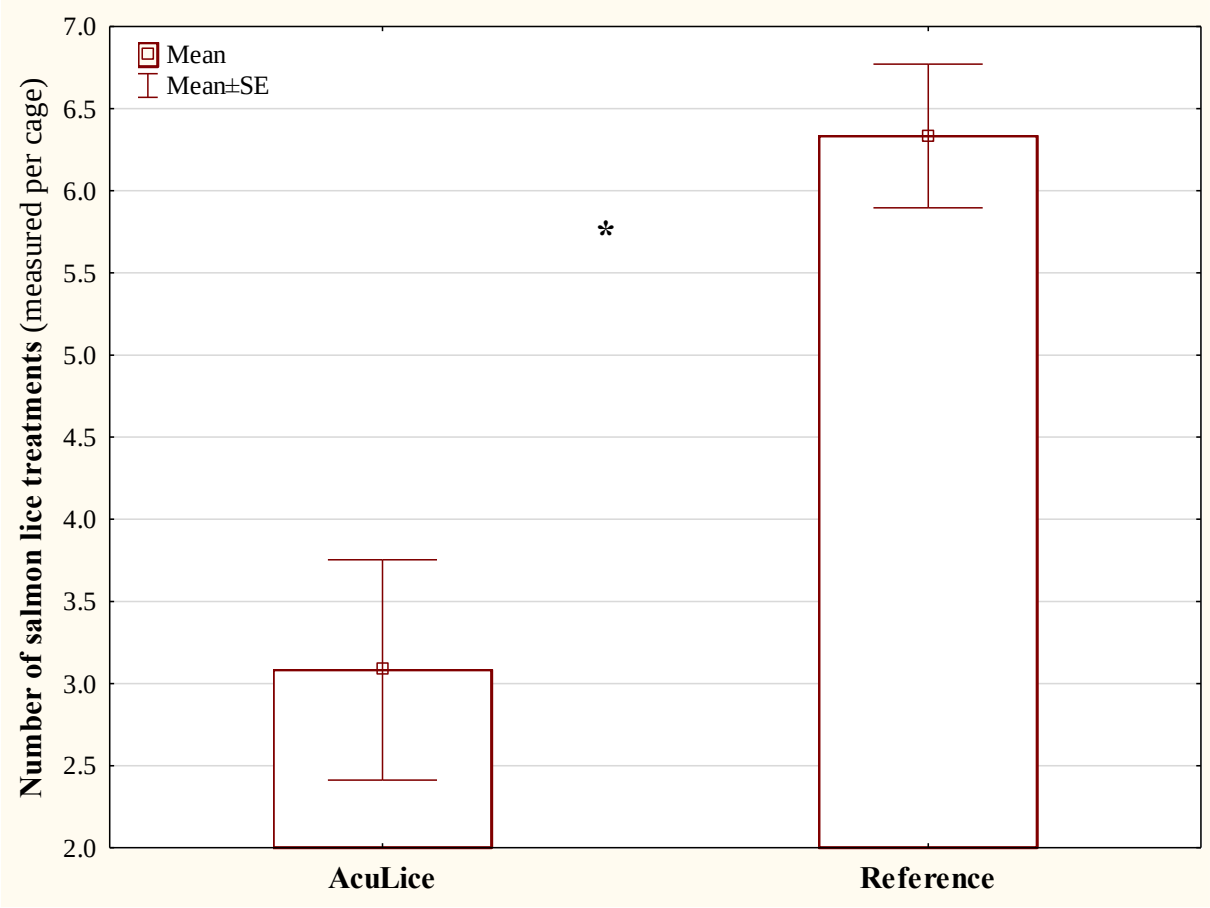

*Figure 23. Mean number of salmon lice treatments during the period (week 30, 2019 to week 20, 2020) measured as treatment per cage between group exposed to AcuLice treatment compared to the reference group. Asterisks indicates the level of significance between the two groups: * $p < 0.05$. Data from each facility is presented as mean ± SEM.*

Number of weeks to first salmon lice treatment

Data collected from Barentswatch indicated the number of weeks from SW transfer of Atlantic salmon until the first salmon lice treatment. For the AcuLice treated facilities the minimum number of weeks was 22 (Grimsholmen) and the maximum number was 40 weeks (Loddetå). The reference group had a period of 16 to 25 weeks (Seglberget, Maradalen) before the first treatment was necessary. See Figure 24.

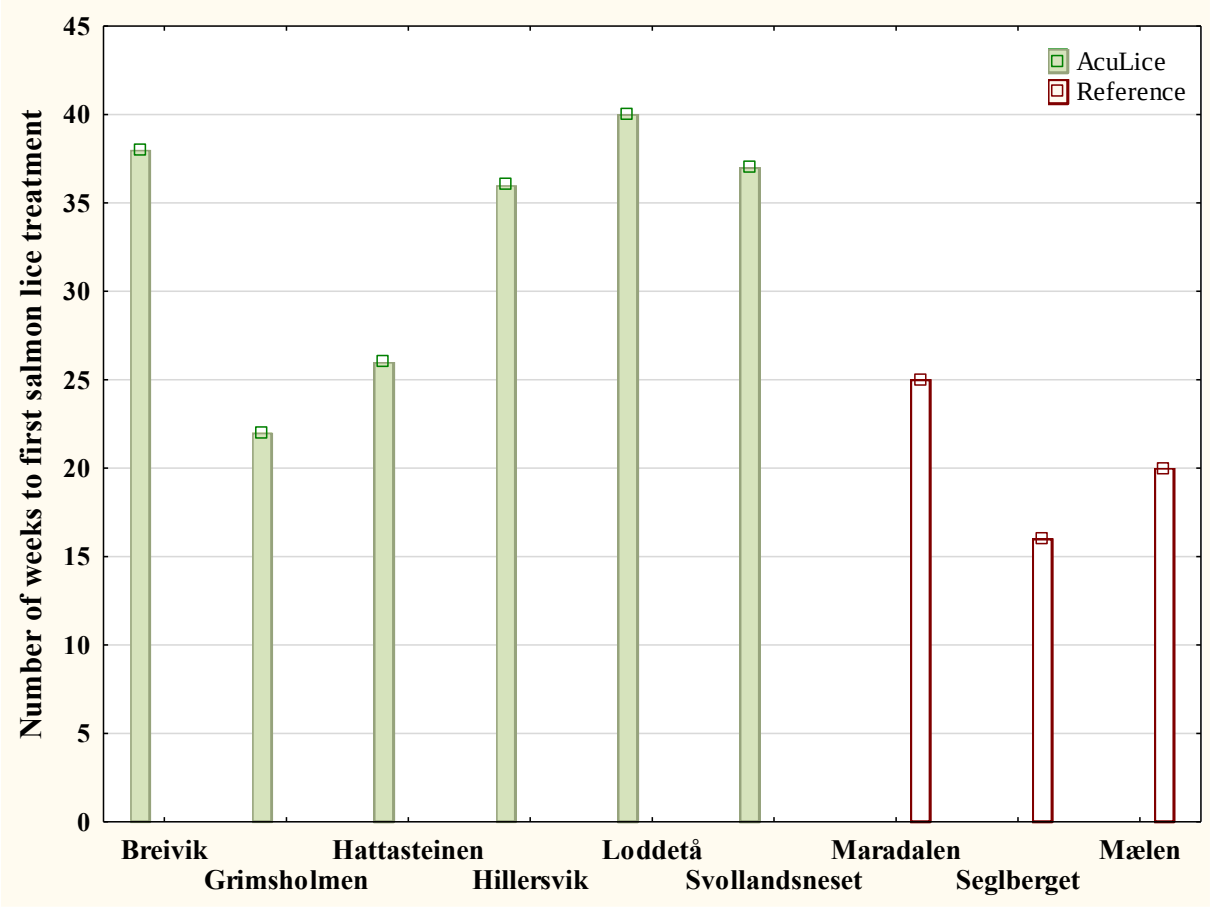

Figure 24. Number of weeks to the first salmon lice treatment at the experimental facilities in Experiment 2. Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities. Data from each facility is presented as mean.

Mean number of weeks until first salmon lice treatment

Overall, the number of weeks until the first salmon lice treatment increased from 20.3 weeks in the reference groups (mean number of weeks), to 33.2 weeks in the AcuLice treated groups (mean number of weeks) (Figure 25). The increase in the number of weeks until first salmon lice treatment was significant (Student's t-test, $p < 0.05$, Table 34 in Appendix III).

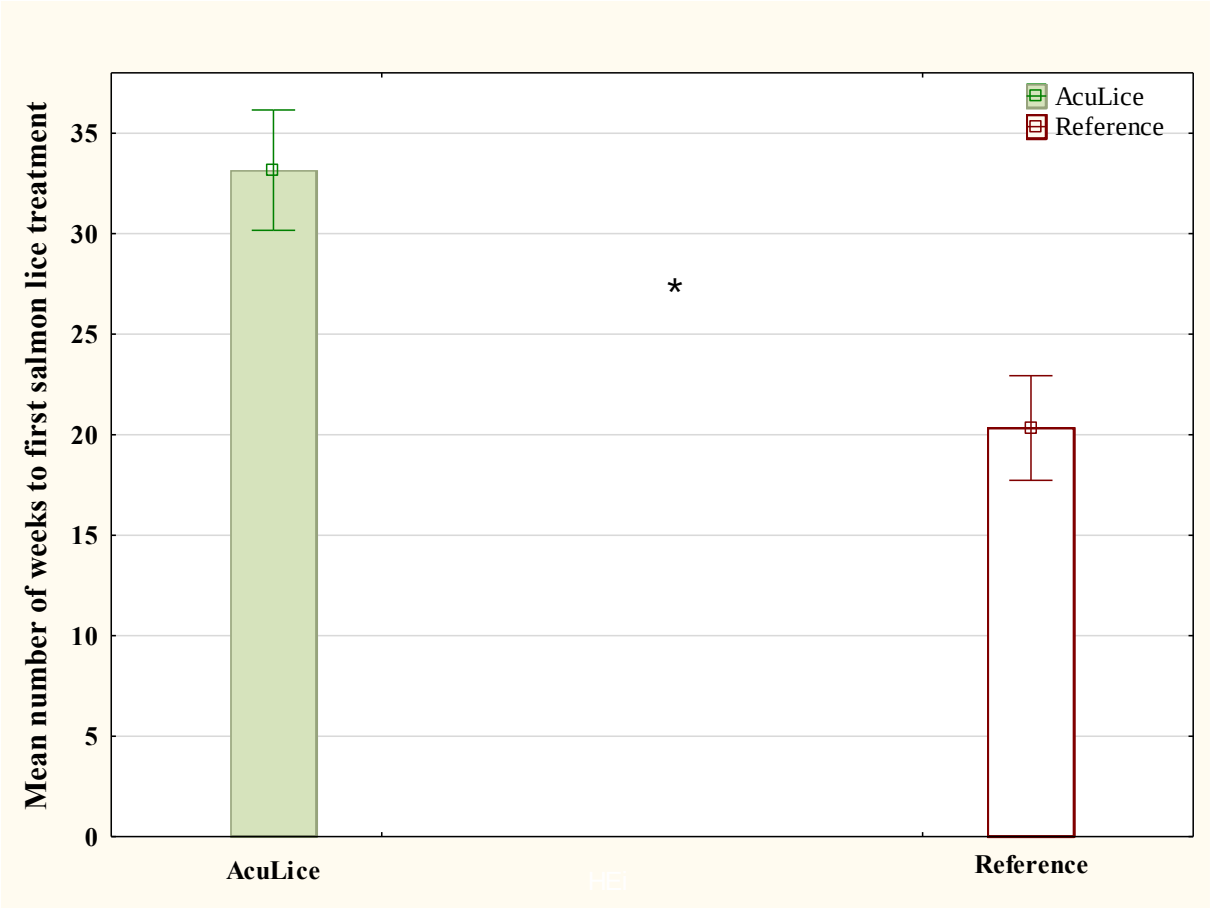

*Figure 25. Mean number of weeks to the first salmon lice treatment. Groups of Atlantic salmon exposed to AcuLice treatment compared to the reference group. Asterisks indicates the level of significance between the two groups: * $p < 0.05$. Data from each treatment group is present as a mean ± SEM.*

4 Discussion

4.1 Discussion of Methodology

The advantage of a large-scale study is that the analyzed effects is in the scale and environment in which it is supposed to be used, but such large-scale trials have some limitations (for example due to differences in SW temperature, production, regulations, different origins of fish and other external factors among the compared facilities). To meet these limitations the experiment was designed to accommodate most of the critical parameters due to the AcuLice equipment. Therefore, the study was divided into two different experiments: Experiment 1 – Acute stress effects of AcuLice treatment and Experiment 2 – Effect of AcuLice treatment in field.

In Experiment 1 and 2 the experimental facilities consist of open sea cages and the fish will be exposed to biological and abiological factors that cannot be overridden. This includes possible presence of pathogens and factors as weather, wind, salinity, current regime and changes in sea-temperature. Even though it is in a scientific context strived for full control of the experimental parameters, the present study represents field results as they would be in a real production situation.

Experiment 1 – Acute stress effects with use of AcuLice

Experiment 1 was carried out at one commercial Atlantic salmon facility with open sea cages. Since the low-frequency sound waves emitted will pass through all the cages at the plant and the absorption of low-frequency sound waves takes place over long distances (at 100 Hz, 99% of the energy is absorbed 10 km), all the cages can be considered as an equal group. Therefore, it was chosen to extract samples exclusively from the cage that is closest to the sound source as semi-parallels. In addition, due to the high number of individuals present in Experiment 1, the number of selected individuals was increased based on calculations established on pathology studies and previous experiments with similar size (n = 30 per sampling) (Haugen, 2020).

In Experiment 1, control sampling and treatment sampling (one hour of AcuLice treatment) were performed on the same day. By having one hour between the two samplings, it was possible to reduce difference in external factors. This was important in order to have a sampling that could be a substitution to a control and thus be comparable to the sampling that was after one hour of treatment. One difference between the samplings was the degree of feeding and food intake. Feeding of the day was first started after the first sampling was conducted and the

fish had thus not received food since the day before. Glucose is a central energy substrate in the metabolism for all vertebrates (Mergenthaler et al., 2013) and food intake can cause higher levels of this plasma glucose (Soengas et al., 2006). Variations in the measured glucose parameter were likely due to the difference in food intake between the two samplings and this can possibly be an influenced factor for our results.

Selection of measured parameters were made based on the relevance of the parameter in relation to an acute stress factor. Cortisol is considered the most central parameter when it comes to stress (Wendelaar Bonga, 1997). An increase in the concentration of cortisol in the blood is part of the primary stress response and affects the regulation of the concentration of the secondary stress parameters (glucose, lactic acid, chloride, calcium and magnesium) in the blood. The stress response of these parameters will therefore reach the highest concentration in blood, after being exposed to a stressor, at different times. Based on previous similar experiments the time period for sampling after treatment start, was chosen to be one hour (Øvrebø, 2020).

The long-term stress was not measured for the fish in Experiment 1. The main reason for this is the uncertainty related to all the stressors the fish could have been exposed to during the AcuLice treatment period, which is throughout the production period at sea. Therefore, many parameters could and will change during this production period. This will lead to lack of a control group resulting in difficulty in compartment and concluding of the results. For the tertiary stress response, it was thus chosen to use the sites in Experiment 2 to measure possible changes in the specific growth rate between facilities with AcuLice treatment and the references without. A field observation was also carried out where registration of the salmon's welfare using the Salmon Welfare Index Model (SWIM) in addition to measurements of the stress parameters (cortisol, glucose, lactic acid, etc., Appendix I) was done in order to strengthen the tertiary stress response (SGR) findings in Experiment 2.

Experiment 2 – Effect of AcuLice treatment in field

The large-scale experiment was chosen to make the study as close to reality as possible. It would have been preferable if all the fish originated and were reared at the same places before transfer to sea. Given the size of the experiment, including multiple different producers, this was not possible. All fish have been produced at RAS or flow through facilities with similar protocols and procedures, but minor differences in the hatchery process will occur. That kind

of variety is what can be expected at regional level in commercial production, when different actors are involved. In addition, the facilities without treatment are referred to as references due to the fact that it is impossible to have control of all variables in a large-scale field study.

In Experiment 2, it has been problematic to find good reference sites. At the start of the project, four facilities were registered in the reference group. Throughout the study, it was shown that at one facility (Ebne) was located in an area with great reports of FW to superficial water. Farming facilities that are exposed to freshwater run-off from rivers have been observed to have lower salmon lice densities than facilities that have salinity around 35‰ (Heuch et al., 2009). Salmon lice thrive poorly in FW, and low salinity in itself can be a delicing method. Therefore, this reference facility was removed from Experiment 2, since the other fjord facilities had a salinity of 30 – 32‰. The study is thus characterized by few references, which is unfortunate for the quality and depth of the experiment, however it is still strong enough due to statistical test and conclusions.

Transfer of salmon to open sea cages occurred at different times in the spring of 2019 from week 11 to week 27. Salmon lice prefers warm temperatures and reproduces faster in SW temperatures at 12°C (Heuch et al., 2000). The difference in transfer times to open SW cages could have had an impact on how exposed the fish were to salmon lice. However, this was done related to large-scale limitations and logistical transfer at the exact same time is unattainable in an experiment of this size.

Variations in the start date of AcuLice treatment were found for the facilities in Experiment 2. Ideally, all installations including start of treatment should take place before the fish are transferred into sea cages. Due to logistics and many facilities and manufacturers involved in Experiment 2, this was not possible. All facilities started treatment in the period from week 28 to week 30 in 2019. Although some facilities had the AcuLice system installed later than others (compared to when the fish were transferred to sea cages), this only affects the analysis of number of weeks before treatment for the AcuLice group. In addition, the start-up problems can be neutralized when it comes to the calculations of number of salmon lice in the 42-week period (week 30, 2019 to week 20, 2020) due to the relatively long-time horizon of Experiment 2. Given that only the AcuLice sites were affected by the start-up problems we chose to continue with the results for all groups.

Due to the use of a large-scale study, this also meant that the fish farmers demanded to have cleaner fish presented in the cages. All facilities in the trial, both with AcuLice treatment and the reference groups, had 3-5% cleaner fish present at release. Because both groups (AcuLice and the reference) had approximately the same amount of cleaner fish present, we chose to disregard this factor as it would apply to both groups and thus could be neutralized.

For analysing the effect of AcuLice treatment on reduction of salmon lice the number of weeks to first salmon lice treatment was measured using data from the database Barentswatch. All the salmon production facilities in Norway are obliged to report to the database with weekly salmon lice numbers as well as when and what kind of salmon lice treatments is used. There will be a possibility for minor differences in sampling procedures as well as the chance for false or incorrect reports for each of the facilities. Nevertheless, protocols created from the authorities in addition to the good practise in the industry will help to reduce the possibility for such differences.

In the present study, a salmon lice treatment is defined as a treatment due to an excessively high density of mature female salmon lice (regulated limit of 0.5 per fish (0.2 in summer)). This means that treatments related to FW as a result of AGD disease in the facility are not included. In addition, chemical treatment will also not be included in our definition, as this often is used to prevent high salmon lice numbers. The mentioned treatments are thus sources of error which were not removed in the present study, but which could potentially have had an impact on the results.

4.2 Discussion of Results

Experiment 1 – Acute stress effects of AcuLice treatment

Primary stress response

The cortisol results in Experiment 1 did not show any difference in concentration between the control sampling and after one hour with treatment. Cortisol has a central role in the stress response and homeostasis related to stress, in addition to impact other processes, such as growth, behavior, reproduction and osmoregulation (Wendelaar Bonga, 1997; Mommsen et al., 1999). Cortisol is involved in the primary stress response and the increase in concentration in blood is involved in the fight or flight response in fish. Consequently, a temporary increase in energy production on behalf of processes that are not required for immediate survival, is a result for the involvement for cortisol (McCormick et al., 1998). The release of cortisol from the internal cells has a delay of several minutes (Wendelaar Bonga, 1997), and minimizing the sampling stress factor on the measurements.

The cortisol concentration will increase rapidly after the fish has been exposed to a stressor (Wendelaar Bonga, 1997) and go towards normal levels within one or more hours. In present experiment, samples were taken 1 hour after AcuLice treatment was started. No behavior alteration was observed via camera in the moment the AcuLice treatment was started, which can support the findings that the fish was not stressed. If the fish were affected in this moment, it would have been a mild stress and there will be a possibility that the cortisol levels had already dropped to the normal levels when the sampling took place. However, present results show no significant difference in the concentration levels in blood between the two samplings. The observation with no behavior alteration can substantiate that the Atlantic salmon in Experiment 1 did not have a primary stress response.

Secondary stress response

There were not observed any differences in most of the analyzed parameters which are related to a secondary stress response. The results did not show effects in the lactic acid, chloride, calcium or magnesium concentrations, but an increase in glucose levels was observed.

In Experiment 1, a significantly higher concentration of glucose was observed when the group had been exposed to AcuLice treatment for one hour. The plasma glucose concentration is affected by an increase in cortisol levels, but can also be influenced by other factors such as

diet and nutrient type (Mommsen et al., 1999). The elevations in plasma cortisol stimulate glycogenolysis (conversion of glycogen stored in the tissue to glucose that is released into the blood) and an increase will be a slow response to a stressor (Fast et al., 2008). According to Olsen et al. (2002), a maximum concentration of glucose in the blood will be achieved after approximately 3 – 6 hours after the salmon has been exposed to a stressor. Since the sampling took place one hour after the start of the treatment, it could indicate that the elevated concentration had either not reached the maximum concentration or that the glucose levels could be influenced by other factors, as feeding.

Studies have shown that Atlantic salmon has a normal concentration of glucose in blood around 3.3 mmol/L (Fast et al., 2008) and values under 6 mmol/L is observed to be in the normal range (Skjervold et al., 2001). The mean values measured in the present study were 5.75 and 6.13 mmol/L for the control and AcuLice groups, respectively, so both can be considered to fall within the normal range for Atlantic salmon. A study by Skjervold et al. (2001) did show that the glucose levels did increase to over 7 mmol/L when Atlantic salmon was exposed to a stressor. Present results show glucose levels of around 6.1 mmol/L, which is just outside the normal range for plasma glucose concentrations, but lower than levels observed for stressed fish. Glucose levels in fish blood is also known to have a great variability and has been considered a poor indicator of secondary stress (Mommsen et al., 1999). In addition, the low values of lactic acid concentration in the plasma support the indication that the increase in glucose that has occurred is due to factors other than stress, such as diet. Based on the mentioned studies, the increase in plasma glucose levels does not have to be directly correlated with the AcuLice treatment.

Furthermore, in the present study no significant difference in the plasma lactic acid concentration between the control and treated group in the Experiment 1 was observed. Lactic acid is a result of a limited amount of oxygen accessible for aerobe cell metabolism and can be achieved by hard physical activity or low oxygen levels in the water (Milligan & Girard, 1993). In relation to a stressor, lactic acid indicates that there has been a high muscle activity which can be correlated with a fish being exposed to a stressor (Iversen et al., 2003). As a result of a stressor the lactic acid concentrations is observed to be over 6 mmol/L in blood plasma (Iversen et al., 2003; Hatløy, 2015). This can indicate that present results, with concentration levels around 2.7 mmol/L, is in the normal range of lactate concentration. It does also correspond to

the schooling behaviour observed trough camera, showing no changes in swimming behaviour during the treatment period.

There were also not shown a significant difference in plasma chloride concentration between the control sampling and one hour after the AcuLice was started in the present study. In SW the plasma chloride concentration will increase when an acute stressor occurs due to leakage through the tight junctions of the epithelium (McDonald & Milligan, 1997). For a non-stressed Atlantic salmon in SW the plasma chloride concentration has been reported to be around 135 ± 2.5 mmol/L (Fivelstad et al., 1998). Present observations are thus lower and indicates no elevated values associated with a stressor.

The calcium uptake trough the gills, has been shown to be stimulated by cortisol in salmonids (Flik & Perry, 1989) and thereafter to influence the intracellular concentration of calcium (Mommsen et al., 1999). In addition, the growth hormone and prolactin, which are hypercalcaemic, and the stanniocalcin and calcitonin, which are hypocalcemic interact in regulating the extracellular calcium ion (Ca^{2+}) levels in salmonids (Copp et al., 1962; Pang et al., 1971). The present findings in Experiment 1 did not show any significant difference in plasma calcium concentration between the two samplings. For sampling 2 the concentration of calcium ions was 2.64 mmol/L, and according to the study by Evans (1979) this concentration is in the normal range of an unstressed fish (around 3.3 mmol/L).

There were not found any differences in the magnesium concentration for the treated group (Sampling 2) compared to the control group (Sampling 1). Previous studies have shown that there is a high connection between increased plasma magnesium and mortality after a fish is undergoing a stressor (Liebert & Schreck, 2006; Iversen & Eliassen, 2009). Changes in the magnesium concentration are a good indicator of acute stress (Stewart et al., 2016). Normal plasma magnesium concentration is typically less than 1 mmol/L for salmonids (Bijvelds et al., 1998; Liebert & Schreck, 2006; Iversen & Eliassen, 2009), which is consistent with the current values in this experiment. Although studies on effects of stress and magnesium (Mg^{2+}) are limited, it has been reported a significant increase in plasma magnesium concentration due to a exposure to a stress factor in Atlantic cod, *Gadus morhua*, (Staurnes et al., 1994), in SW Coho salmon, *Oncorhynchus kisutch*, (Redding & Schreck, 1983) and in gilthead sea bream, *Sparus aurata*, (Arend et al., 1999). In SW the concentration of magnesium is 50 – 100 times higher

than it is in fish plasma. Therefore, a small increase in gills permeability during stress can lead to large influx of magnesium (Redding & Schreck, 1983). There is no indication of increased magnesium concentration in this experiment and thus no indication of increased gill permeability due to a stress factor.

Overall, the findings of current trial indicates that the secondary stress response was not activated during the one-hour treatment with AcuLice. The glucose levels did increase during the experiment, but in relation to the other parameters as well as results from previous studies, this is potentially based on factors other than the treatment.

Experiment 2 – Effect of AcuLice treatment in field

Tertiary stress response

The Atlantic salmon present in Experiment 2 did not show any differences due to the specific growth rate and this, including the results from the field observation (Appendix I), suggest that the fish did not have a tertiary stress response when exposed to AcuLice treatment. Atlantic salmon that has been exposed to a stress factor over time, will activate the tertiary stress response who is built over time (Schreck & Tort, 2016). A chronic stress factor will negatively affect the growth, reproductive ability and immune system (Schreck, 2010). In the field observation where SWIM parameters were analyzed at two different facilities with AcuLice treatment (Breivik S and Ihlholmen), the results suggest no or low impact of AcuLice treatment on chronic stress response (Appendix I). The results from the field observation supports the SGR results which indicates that the fish has not been exposed to a chronic stressor and thus has not had a tertiary stress response. This can also be substantiated by the fact that no changes have been found in the primary stress response and little that indicates that the secondary stress response has been present in the study.

Salmon lice population composition

To find out if AcuLice treatment has a salmon lice removal effect, the number of salmon lice was counted weekly and categorized. In the salmon farming context, the two categories small salmon lice and mature female salmon lice, are the most relevant in connection with accumulation and the delicing limit (Torrissen et al., 2013; Mattilsynet, 2021). Therefore, these main categories were analyzed. Numbers on large salmon lice (salmon lice in the stages preadult 1 and 2 for male and female, and adult male) are given in Appendix III.

The results showed that there was a difference in the number of salmon lice between the two groups (i.e. AcuLice treatment vs. no AcuLice treatment) in the period from week 30, 2019 to week 20, 2020. The AcuLice sites had a significantly larger proportion of small salmon lice in their facilities. This may indicate that the salmon lice pressure at the sites with AcuLice treatment was higher and thus has a significantly greater salmon lice impact on these facilities compared to the reference group.

Based on the results that the AcuLice sites had a significantly higher number of small salmon lice, this would lead to that the other salmon lice stages would be accumulated in a larger number than at the reference sites (Stien et al., 2005). However, the results showed that the AcuLice sites had a significantly lower number of mature female salmon lice than the reference sites. This is contrary to the expected development where a larger number of small salmon lice should lead to more mature female salmon lice (Kristoffersen et al., 2014). The lower proportion of mature female salmon lice thus indicates that salmon lice are removed or disappeared during the salmon lice life cycle at the localities using AcuLice.

A previous study by Heuch and Karlsen (1997) has observed the anterolateral flow field from a swimming salmonids is one of the most important factors for successful infestation with a host for a salmon lice. The flow field is derived from water being moved when the salmonid is swimming and is in a low frequency range of 1 – 5 Hz (Kalmijn 1988, 1989; Heuch and Karlsen 1997). Therefore, low frequencies in this range can be used to mask the water pressure signature from a potential host. As shown in the present study, some of the salmon lice had disappeared during the AcuLice treatment and it is unclear exactly why this occurs. It is conceivable that salmon lice that have infected the salmon become unsure whether it is in the right species and therefore choose to jump off while waiting for the apparently correct host where the sound frequency comes from. Another possible reason is that the salmon lice were disturbed by the constant frequency which causes them to stop eating on the salmon skin and thus end up dying.

The results indicated that salmon lice disappeared in the period from when they are defined as small salmon lice to the stage of mature female salmon lice. Due to the fact that the study includes localities that produce fish during ordinary operation, these must follow national regulations with delice at the limit of 0.5 mature female salmon lice. An average of 3.1 delice

operations per cage has been carried out in the AcuLice facilities, which is a significant lower number of treatments compared to the reference group with an average of 6.3 delice during the period from week 30, 2019 to week 20, 2020 (Figure 23). This suggests that delicing is not the cause of lower number of mature female salmon lice in AcuLice facilities. Furthermore, it supports previous findings that AcuLice has a lower number of mature female lice which leads to fewer salmon lice treatments.

Overall, the results indicate that the AcuLice sites have had a greater salmon lice pressure with a significantly larger number of small lice during the period. In addition, the results suggest that salmon lice are removed from the fish during the salmon lice life cycle at the AcuLice sites and that the number of delice treatments compared to the reference sites is significantly lower. Based on these results, it appears that AcuLice influences the removal of salmon lice.

Number of weeks until first required salmon lice treatment

Assuming that AcuLice removes salmon lice before developing to mature female salmon lice, it will be expected that it will take longer time before facilities with this treatment have to delice. To substantiate the results related to the effects on salmon lice population composition, number of weeks until the first salmon lice treatment occurred was measured on data collected from BarentsWatch. In order for the result of the measurement of weeks until the first salmon lice treatment to be able to give valid results, some requirements should be fulfilled.

First, it will be important that AcuLice and the reference group have some of the same infestation pressure. Since the facilities were located in different locations in the sea, it was not possible to achieve complete equal salmon lice pressure. Results from Experiment 2 indicate that the reference group may have a lower salmon lice pressure, which means that AcuLice at least does not have an advantage when it comes to this factor. Therefore, the salmon lice pressure requirement is partly fulfilled.

Secondly, there should preferably not have been other forms of salmon lice treatment in the period before the first required treatment (defined in the present study as mechanical salmon lice treatment). Based on Barentswatch data, cases of medicated feed treatment can be found at the two reference sites Maradalen and Mælen, in addition to one AcuLice facility, Hillersvik (Table 33, Appendix III). This could help to extend the production time at these facilities until

the first salmon lice treatment is required. This thus applies to 1 of 6 AcuLice sites and to 2 of 3 reference sites. The factor thus occurred in both treatment groups (several facilities in the reference group, but several times in Hillersvik) and it was decided to continue with the results. During the period, AGD FW treatments were also carried out at some facilities. These treatments were performed on the basis of a disease problem and were not initiated by high densities of salmon lice and are not registered as a lice infestation in Barentswatch. Therefore, it was decided to continue with the results despite the possible delicacy of such treatment. Both of the mentioned treatments may have had an effect on the results when it comes to the number of weeks until the first required delice took place. Nevertheless, in a field study, such sources of error will occur as a result of national regulations and because it has occurred in both treatment groups, the effect will potentially be minimized.

A third criterion is that the facilities deliced at the same maximum values for mature female salmon lice. As shown in Table 4 (in 2.2.6 Data Collection) the AcuLice group did have a number of mature female salmon lice at 0.38 the week before the first salmon lice treatment compared to the average of 0.70 in the reference group. Since the AcuLice group delice at a lower number of mature female salmon lice, this factor will not provide an advantage for the AcuLice treatment. The criterion is thus partially met.

The data material related to the production period before the first de-lice contains some noise, especially associated with the reference group, which may have had an impact on the results. Nevertheless, these results do indicate that there was a significant longer production time before a salmon lice treatment occur and may indicate that the AcuLice system has a positive effect on duration of production period before delicing when used in commercial salmon farming facilities.

Overall, the results in Experiment 2, related to the salmon lice composition, number of salmon lice treatments and to the number of weeks until the first treatment, indicated that AcuLice treatment had a significant effect on reduction of the salmon lice burden in Atlantic salmon commercial production.

5 Conclusion

The Atlantic salmon group reared with low frequent sound treatment (AcuLice) for one hour in commercial open sea cages showed few significant effects to an acute stress response compared to the control. The AcuLice equipment did show significant effects on the salmon lice composition, number of salmon lice treatments and number of weeks until first salmon lice treatment.

Experiment 1

- **$H0_1$:** AcuLice treatment for one hour has no significant effect on plasma cortisol concentrations in Atlantic salmon (Primary stress response) **is not rejected.**
- **$H0_2$:** AcuLice treatment for one hour has no significant effect on plasma glucose concentration in Atlantic salmon (Secondary stress response) **is rejected.** A significant difference in plasmatic glucose concentration was observed between the control group and the group with one hour with AcuLice treatment and therefore the **$H1_2$ is accepted**.
- **$H0_3$:** AcuLice treatment for one hour has no significant effect on plasma lactic acid concentration in Atlantic salmon (Secondary stress response) **is not rejected.**
- **$H0_4$:** AcuLice treatment for one hour has no significant effect on plasma chloride concentration in Atlantic salmon (Secondary stress response) **is not rejected.**
- **$H0_5$:** AcuLice treatment for one hour has no significant effect on plasma calcium concentration in Atlantic salmon (Secondary stress response) **is not rejected.**
- **$H0_6$:** AcuLice treatment for one hour has no significant effect on plasma magnesium concentration in Atlantic salmon (Secondary stress response) **is not rejected.**

Experiment 2

- **$H0_7$:** AcuLice treatment has no significant effect on specific growth rate in Atlantic salmon (Tertiary stress response) **is not rejected.**
- **$H0_8$:** AcuLice treatment has no significant effect on small salmon lice counts on Atlantic salmon **is rejected.** A significant difference in numbers of small salmon lice was observed between the reference group and the AcuLice treated group and therefore the **$H1_8$ is accepted**.
- **$H0_9$:** AcuLice treatment has no significant effect on mature female salmon lice counts on Atlantic salmon **is rejected.** A significant difference in numbers of mature female

salmon lice was observed between the reference group and the AcuLice treated group and therefore the **$H1_9$ is accepted**.

- **$H0_{10}$:** AcuLice treatment has no significant effect on numbers of salmon lice treatments during the treatment period **is rejected**. A significant difference on numbers of salmon lice treatments during the treatment period was observed between the reference group and the AcuLice treated group and therefore **$H1_{10}$ is accepted.**
- **$H0_{11}$:** AcuLice treatment has no significant effect on the production time of Atlantic salmon in open sea cages before the first salmon lice treatment **is rejected.** A significant difference in numbers of weeks before the first salmon lice treatment was observed between the reference group and the AcuLice treated group and therefore the **$H1_{11}$ is accepted**.

Future perspective

The present study was conducted as a part of the overall project of conducting effects of AcuLice treatment. The AcuLice treatment shows promising results with no observed signs of the treatment leading to stress response in Atlantic salmon. Further analyses on nearby environment and other species, e.g. the harbour porpoise (*Phocoena phocoena*), ballan wrasse, lumpfish and invertebrates, should be investigated for a deeper understanding of the low-frequent sound effects. Positive results in reduced number of mature female salmon lice on Atlantic salmon, lower number of salmon lice treatments and a longer production period before the first salmon lice treatment occurred, was also shown in the present study when comparing AcuLice facilities to references. Due to some influencing factors associated with lice removal, the study should be repeated to strengthen the results. In addition, it would be preferable to conduct a study where the parameters that can influence the removal of salmon lice, such as cleaner fish and the use of drug treatment, were completely absent. Because of a large-scale study where the facilities produced salmon for commercial consumption this was not possible to override in the present study. Furthermore, it would be interesting to further study which effect the low frequent sound has on the salmon lice individual and which stages have the greatest effect of the treatment method. Moreover, the effects of AcuLice treatment compared to other long-term salmon lice treatment methods such as biological delice (use of cleaner fish) and laser treatment would be areas of interest for future research. It would be interesting to look at the effect between the different treatment methods in addition to the overall situation of the treatment methods' costs and need for maintenance.

Bibliography

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K., & Horsberg, T. E. (2015). Drug resistance in sea lice: A threat to salmonid aquaculture. *Trends in Parasitology* 31, 72-81. doi:10.1016/j.pt.2014.12.006

Abolofia, J., Wilen, J. E., & Asche, F. (2017). The cost of lice: Quantifying the impacts of parasitic sea lice on farmed salmon. *Marine Resource Economics*, 32, 329–349. doi:10.1086/691981

Arends, R. J., Mancera, J. M., Muñ Oz, J. L., Bonga, W., & Flik, G. (1999). The stress response of the gilthead sea bream *(Sparus aurata L.)* to air exposure and confinement. *Journal of Endocrinology*, 163, 149–157. doi:10.1677/joe.0.1630149.

Austreng, E., Storebakken, T., & Åsgård, T. (1987). Growth rate estimates for cultured Atlantic salmon and rainbow trout. *Aquaculture*, 60, 157–160. doi:10.1016/0044-8486(87)90307-3

Barentswatch.no. (2021). Retrieved 28.05.21 from https://www.barentswatch.no/fiskehelse/

Berge, A. (2014). Pionérene: Ove og Sivert Grøntvedt. *Ilaks (15. October 2014).* Retrieved from https://ilaks.no/pionerene-ove-og-sivert-grontvedt/

Bijvelds, M., Velden, J., Kolar, Z., & Flik, G. (1998). Magnesium transport in freshwater teleosts. *Journal of Experimental Biology*, 201, 1981–1990. doi:10.1242/jeb.201.13.1981

Bowers, J. M., Mustafa, A., Speare, D. J., Conboy, G. A., Brimacombe, M., Sims, D. E., & Burka, J. F. (2000). The physiological response of Atlantic salmon, *Salmo salar L*., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. *Journal of Fish Diseases*, 23, 165–172. doi:10.1046/j.1365-2761.2000.00225.x

Brattelid, T., Forsgren, E. , Hansen, T., Aas-Hansen, Ø., Fivelstad, S. Bæverfjord, G. … Stenevik, I. H. (2009). *Fisk i forskning – miljøkrav og velferdsindikatorer hos fisk* (ISBN 978-82-12-02726-8, pp. 106). Oslo: Norges Forskningsråd.

Bricknell, I. R., Dalesman, S. J., O'Shea, B., Pert, C. C., & Luntz, A. J. M. (2006). Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. *Diseases of Aquatic Organisms,* 71, 201–212. doi:10.3354/dao071201

Burrin, J. M., & Price, C. P. (1985). Measurement of blood glucose. *Annals of Clinical Biochemistry,* 22, 327–342. doi:10.1177/000456328502200401

Chrousos, G. P. (1998). Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 Hans Selye Memorial Lecture. *Annals of the New York Academy of Sciences,* 851, 311-335. doi:10.1111/j.1749-6632.1998.tb09006.x

Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F., & Henze, K. G. (1962). Evidence for Calcitonin—A New Hormone from the Parathyroid That Lowers Blood Calcium. *Endocrinology,* 70, 638–649. doi:10.1210/endo-70-5-638

Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M., Free, C. M., Froehlich, H. E., … Lubchenco, J. (2020). The future of food from the sea. *Nature,* 588, 95–100. doi:10.1038/s41586-020-2616-y

Dalvin, S. (2020). Generell biologi lakselus. *Institute of Marine Research (IMR) (17 December 2018).* Retrieved from https://www.hi.no/hi/temasider/arter/lakselus/generell-biologi

Dalvin, S., Karlsen, Ø., & Samuelsen, O. (2020). Lakselus. *Institute of Marine Research (IMR) (17 December 2018).* Retrieved 23.03.21 from https://www.hi.no/hi/temasider/arter/lakselus

Dawson, L. H. J., Pike, A. W., Houlihan, D. F., & McVicar, A. H. (1999). Changes in physiological parameters and feeding behaviour of Atlantic salmon (*Salmo salar*) infected with sea lice (*Lepeophtheirus salmonis*). *Diseases of Aquatic Organisms*, 35, 89–99. doi:10.3354/dao035089

Directorate of Fisheries. (2010). For stor merd eller for mange fisk? *Fiskeridirektoratet.* Fiskeridirektoratets og Mattilsynets anbefalinger. Retrieved from

https://www.fiskeridir.no/Akvakultur/Dokumenter/Rapporter/For-stor-merd-eller-for-mange-fisk

Directorate of Fisheries. (2021a). Akvakulturstatistikk: matfiskproduksjon av laks, regnbueørret og ørret. *Fiskeridirektoratet.* Retrieved 19.05.21 from https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Laks-regnbueoerret-og-oerret/Matfiskproduksjon

Directorate of Fisheries. (2021b). Rømmingsstatistikk. *Fiskeridirektoratet (fiskeridir.no).* Retrieved 19.05.21 from https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Roemmingsstatistikk

Diserud, O. H., Fiske, P., Sægrov, H., Urdal, K., Aronsen, T., Lo, H., … Hindar, K. (2019). Escaped farmed Atlantic salmon in Norwegian rivers during 1989-2013. *ICES Journal of Marine Science*, 76, 1140–1150. doi:10.1093/icesjms/fsy202

Evans D.H. (1980). *Osmotic and Ionic Regulation by Freshwater and Marine Fishes*. In *Environmental Physiology of Fishes* (pp. 93-122), Ali M.A. (Ed.). Boston: Springer. doi:10.1007/978-1-4899-3659-2_5

Fast, M. D., Hosoya, S., Johnson, S. C., & Afonso, L. O. B. (2008). Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. *Fish and Shellfish Immunology*, 24, 194–204. doi:10.1016/j.fsi.2007.10.009

Finstad, B., Bjørn, P. A., & Nilsen, S. (1995). Survival of salmon lice, *Lepeophtheirus salmonis* Krøyer, on Arctic charr, *Salvelinus alpinus* (L.), in fresh water. *Aquaculture Research*, 26, 791–795. doi:10.1111/j.1365-2109.1995.tb00871.x

Fivelstad, S., Haavik, H., Løvik, G., & Olsen, A. B. (1998). Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.*):* Ion regulation and growth. *Aquaculture*, 160, 305–316. doi:10.1016/S0044-8486(97)00166-X

Flik, G., & Perry, S. F. (1989). Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater rainbow trout. *Journal of Endocrinology*, 120, 75–82. doi:10.1677/joe.0.1200075

Forseth, T., Barlaup, B. T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., ... & Wennevik, V. (2017). The major threats to Atlantic salmon in Norway. *ICES Journal of Marine Science*, 74, 1496-1513.

Gallardo-Escárate, C., Valenzuela-Muñoz, V., Núñez-Acuña, G., Carrera, C., Gonçalves, A. T., Valenzuela-Miranda, D., … Roberts, S. (2019). Catching the complexity of salmon-louse interactions. *Fish and Shellfish Immunology*, 90, 199–209. doi:10.1016/j.fsi.2019.04.065

Grefsrud, E. S., Karlsen, Ø., Kvamme, B. O., Glover, K. A., Husa, V., Hansen, P. K., ... & Svåsand, T. (2021). *Risikorapport norsk fiskeoppdrett 2021 - Kunnskapsstatus effekter av norsk fiskeoppdrett* (Report nr. 2021-7). Norway: Institute of Marine Research. Retrieved from: https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2021-7

Grimnes, A., & Jakobsen, P. J. (1996). The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology*, 48, 1179-1194. doi:10.1111/j.1095-8649.1996.tb01813.x

Hamre, L. A., Eichner, C., Caipang, C. M. A., Dalvin, S. T., Bron, J. E., Nilsen, F., … Skern-Mauritzen, R. (2013). The Salmon Louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) Life Cycle Has Only Two Chalimus Stages. *PLoS ONE*, 8, e73539. doi:10.1371/journal.pone.0073539

Handeland, S. O., Järvi, T., Fernö, A., & Stefansson, S. O. (1996). Osmotic stress, antipredatory behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 2673-2680. doi:10.1139/cjfas-53-12-2673

Handeland, S. O., & Stefansson, S. O. (2001). Photoperiod control and influence of body size on off-season parr-smolt transformation and post-smolt growth. *Aquaculture*, 192, 291–307. doi:10.1016/S0044-8486(00)00457-9

Hatløy, T. (2015). *Effekten av akutt allostatisk belastning på hypothalamus-hypofyse-interrenal aksen, og dets betydning på dyrevelferden hos diploid og triploid atlantisk laksesmolt (Salmo salar L.)* (Master thesis). University of Nordland. Retrieved from https://nordopen.nord.no/nord-xmlui/handle/11250/2353116

Haugen, T. S. (2020). *The effect of winter-signal photoperiod manipulations on smoltification of Atlantic salmon (Salmo salar) reared in commercial Recirculating Aquaculture Systems (RAS)* (Master thesis). University of Bergen.

Heuch, P. A., Nordhagen, J. R., & Schram, T. A. (2000). Egg production in the salmon louse (*Lepeophtheirus salmonis* (Kroyer)) in relation to origin and water temperature. *Aquaculture Research*, 31, 805–814. doi:10.1046/j.1365-2109.2000.00512.x

Heuch, P. A., Olsen, R. S., Malkenes, R., Revie, C. W., Gettinby, G., Baillie, M., … Finstad, B. (2009). Temporal and spatial variations in lice numbers on salmon farms in the Hardanger fjord 2004-06. *Journal of Fish Diseases*, 32, 89–100. doi:10.1111/j.1365-2761.2008.01002.x

Heuch, Peter Andreas, & Karlsen, H. E. (1997). Detection of infrasonic water oscillations by copepodids of *Lepeophtheirus salmonis* (Copepoda: Caligida). *Journal of Plankton Research*, 19, 735–747. https://academic.oup.com/plankt/article/19/6/735/1521406

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E. & Elvegård, T.A. (2014). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 425-426, 18-23. doi:10.1016/j.aquaculture.2013.12.033.

Imsland, A.K., Hanssen, A., Reynolds, P., Nytrø, A.V., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C. & Mikalsen, B. (2018). It works! Lumpfish can significantly lower sea lice infections in large scale salmon farming. *Biology Open*, 7, 9. bio036301. doi:10.1242/bio.036301

Iversen, A., Hermansen, Ø., Nystøyl, R., & Hess, E. J. (2017). *Kostnadsutvikling i lakseoppdrett–med fokus på fôr-og lusekostnader* (Report nr. 24/2017). Tromsø: Nofima. Retrieved from https://nofima.no/publikasjon/1523319/

Iversen, M., & Eliassen, R. A. (2009). The effect of AQUI-S® sedation on primary, secondary, and tertiary stress responses during salmon smolt, *Salmo salar* L., transport and transfer to sea. *Journal of the World Aquaculture Society*, 40, 216–225. doi:10.1111/j.1749-7345.2009.00244.x

Iversen, M., Finstad, B., McKinley, R. S., & Eliassen, R. A. (2003). The efficacy of metomidate, clove oil, Aqui-STM and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. *Aquaculture*, 221, 549–566. doi:10.1016/S0044-8486(03)00111-X

Iversen, M. H., & Eliassen, R. A. (2014). The effect of allostatic load on hypothalamic-pituitary-interrenal (HPI) axis before and after secondary vaccination in Atlantic salmon postsmolts (*Salmo salar* L.). *Fish Physiology and Biochemistry*, 40, 527–538. doi:10.1007/s10695-013-9863-x

Iwama, G. (2006). Stress in fish. *Annals of the New York Academy of Sciences*, 851, 304–310. doi:10.1111/j.1749-6632.1998.tb09005.x

Johannessen, A. (1975). *Lakselus, Lepeophtheirus salmonis Krøyer (Copepoda caligidae). Frittlevende larvestadier, vekst og infeksjon på laks (Salmo salar L.) fra oppdrettsanlegg og kommersielle fangster i vestnorske farvann 1973-1974* (Master Thesis, University of Bergen). Retrieved from https://imr.brage.unit.no/imr-xmlui/handle/11250/2394233

Johnson, S. C., & Albright, L. J. (1991). Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom*, 71, 425–436. doi:10.1017/S0025315400051687

Jones, M. W., Sommerville, C., & Wootten, R. (1992). Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. *Journal of Fish Diseases*, 15, 197–202. doi:10.1111/j.1365-2761.1992.tb00654.x

Kalmijn, A.J. (1988) Hydrodynamic and Acoustic Field Detection. In Atema J., Fay R.R., Popper A.N., Tavolga W.N. (Eds), *Sensory Biology of Aquatic Animals* (pp. 83–130). New York: Springer. doi:10.1007/978-1-4612-3714-3_4

Kalmijn, A.J. (1989) Functional Evolution of Lateral Line and Inner Ear Sensory Systems. In *The Mechanosensory Lateral Line* (pp. 187–215), Coombs, S., Görner, P., Münz, H. (Eds). New York: Springer. doi:10.1007/978-1-4612-3560-6_9

Komisarczuk, A. Z., Grotmol, S., & Nilsen, F. (2017). Ionotropic receptors signal host recognition in the salmon louse (*Lepeophtheirus salmonis*, copepoda). *Plos One*, 12, e0178812. doi:10.1371/journal.pone.0178812

Kragesteen, T. J., Simonsen, K., Visser, A. W., & Andersen, K. H. (2019). Optimal salmon lice treatment threshold and tragedy of the commons in salmon farm networks. *Aquaculture*, 512, 734329. doi:10.1016/j.aquaculture.2019.734329

Kristoffersen, A. B., Jimenez, D., Viljugrein, H., Grøntvedt, R., Stien, A., & Jansen, P. A. (2014). Large scale modelling of salmon lice (*Lepeophtheirus salmonis*) infection pressure based on lice monitoring data from Norwegian salmonid farms. *Epidemics*, 9, 31–39. doi:10.1016/j.epidem.2014.09.007

Kvadsheim, P. H., Sivle, L. D., Hansen, R. R., & Karlsen, H. E. (2017). *Effekter av menneskeskapt støy på havmiljø* (Report nr. 17/00075). Kjeller: Norwegian Defence Research Establishment (FFI). Retrieved from https://publications.ffi.no/nb/item/asset/dspace:2657/17-00075.pdf

Liebert, A. M., & Schreck, C. B. (2006). Effects of acute stress on osmoregulation, feed intake, IGF-1, and cortisol in yearling steelhead trout (*Oncorhynchus mykiss*) during seawater

adaptation. *General and Comparative Endocrinology*, 148, 195–202. doi:10.1016/j.ygcen.2006.03.002

Mackinnon, B. M. (1998). Host factors important in sea lice infections. *ICES Journal of Marine Science*, 55, 188–192. doi:10.1006/jmsc.1997.0361

Madaro, A., Olsen, R. E., Kristiansen, T. S., Ebbesson, L. O. E., Nilsen, T. O., Flik, G., & Gorissen, M. (2015). Stress in Atlantic salmon: Response to unpredictable chronic stress. *Journal of Experimental Biology*, 218, 2538–2550. doi:10.1242/jeb.120535

Mattilsynet. (2021). Lakselus. Mattilsynet (29 January 2018). Retrieved 10.05.21 from https://www.mattilsynet.no/fisk_og_akvakultur/fiskehelse/fiske_og_skjellsykdommer/lakselus/

McDonald, G., & Milligan, L. (1997). Ionic, osmotic and acid-base regulation in stress. In *Stress and Health in Aquaculture* (pp. 119–145), Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B. (Eds.). Cambridge: Cambridge University Press.

McGinnity, P., Stone, C., Taggart, J. B., Cooke, D., Cotter, D., Hynes, R., … Ferguson, A. (1997). Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES Journal of Marine Science*, 54, 998–1008. doi:10.1016/S1054-3139(97)80004-5

McLean, P. H., Smith, G. W., & Wilson, M. J. (1990). Residence time of the sea louse, *Lepeophtheirus salmonis* K., on Atlantic salmon, *Salmo salar* L., after immersion in fresh water. *Journal of Fish Biology*, 37, 311–314. doi:10.1111/j.1095-8649.1990.tb05861.x

Mergenthaler, P., Lindauer, U., Dienel, G. A., & Meisel, A. (2013). Sugar for the brain: The role of glucose in physiological and pathological brain function. *Trends in Neurosciences*, 36, 587–597. doi:10.1016/j.tins.2013.07.001

Milligan, C. L., & Girard, S. S. (1993). Lactate metabolism in rainbow trout. *Journal of Experimental Biology*, 180, 175–193. doi:10.1242/jeb.180.1.175

Mommsen, T. P., Vijayan, M. M., & Moon, T. W. (1999). Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9, 211–268. doi:10.1023/A:1008924418720

Murray, A. G. (2013). Epidemiology of the spread of viral diseases under aquaculture. *Current Opinion in Virology*, 3, 74–78. doi:10.1016/j.coviro.2012.11.002

Mustafa, A., MacWilliams, C., Fernandez, N., Matchett, K., Conboy, G. A., & Burka, J. F. (2000). Effects of sea lice (*Lepeophtheirus salmonis*) infestation non-specific defence mechanisms in Atlantic salmon (*Salmo salar* L.). *Fish and Shellfish Immunology*, 10, 47–59. doi:10.1006/fsim.1999.0229

Myksvoll, M. S., Sandvik, A. D., Albretsen, J., Asplin, L., Johnsen, I. A., Karlsen, Ø., … Ådlandsvik, B. (2018). Evaluation of a national operational salmon lice monitoring system—From physics to fish. *PLoS ONE*, 13, e0201338. doi:10.1371/journal.pone.0201338

Latremouille, D. N. (2003). Fin erosion in aquaculture and natural environments. *Reviews in Fisheries Science*, 11, 315-335. doi: 10.1080/10641260390255745

Olafsen, T., Winther, U., Olsen, Y., & Skjermo, J. (2012). *Verdiskaping basert på produktive hav i 2050*. Trondheim: Det Kongelige Norske Videnskabers Selskab (DKNVS) og Norges Tekniske Vitenskapsakademi (NTVA). Retrieved from https://www.regjeringen.no/no/dokumenter/verdiskaping-basert-pa-produktive-hav-i-/id697596/

Olsen, R. E., Sundell, K., Hansen, T., Hemre, G. I., Myklebust, R., Mayhew, T. M., & Ringø, E. (2002). Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. *Fish Physiology and Biochemistry*, 26, 211–221. doi:10.1023/A:1026217719534

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T. S., Gismervik, K., & Stien, L. H. (2019). Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in Aquaculture*, 11, 1398–1417. doi:10.1111/raq.12299

Øvrebø, T. K. (2020). *Growth performance and welfare of post-smolt (Salmo salar L.) reared in semi closed containment systems (S-CCS) – a comparative study* (Master thesis). University of Bergen. Retrieved from https://bora.uib.no/bora-xmlui/handle/1956/22858

Pang, P. K. T., Griffith, R. W., & Pickford, G. E. (1971). Hypocalcemia and Tetanic Seizures in Hypophysectomized Killifish, *Fundulus heteroclitus*. *Proceedings of the Society for Experimental Biology and Medicine*, 136, 85–87. doi:10.3181/00379727-136-35199

Pike, A. W., & Wadsworth, S. L. (1999). Sealice on salmonids: Their biology and control. *Advances in Parasitology*, 44, 233–337. doi:10.1016/s0065-308x(08)60233-x

Produksjonsområdeforskriften. (2017). *Forskrift om produksjonsområde for akvakultur av matfisk i sjø av laks, ørret og regnbueørret* (FOR-2017-01-16-61). Retrieved from https://lovdata.no/dokument/SF/forskrift/2017-01-16-61

Randall, D. J., & Ferry, S. F. (1992). 4 Catecholamines. In *Fish Physiology* (pp. 255-300), Hoar, W.S., Randall, D.J., Farrell, A.P. (Eds.). Academic press. doi:10.1016/S1546-5098(08)60011-4.

Redding, J. M., & Schreck, C. B. (1983). Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. *Transactions of the American Fisheries Society*, 112, 800-807. doi:10.1577/1548-8659(1983)112<800:IOASOO>2.0.CO;2

Rodger, H. D. (2014). Amoebic gill disease (AGD) in farmed salmon (*Salmo salar*) in Europe. *Fish Veterinary Journal*, 14, 16-27.

Saligaut, C., Linard, B., Breton, B., Anglade, I., Bailhache, T., Kah, O., & Jego, P. (1999). Brain aminergic systems in salmonids and other teleosts in relation to steroid feedback and gonadotropin release. *Aquaculture*, 177, 13–20. doi:10.1016/S0044-8486(99)00065-4

Schreck, C. B. (2010). Stress and fish reproduction: The roles of allostasis and hormesis. *General and Comparative Endocrinology*, 165, 549–556. doi:10.1016/j.ygcen.2009.07.004

Schreck, C. B., & Tort, L. (2016). The Concept of Stress in Fish. In Schreck, C. B., Tort, L., Farrell, A.P., Brauner, C. J. (Eds.), *Fish Physiology* (pp. 1-34). Academic press. doi:10.1016/B978-0-12-802728-8.00001-1

Selye, H. (1950). Stress and the general adaptation syndrome. *British Medical Journal*, 1, 1383–1392. doi:10.1136/bmj.1.4667.1383

Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S. & Browman, H.I., 2013. Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). *Aquaculture*, 402-403, 113-118. doi:10.1016/j.aquaculture.2013.03.032

Skilbrei, O. T. (2013). Migratory behaviour and ocean survival of escaped out-of-season smolts of farmed Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions*, 3, 213–221. doi:10.3354/aei00062

Skjervold, P. O., Fjæra, S. O., Østby, P. B., & Einen, O. (2001). Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture*, 192, 265–280. doi:10.1016/S0044-8486(00)00447-6

The Sea Lice Research Centre (2020). The Atlantic Salmon Louse. Retrieved 20.11.20 from https://slrc.w.uib.no/about-sea-lice/the-atlantic-salmon-louse/

Smith, M. E., Kane, A. S., & Popper, A. N. (2004). Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). *Journal of Experimental Biology*, 207, 427-435. doi:10.1242/jeb.00755

Soengas, J. L., Polakof, S., Chen, X., Sangiao-Alvarellos, S., & Moon, T. W. (2006). Glucokinase and hexokinase expression and activities in rainbow trout tissues: Changes with

food deprivation and refeeding. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 291, R810-R821. doi:10.1152/ajpregu.00115.2006

Statistics Norway, (2020). Akvakultur - årlig, foreløpige tall (Table 1/07326). Retrieved 10.11.20 from https://www.ssb.no/fiskeoppdrett

Staurnes, M., Rainuzzo, J. R., Sigholt, T., & Jøgensen, L. (1994). Acclimation of Atlantic cod (*Gadus morhua*) to cold water: Stress response, osmoregulation, gill lipid composition and gill Na-K-ATPase activity. *Comparative Biochemistry and Physiology - Part A: Physiology*, 109, 413–421. doi:10.1016/0300-9629(94)90145-7

Sterling, P. (2012). Allostasis: A model of predictive regulation. *Physiology and Behavior*, 106, 5–15. doi:10.1016/j.physbeh.2011.06.004

Stewart, H. A., Noakes, D. L. G., Cogliati, K. M., Peterson, J. T., Iversen, M. H., & Schreck, C. B. (2016). Salinity effects on plasma ion levels, cortisol, and osmolality in Chinook salmon following lethal sampling. *Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology*, 192, 38–43. doi:10.1016/j.cbpa.2015.11.011

Stien, A., Bjørn, P. A., Heuch, P. A., & Elston, D. A. (2005). Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. *Marine Ecology Progress Series*, 290, 263-275. doi:10.3354/meps290263

Stien, L. H., Bracke, M. B. M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., … Kristiansen, T. S. (2013). Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Reviews in Aquaculture*, 5, 33–57. doi:10.1111/j.1753-5131.2012.01083.x

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., … Takle, H. (2016). Impact of fish density and specific water flow on skin properties in Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture*, 464, 629–637. doi:10.1016/j.aquaculture.2016.08.012

Taranger, G. L., Karlsen, Ø., Bannister, R. J., Glover, K. A., Husa, V., Karlsbakk, E., … Svåsand, T. (2015). Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. *ICES Journal of Marine Science*, 72, 997–1021. doi:10.1093/icesjms/fsu132

The Veterinary Institute. (2009). Veileder for nasjonal bekjempelse av lakselus. Retrieved from https://sjomatnorge.no/wp-content/uploads/importedmedia/Lakselusveilederen_web_231109.pdf

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., … Jackson, D. (2013). Salmon lice - impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases*, 36, 171–194. doi:10.1111/jfd.12061

Tort, L. (2011). Stress and immune modulation in fish. *Developmental and Comparative Immunology*, 35, 1366–1375. doi:10.1016/j.dci.2011.07.002

Tsatsakis, A. M., Nawaz, M. A., Tutelyan, V. A., Golokhvast, K. S., Kalantzi, O.-I., Chung, D. H., … Chung, G. (2017). Impact on environment, ecosystem, diversity and health from culturing and using GMOs as feed and food. *Food and Chemical Toxicology*, 107, 108–121. doi:10.1016/J.FCT.2017.06.033

Tully, O. (1989). The succession of generations and growth of the caligid copepods *Caligus elongatus* and *Lepeophtheirus salmonis* parasitising farmed Atlantic salmon smolts (*Salmo salar* l.). *Journal of the Marine Biological Association of the United Kingdom*, 69, 279–287. doi:10.1017/S0025315400029404

Tully, O. (1992). Predicting infestation parameters and impacts of caligid copepods in wild and cultured fish populations. *Invertebrate Reproduction and Development*, 22, 91–102. doi:10.1080/07924259.1992.9672261

Vollset, K. W., Dohoo, I., Karlsen, Ø., Halttunen, E., Kvamme, B. O., Finstad, B., … Barlaup, B. T. (2017). Disentangling the role of sea lice on the marine survival of Atlantic salmon. *ICES Journal of Marine Science*, 75, 50–60. doi:10.1093/icesjms/fsx104

Wedemeyer, G.A., (1996). Physiology of Fish in Intensive Culture Systems. Chapman & Hall, ITP, New York, p. 232.

Wendelaar Bonga, S. E. (1997). The Stress Response in Fish. *Physiological Reviews*, 77, 591–625. doi:10.1152/physrev.1997.77.3.591

Wendelaar Bonga, S. E. (2011). Hormonal Responses to Stress. *Encyclopedia of Fish Physiology*, 2, 1515–1523.

Ytrestøyl, T., Aas, T. S., & Åsgård, T. (2015). Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture*, 448, 365–374. doi:10.1016/J.AQUACULTURE.2015.06.023

Appendix I

Appendix I includes a descriptive field observation. The aim of this observation is to reveal the effects of long-time stress with use of AcuLice treatment.

Field observation – Long-time stress effects with AcuLice treatment

Fish Material and Rearing Conditions

The fish (n = 120) used in this felt study was Atlantic salmon. Group 1 includes fish at Breivik S (n = 60) originally comes from the Salmobreed stain and was reared from hatching to smolt is at the flow-through facility drifted by Sævareid Fiskeanlegg AS. Fish in facility Ihlholmane (n = 60, Group 2) was reared from hatching to smolt at a flow-through facility drifted by Alsaker Fjordbruk AS. All the fish were a part of the commercial production lines at the hatcheries Sævareid Fiskeanlegg AS and Alsaker Fjordbruk AS. The fish had a weight from 200 to 350 g when transferred to sea.

Field study Facilities

The field study took place at the full-scale facility Breivik S (59.671 °N, 5.312 °E, location number 11574) drifted by Kobbevik & Furuholmen Oppdrett AS and Ihlholmen (59.721 °N, 5.586 °E, location number 27095) drifted by Fjeldberg Fjordbruk AS, Nordsjø Fjordbruk AS, Sunnhordaland fjordbruk AS, Tysnes Fjordbruk AS (Figure 26, Appendix I). The facilities had the AcuLice treatment during the entire production period.

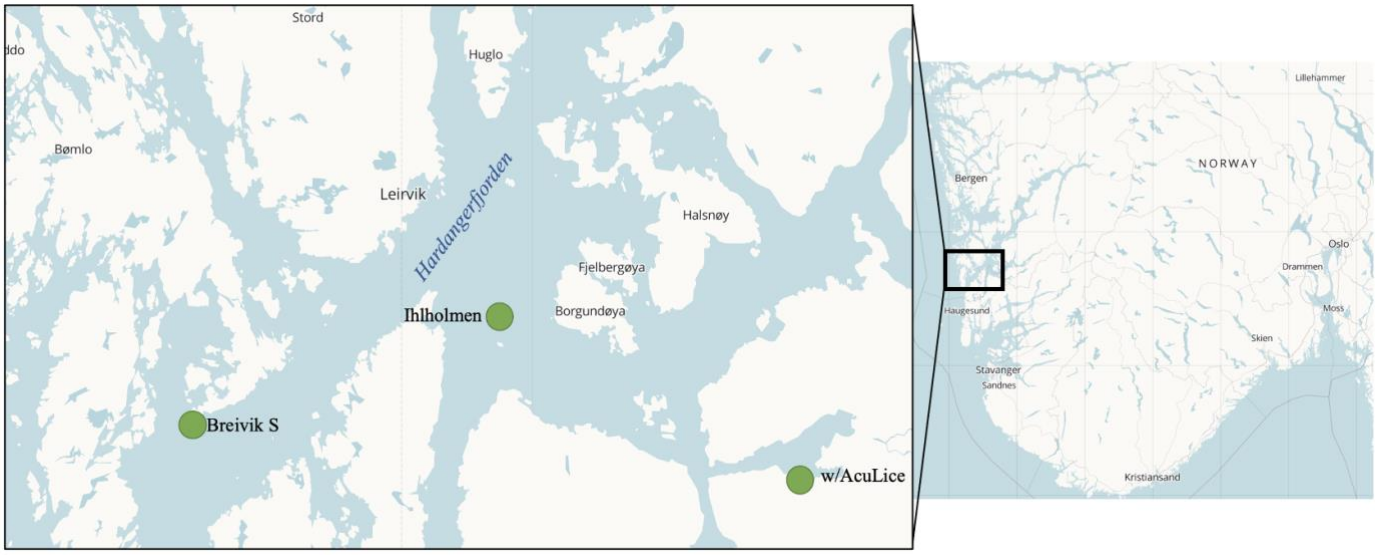

Figure 26. Location of the experimental facilities Breivik S and Ihlholmen marked in green.

Both the facilities produce Atlantic salmon for commercial consumption following a standard protocol. The daily and weekly husbandry was conducted by the facility' own employees.

Sampling Protocol

The samplings took place 24 July 2019 (Breivik S and Ihlholmen) and 23 – 24 January 2020 (Ihlholmen – Breivik S). A total of 120 fish were a part of the samplings. In each sampling there was 3 subsets (n = 10) and all fish were selected randomly from three different sea pens at the two facilities (Breivik and Ihlholmen. Tables 5-6, Appendix I).

Table 5. Overview of the samplings and subsets for the long-time effects experiment at Breivik S on 24 July 2019 and 24 January 2020.

Sampling number	Date	Subset	Cage number	Treatment	Fish N
1	24.07.2019	1	1	AcuLice	10
		2	2	AcuLice	10
		3	4	AcuLice	10
2	24.01.2020	4	1	AcuLice	10
		5	2	AcuLice	10
		6	4	AcuLice	10

Table 6. Overview of the samplings and subsets for the long-time effects experiment at Ihlholmen on 24 July 2019 and 23 January 2020.

Sampling number	Date	Subset	Cage number	Treatment	Fish N
1	24.07.2019	1	1	AcuLice	10
		2	3	AcuLice	10
		3	4	AcuLice	10
2	23.01.2020	4	1	AcuLice	10
		5	3	AcuLice	10
		6	4	AcuLice	10

The blood samplings and analyses did follow the same protocol as described in Material and Method in Exp1. In addition, the fish was examined for external morphology (SWIM, (L. H. Stien et al., 2013)). Swim 1.0 includes welfare indicators for individual fish evaluation: fin condition (1 – 4), skin status (1 – 7), vertebral deformation state (1 – 3), gill status (1 – 5), opercula (1 – 3), mouth jaw wound (1 – 3), upper jaw deformation (1 – 3), lower jaw deformation (1 – 3), condition factor state (1 – 3), emaciation state (1 – 3), smoltification stage (1 – 6) and sexual maturity stage (1 – 4). For each of the observed welfare indicators, an integer score of between 1 (best) and N (worst, N depends on the number of levels for the welfare indicator) is given. To calculate condition factor the formula: $CF = 100 * \frac{W}{L^3}$, where W is individual weight in grams, and L is the corresponding length in centimetres was used.

Table 7. Welfare indicators according to the Salmon Welfare Index Model (SWIM 1.0) for evaluate the individual fish welfare status (Stien et al., 2013). The SWIM gives a mean number of the individual welfare indicators grounded on the assumed impact on the individual fish. The welfare indicators sexual maturity status and smoltification status are not included in the table due to the non-relevance in this felt study.

Welfare indicators	Score	Description
Fin condition	1	Normal healthy fins, nothing to comment
	2	Scar tissue or slight necrosis
	3	Moderate current skin damage to the fins
	4	Severe current skin damage to the fins
Skin condition	1	Normal healthy skin, nothing to comment
	2	Scar tissue, healed
	3	Scale loss (dislocated or missing scales)
	4	Superficial wound or ulcer <1 cm2
	5	Superficial wound or ulcer >1 cm2
	6	Multiple wounds or ulcers
	7	Large open wounds or ulcers, life threatening
Vertebral deformation	1	Signs of deformed spine
	2	Clearly visible spinal deformity
	3	Extreme deformity
Gill status	1	Very light: one withe spot
	2	Light: 2-3 spots/small mucus patch
	3	Moderate: Established thickened mucus patch (up to 20% of gill area)
	4	Advanced: Established lesions covering up to 50% of gill area
	5	Heavy: Extensive lesions covering most of the gill surface (50%+)
Opercula	1	Opercula only partly covering gills
	2	Opercula absent on one of the fills (Gills exposed)
	3	Both opercula absent (Both gills exposed)
Mouth jaw wound	1	Minor wound on jaw
	2	Moderate wound
	3	Large deep and extensive wound
Upper jaw deformation	1	Suspected malformation
	2	Distinct malformation
	3	Major malformation (jaw pointing backwards)
Lower jaw deformation	1	Suspected malformation
	2	Distinct malformation
	3	Major malformation (jaw pointing backwards)
Condition factor	1	Fat: 1.0 – 1.5
	2	Intermediate: 0.9 – 1.0
	3	Lean: < 0.9
Emaciation state	1	Not emaciated, healthy looking
	2	Potentially emaciated
	3	Distinctly emaciated, clearly diseased, and moribund

Results

Results from physiological welfare surveillance

In the field observation conducted at Breivik S. and Ihlholmen the plasma cortisol, - glucose, - lactic acid, - chloride, - calcium and - magnesium were analysed to find out long-term physiological effects of AcuLice treatment. There were not shown any difference in the plasma cortisol concentrations at both facilities between the two samplings. At Breivik S a decrease in glucose, lactic acid, chloride, calcium and magnesium was observed between sampling 1 (24 July 2019) and sampling 2 (24 January 2020). A decrease in the plasma lactic acid, calcium and magnesium was also shown at the facility Ihlholmen, between the two samplings. No difference was shown in the plasma glucose or chloride concentration at Ihlholmen between the samplings. See Table 8, Appendix I (Statistics in Appendix IV).

Table 8. Ion concentrations, glucose, lactic acid and cortisol in Atlantic salmon exposed to AcuLice at Breivik S and Ihlholmen measured 24th July 2019 and 23-24th January 2020. n=30 for all measured variables. Asterisk indicates the degree of significance ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between the samplings, NS indicates no-significant between the groups.*

	Breivik S			Ihlholmen		
	Sampling 1 24.07.2019	Sampling 2 24.01.2020	*Significance*	Sampling 1 24.07.2019	Sampling 2 23.01.2020	*Significance*
Cortisol	9.48	9.15	*NS*	7.62	11.14	*NS*
Glucose	5.27	4.52	** *($p \approx 0.005$)*	4.96	4.71	*NS*
Lactic Acid	5.35	3.78	*** *($p < 0.001$)*	6.25	4.27	*** *($p < 0.001$)*
Chloride	128.52	124.60	* *($p \approx 0.029$)*	120.21	124.74	*NS*
Calcium	3.48	2.51	*** *($p < 0.001$)*	3.26	2.52	*** *($p < 0.001$)*
Magnesium	1.25	0.96	*** *($p < 0.001$)*	1.44	0.90	*** *($p < 0.001$)*

Results for morphological welfare surveillance

Growth

In both facilities a significant ($p < 0.001$) were observed for the increase in length and weight from sampling 1 to sampling 2. At Breivik the fish had increase in average weight and length from 773.4 g and 39.0 cm (Sampling 1) to 2366.3 g and 56.7 cm (Sampling 2). The mean weight and length at Ihlholmen did increase from 1204.5 g and 45.0 cm (Sampling 1) to 3471.8 g and 64.1 cm (Sampling 2) (Table 9, Appendix I). The differences in condition factor were not significant for the two facilities.

Salmon Welfare Index Model (SWIM)

For the SWIM status at the two facilities both did have a significant difference ($p < 0.05$) in fin condition, skin state and mouth jaw wound status between the two samplings. See Table 9, Appendix I (Statistics in Appendix IV).

Table 9. Morphological welfare surveillance for Atlantic salmon exposed to AcuLice at Breivik S and Ihlholmen measured 24th July 2019 and 23/24th January 2020. Asterisk indicates the degree of significance (p < 0.05, ** p < 0.01, *** p < 0.001), NS indicates no-significant differences between the groups.*

	Breivik S			Ihlholmen		
	Sampling 1 24.07.2019	Sampling 2 24.01.2020	*Significance*	Sampling 1 24.07.2019	Sampling 2 24.01.2020	*Significance*
Length (cm)	39.0	56.7	*** ($p < 0.001$)	45.0	64.1	*** ($p < 0.001$)
Weight (g)	773.4	2366.3	*** ($p < 0.001$)	1204.5	3471.8	*** ($p < 0.001$)
Condition factor (g cm^{-3})	1.3	1.3	*NS*	1.3	1.3	*NS*
Emaciation	1.0	1	*NS*	1.0	1.0	*NS*
Vertebral deformation	1.0	1	*NS*	1.0	1.0	*NS*
Sexual mature	1.0	1.0	*NS*	1.0	1.0	*NS*
Smoltification state	1.0	1.0	*NS*	1.0	1.0	*NS*
Fin condition	3.6	3.8	* ($p = 0.02$)	3.4	3.8	*** ($p < 0.001$)
Skin state	3.3	3.6	* ($p = 0.03$)	3.2	3.5	*** ($p < 0.001$)
Eye status	1.3	1.4	*NS*	1.8	1.7	*NS*
Gill status	1.0	1.0	*NS*	1.0	1.0	*NS*
Opercula	1.1	1.1	*NS*	1.2	1.1	*NS*
Mouth jaw wound	1.0	1.7	*** ($p < 0.001$)	1.1	1.9	*** ($p < 0.001$)
Upper jaw deformation	1.0	1.0	*NS*	1.0	1.0	*NS*
Lower jaw deformation	1.0	1.0	*NS*	1.0	1.0	*NS*

Conclusion

Based on the field observation significant changes in some of the measured parameters, including expected weight and length changes, was shown. When it comes to physiological welfare monitoring, some of the parameters had a decreased plasma concentration between 24 July 2019 and 23/24 January 2020. For a stressed salmon in SW there would be an increase in plasmatic ion concentration (McDonald & Milligan, 1997; Wendelaar Bonga, 1997), in addition to increased levels of cortisol, glucose and lactic acid. In the field observation there was shown a decrease between the two samplings. However, the concentration levels in both samplings at both facilities are in the normal range for each parameter in an unstressed SW teleost (Evans, 1979; Wendelaar Bonga, 1997; Bijvelds et al., 1998; Fivestad et al., 1998; Skjervold et al., 2001; Iversen et al., 2003; Liebert & Schreck, 2006; Fast et al., 2008; Hatløy,

2015). For the morphological welfare factors, there is an increase in three parameters in both groups (Fin condition, skin status, mouth jaw wound). These differences are typically seen in relation to farming of salmon and aquaculture processes (Latremouille, 2010; Sveen et al., 2016). For the condition factor there was not observed any difference at the two facilities during the treatment period.

The field observation had a lack of controls, and it is thus difficult to be able to compare the results and get a concrete conclusion. In addition, it was not possible to have control over all external factors that may affect the result in this field observation. Nevertheless, there is no indications that the fish that participated in this field observation had a tertiary stress response as a result of AcuLice treatment.

Appendix II

Appendix II includes statistical analyses and other results that were not included for Experiment 2. Significant differences are presented in bold font.

Experiment 1 – Acute stress effects of AcuLice treatments

Plasma Cortisol Concentration

Table 10. Cortisol plasma concentration: Two-way nested ANOVA on cortisol concentration between control sampling and one hour with AcuLice treatment at Brattavika.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	58120.75	1	58120.75	334.3967	0.000040
Sampling	474.38	1	474.38	2.7293	0.171345
Subsampling(Sampling)	689.20	4	172.30	0.5130	0.726466
Error	16458.76	49	335.89		

Table 11. Mean cortisol plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	29.719	2.736
AcuLice	35.498	4.210

Plasma Glucose Concentration

Table 12. Glucose plasma concentration: Two-way nested ANOVA on glucose concentration between control sampling and one hour with AcuLice treatment at Brattavika. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1961.662	1	1961.662	9267.633	0.000000
Sampling	1.959	1	1.959	9.253	**0.036581**
Subsampling(Sampling)	0.837	4	0.209	0.337	0.851583
Error	31.015	50	0.620		

Table 13. Mean glucose plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	5.751	0.144
AcuLice	6.133	0.146

Plasma Lactic acid Concentration

Table 14. Lactic acid plasma concentration: Two-way nested ANOVA on lactic acid concentration between control sampling and one hour with AcuLice treatment at Brattavika.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	418.0648	1	418.0648	409.7548	0.000035
Sampling	0.0034	1	0.0034	0.0033	0.956652
Subsampling(Sampling)	4.0846	4	1.0211	1.8334	0.136425
Error	28.9615	52	0.5570		

Table 15. Mean lactic acid plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	2.696	0.126
AcuLice	2.681	0.160

Plasma Chloride Concentration

Table 16. Chloride plasma concentration: Two-way nested ANOVA on chloride concentration between control sampling and one hour with AcuLice treatment at Brattavika.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	930983.0	1	930983.0	32585.92	0.000000
Sampling	17.3	1	17.3	0.60	0.480302
Subsampling(Sampling)	114.3	4	28.6	0.90	0.472717
Error	1656.5	52	31.9		

Table 17. Mean chloride plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	127.397	0.996
AcuLice	126.275	1.096

Plasma Calcium Concentration

Table 18. Calcium plasma concentration: Two-way nested ANOVA on calcium concentration between control sampling and one hour with AcuLice treatment at Brattavika. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	406.5888	1	406.5888	32009.70	0.000000
Sampling	0.0017	1	0.0017	0.14	0.731533
Subsampling(Sampling)	0.0509	4	0.0127	2.63	**0.044934**
Error	0.2468	51	0.0048		

Table 19. Mean calcium plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	2.669	0.013
AcuLice	2.680	0.015

Plasma Magnesium Concentration

Table 20. Magnesium plasma concentration: Two-way nested ANOVA on magnesium concentration between control sampling and one hour with AcuLice treatment at Brattavika.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	44.14700	1	44.14700	1559.793	0.000000
Sampling	0.02507	1	0.02507	0.886	0.351007
Subsampling(Sampling)	0.11950	4	0.02987	1.056	0.387875
Error	1.47176	52	0.02830		

Table 21. Mean magnesium plasma concentration (mmol/L) and SEM for each sampling group (Control and AcuLice).

Sampling	Mean (mmol/L)	± Str. Error (mmol/L)
Control	0.894	0.040
AcuLice	0.853	0.016

Appendix III

Appendix III includes statistical analyses and other results that were not included for Experiment 1. Significant differences are presented in bold font.

Experiment 2 – Effect of AcuLice treatments in field

Specific growth rate (SGR)

Table 22. Specific growth rate (SGR): Two-way nested ANOVA on specific growth rate (SGR) in the AcuLice group compared to the reference group in the period from week 30, 2019 to week 20, 2020. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	6.532797	1	6.532797	1256.753	0.000000
Facility (Treatment type)	0.191768	6	0.031961	6.149	**0.000301**
Treatment type	0.005022	1	0.005022	0.966	0.333760
Error	0.150747	29	0.005198		

Table 23. Specific growth rate (SGR): Mean and standard error of mean (SEM) for analyzed parameters at each sampling point in the period from week 30, 2019 to week 20, 2020.

	Mean	Std. Error	-Str. Error	+Std. Error
AcuLice	0.4491	0.0181	0.4310	0.4672
Reference	0.4321	0.0344	0.3977	0.4664

Effect on salmon lice dynamics

Small salmon lice

Table 24. Small salmon lice: Two-way nested ANOVA on small salmon lice in the AcuLice group compared with the Reference group in the period from week 30, 2019 to week 20, 2020. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7.138095	1	7.138095	466.7777	0.000000
Facility (Treatment type)	2.474763	7	0.353538	23.1187	**< 0.001**
Treatment type	2.609569	1	2.609569	170.6462	**< 0.001**
Error	0.519937	34	0.015292		

Table 25. Mean number of lice in the groups: Small salmon lice. Mean and standard error of mean (SEM) for analyzed parameters at each sampling point.

	Mean	Std. Error	-Str. Error	+Std. Error
AcuLice	0.6749	0.0667	0.6082	0.7415
Reference	0.1381	0.0197	0.1184	0.1578

Mature female salmon lice

Table 26. Mature female salmon lice: Two-way nested ANOVA on mature female salmon lice in the AcuLice group compared with the Reference group in the period from week 30, 2019 to week 20, 2020. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4.482108	1	4.482108	719.2157	0.000000
Facility (Treatment type)	0.199998	7	0.028571	4.5846	**0.001078**
Treatment type	0.383489	1	0.383489	61.5361	**< 0.001**
Error	0.211886	34	0.006232		

Table 27. Mean number of lice in the groups Mature female salmon lice. Mean and standard error of mean (SEM) for analyzed parameters at each sampling point

	Mean	Std. Error	-Str. Error	+Std. Error
AcuLice	0.2424	0.0206	0.2218	0.2630
Reference	0.4339	0.0223	0.4116	0.4561

Large salmon lice

Table 28. Large salmon lice: Two-way nested ANOVA on large salmon lice in the AcuLice group compared with the Reference group in the period from week 30, 2019 to week 20, 2020. Bold font is indicating significant difference.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	27.92038	1	27.92038	727.9448	0.000000
Facility (Treatment type)	1.82550	7	0.26079	6.7993	**0.000045**
Treatment type	4.31404	1	4.31404	112.4764	**< 0.001**
Error	1.30407	34	0.03836		

Table 29. Mean number of lice in the groups: Large salmon lice. Mean and standard error of mean (SEM) for analyzed parameters at each sampling point

	Mean	Std. Error	-Str. Error	+Std. Error
AcuLice	0.5144	0.0386	0.4757	0.5530
Reference	1.1651	0.0477	1.1174	1.2127

Summaries of data

Table 30. Specific growth rate (SGR) and mean number of salmon lice categorized as mature female -, small - and large salmon lice at each production facility in Experiment 2 in the period from week 30, 2019 to week 20, 2020.

	Facility	SGR	Mature female salmon lice	Small salmon lice	Large salmon lice
AcuLice	Breivik S	0.426	0.122	0.401	0.377
	Grimsholmen	0.537	0.278	0.419	0.514
	Hattasteinen	0.535	0.124	0.740	0.267
	Hillersvik	0.490	0.306	1.216	0.856
	Loddetå	0.411	0.290	0.388	0.460
	Svollandsneset	0.319	0.303	0.736	0.612
Reference	Maradalen	0.374	0.386	0.239	0.834
	Mælen	0.481	0.491	0.066	1.341
	Seglberget		0.416	0.126	1.319

Salmon lice treatments during the experimental period (week 30, 2019 to week 20, 2020)

Table 31. Number of salmon lice treatments at each facility in Experiment 2 during the period (from week 30, 2019 to week 20, 2020).

Number of salmon lice treatments during the 42-week period				
	Facilities	Number of cages	Number of salmon lice treatments	Salmon lice treatments per cage
AcuLice	Breivik S.	4	12	3.0
	Grimsholmen	4	24	6.0
	Hattasteinen	4	4	1.0
	Hillersvik	5	15	3.0
	Loddetå	4	9	2.3
	Svollandsneset	5	16	3.2
Reference	Maradalen	5	36	7.2
	Mælen	6	36	6.0
	Seglberget	6	34	5.7
Mean number of treatments in AcuLice group				3.1
Mean number of treatments in the reference group				6.3

The number of salmon lice treatments during the 43-week period (week 30, 2019 to week 20, 2020) was counted on the basis of the data material obtained in Experiment 2. In this connection, calculations were also performed for chemical treatments where no significant difference between AcuLice and the Reference groups were observed (Table 32, Appendix III). However, the number of total delice treatments (salmon lice treatments and chemical) indicated a lower number of delice treatments for the AcuLice facilities compared to the reference group (Table 32, Appendix III).

Table 32. Salmon lice treatments during the period form week 30, 2019 to week 20, 2020: Test results from a student t-test on numbers of salmon lice treatments, chemical salmon lice treatments and total numbers of salmon lice treatments (salmon lice treatments and chemical salmon lice treatments) measured as treatments per cage. Data source: project data collected from each facility. Bold font is indicating significant difference.

	Mean (AcuLice)	Mean (Reference)	t-value	df	p
Salmon lice treatment	3.083333	6.33333	-3.178711	7	**0.015518**
Chemical treatment	0.100000	0.66667	-2.156519	7	0.067956
Total treatment	3.183333	7.00000	-3.058398	7	**0.009881**

Number of weeks to first salmon lice treatment

Table 33. Week number for transfer of the fish into sea cages and when the first salmon lice treatment did occur in 2019, in addition, numbers of weeks util first treatment was calculated for Experiment 2. Data were retrieved from BarentsWatch, and errors were corrected, see the comments.

	Facilities	Transfer to sea	First salmon lice treatment	Weeks until first salmon lice treatment	*Comments*
AcuLice	Breivik S	15	53	38	*Error registration in BW done for week 45 (this was a freshwater treatment as a result of AGD).*
	Grimsholmen	14	36	22	
	Hattasteinen	27	53	26	*Error registration in BW done for week 45 (this was a FW treatment as a result of AGD).*
	Hillersvik		54	36	*4 feed treatments in advance of the first mechanical treatment*
	Loddetå	14	54	40	
	Svollandsneset	12	49	37	
Reference	Maradalen	11	36	25	*2 feed treatments in advance of the first mechanical treatment.*
	Seglberget	14	30	16	
	Mælen	11	31	20	*1 feed treatments in advance of the first mechanical treatment.*

Table 34. Number of weeks before first salmon lice treatment: Test results from a student t-test on duration before first salmon lice treatment (numbers of weeks) in AcuLice group compared with Reference group in the period from Atlantic salmon was transferred to open sea cages to first salmon lice treatment occurred. Data collected from 'barentswatch.no'.

	Mean		t-value	df	p
	AcuLice	Reference			
Weeks until first salmon lice treatment	33.17	20.33	2.73	7	0.029

Appendix IV

Appendix IV includes statistical analyses for the Field observation.

Field observation

Students t-test

Table 35. SWIM (Breivik S): Students t-test on SWIM parameters at the facility Breivik S between the sampling on 24 June and 24 January. Bold font is indicating significant difference.

Variable	Mean 1	Mean 2	t-value	df	p	N1	N2	SD1	SD2	F-ratio	p-variance
Length	**39.02**	**56.67**	**-17.97**	**124**	**<0.001**	**64**	**62**	**5.18**	**5.84**	**1.27**	**0.35**
Weight	**773.44**	**2366.29**	**-15.87**	**124**	**<0.001**	**64**	**62**	**273.25**	**753.64**	**7.61**	**0.00**
Condition factor (CF)	1.25	1.26	-0.44	124	0.66	64	62	0.12	0.21	3.33	0.00
Emaciation	1.03	1	0.98	124	0.33	64	62	0.25	0.00	0.00	1.00
Vertebral deformation	1.00	1		124		64	62	0.00	0.00		
Sexual mature	1.00	1.00		124		64	62	0.00	0.00		
Smoltification state	1.00	1.00		124		64	62	0.00	0.00		
Fin condition	**3.64**	**3.82**	**-2.33**	**124**	**0.02**	**64**	**62**	**0.48**	**0.39**	**1.58**	**0.08**
Skin condition	**3.25**	**3.57**	**-2.23**	**124**	**0.03**	**64**	**62**	**0.44**	**1.03**	**5.62**	**0.00**
Eye status	1.30	1.36	-0.46	124	0.65	64	62	0.61	0.79	1.69	0.04
Gill status	1.03	1.03	-0.03	124	0.98	64	62	0.18	0.18	1.03	0.90
Opercula	1.13	1.05	1.39	124	0.17	64	62	0.38	0.22	3.05	0.00
Mouth jaw wound	**1.00**	**1.71**	**-8.57**	**124**	**<0.001**	**64**	**62**	**0.00**	**0.66**	**0.00**	**1.00**
Upper jaw deformation	1.02	1.00	0.98	124	0.33	64	62	0.13	0.00	0.00	1.00
Lower jaw deformation	1.02	1.02	0.00	124	1.00	64	62	0.20	0.27	1.81	0.00

Table 36. SWIM (Ihlholmen): Students t-test on SWIM parameters at the facility Ihlholmen between the sampling on 24 June and 24 January. Bold font is indicating significant difference.

Variable	Mean 1	Mean 2	t-value	df	p	N1	N2	SD1	SD2	F-ratio	p-variance
Length	**45.00**	**64.12**	**-22.67**	**120**	**< 0.001**	**60**	**62**	**3.87**	**5.31**	**1.88**	**0.02**
Weight	**1204.50**	**3471.77**	**-18.35**	**120**	**< 0.001**	**60**	**62**	**3.24.20**	**902.00**	**7.74**	**0.00**
Condition factor (CF)	1.29	1.28	0.32	120	0.75	60	62	0.10	0.17	2.90	0.00
Emaciation	1.03	1.00	1.45	120	0.15	60	62	0.18	0.00	0.00	1.00
Vertebral deformation	1.02	1.03	-0.55	120	0.58	60	62	0.13	0.18	1.90	0.01
Sexual mature	1.00	1.00		120		60	62	0.00	0.00		
Smoltification state	1.00	1.00		120		60	62	0.00	0.00		
Fin condition	**3.43**	**3.76**	**-3.71**	**120**	**< 0.001**	**60**	**62**	**0.53**	**0.43**	**1.52**	**0.11**
Skin condition	**3.20**	**3.55**	**-3.38**	**120**	**< 0.001**	**60**	**62**	**0.44**	**0.67**	**2.28**	**0.00**
Eye status	1.77	1.68	0.48	120	0.63	60	62	0.91	1.14	1.58	0.08
Gill status	1.02	1.05	-0.98	120	0.33	60	62	0.13	0.22	2.81	0.00
Opercula	1.20	1.08	1.53	120	0.13	60	62	0.55	0.27	3.96	0.00
Mouth jaw wound	**1.03**	**1.85**	**-10.22**	**120**	**< 0.001**	**60**	**62**	**0.26**	**0.57**	**4.84**	**0.00**
Upper jaw deformation	1.00	1.00		120		60	62	0.00	0.00		
Lower jaw deformation	1.00	1.05	-0.98	120	0.33	60	62	0.00	0.38	0.00	1.00

Table 37. Fish Welfare (Breivik S): Student t-test on the parameters (cortisol, glucose, lactic acid, chloride, calcium and magnesium) measured at the facility Breivik S between the two samplings 24 June and 24 January. Bold font is indicating significant difference.

Variable	Mean 1	Mean 2	t-value	df	p	N1	N2	SD1	SD2	F-ratio	p-variance
Cortisol	9.482	9.154	0.140	54	0.889122	30	26	4.064	12.088	8.848	0.000
Glucose	**5.269**	**4.524**	**2.941**	**53**	**0.004837**	**30**	**25**	**1.142**	**0.596**	**3.678**	**0.002**
Lactic Acid	**5.351**	**3.777**	**5.996**	**47**	**0.000000**	**20**	**29**	**0.792**	**0.971**	**1.505**	**0.358**
Chloride	**128.517**	**124.600**	**2.244**	**57**	**0.028710**	**30**	**29**	**5.510**	**7.744**	**1.975**	**0.074**
Calcium	**3.480**	**2.510**	**22.789**	**57**	**0.000000**	**30**	**29**	**0.166**	**0.161**	**1.067**	**0.866**
Magnesium	**1.249**	**0.958**	**5.371**	**57**	**0.000002**	**30**	**29**	**0.176**	**0.237**	**1.808**	**0.119**

Table 38. Fish Welfare (Ihlholmen): Student t-test on the parameters (cortisol, glucose, lactic acid, chloride, calcium and magnesium measured at the facility Ihlholmen between the two samplings 24 June and 23 January. Bold font is indicating significant difference.

Variable	Mean 1	Mean 2	t-value	df	p	N1	N2	SD1	SD2	F-ratio	p-variance
Cortisol	7.617	11.144	-1.623	45	0.111611	30	17	7.111	7.250	1.039	0.897
Glucose	4.964	4.713	1.158	54	0.251969	28	28	0.857	0.768	1.246	0.572
Lactic Acid	**6.246**	**4.268**	**6.385**	**46**	**0.000000**	**19**	**29**	**0.757**	**1.201**	**2.515**	**0.045**
Chloride	120.211	124.741	-0.933	55	0.354748	28	29	24.561	8.816	7.761	0.000
Calcium	**3.257**	**2.522**	**11.937**	**55**	**0.000000**	**28**	**29**	**0.250**	**0.214**	**1.366**	**0.417**
Magnesium	**1.435**	**0.898**	**5.935**	**55**	**0.000000**	**28**	**29**	**0.339**	**0.344**	**1.031**	**0.938**